Biochimica et Biophysica Acta 1803 (2010) 191–200

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

The role of formins in filopodia formation

Harry Mellor *

Department of Biochemistry, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK

ARTICLE INFO

Article history:
Received 7 May 2008
Received in revised form 17 December 2008
Accepted 17 December 2008
Available online 3 January 2009

Keywords:
Actin
Cytoskeleton
Formin
Filopodia
Cell migration

ABSTRACT

Filopodia are highly dynamic cell-surface protrusions used by cells to sense their external environment. At the core of the filopodium is a bundle of actin filaments. These give form to the filopodia and also drive the cycle of elongation and retraction. Recent studies have shown that two very different actin nucleating proteins control the formation of filopodial actin filaments — Arp2/3 and Formins. Although the actin filaments produced by these two nucleators have very different structures and properties, recent work has begun to piece together evidence for co-operation between Arp2/3 and formins in filopodia formation, leading to a deeper understanding of these sensory organelles.

1. Introduction

Filopodia are needle-like, actin-rich protrusions from the cell surface, first observed in early studies of the morphology of neural growth cones [1], epithelial cells undergoing wound closure [2] and chick embryo cells in culture [3]. Filopodia are highly dynamic structures that extend and retract over a timeframe of 10s of seconds. Their function is not precisely defined; however, the consensus view is that they act as sensory organelles — exploring the external environment and probing for directional cues, matrix composition and the presence of other cells. Proving this is extremely difficult — treatments that perturb filopodia formation cause a loss of direction sensing in a number of conditions [4–9]; however, these perturbations also have general effects on the actin cytoskeleton and/or cell polarity, making it difficult to be sure that filopodial function is being examined directly.

Despite these experimental complications, much can be inferred from the cells types that produce filopodia and the contexts in which this happens. In almost all cases, these are cells that are actively exploring their environment and making tentative contacts with other cells and/or the substratum. The best-studied filopodia are those of nerve growth cones (Fig. 1). These extend towards the direction of chemotactic signals prior to turning of the growth cone and are therefore presumed to have a sensory, pathfinding function [4,10]. A similar situation exists at the tip of endothelial sprouts in angiogenesis, where the leading cell extends filopodia in the direction of the pro-angiogenic signal [7]. Growth cone filopodia can exert tension on the substratum and this has been proposed to contribute to growth cone navigation [11,12]. Growth cone filopodia contain integrins, allowing them to make adhesive contacts with the extracellular matrix and hence apply a traction force [13,14]. Filopodia are also produced during phagocytosis where they make initial contacts with the pathogen [15]. Like growth cone filopodia, phagocytic filopodia are able to exert contractile force and can use this to drag pathogens towards the phagocytic cell [16,17]. Filopodia are also found at the interface between neighbouring epithelial cells during the formation of adherens junctions [18,19]. These filopodia cluster the adhesion protein cadherin [18,19] and interdigitate with filopodia on the opposing cell to form a zipper-like structure that then resolves into a mature junction [19]. This zippering function is important during embryonic development for the closure of epithelial sheets — for example, during ventral enclosure in *C. elegans* [18] and dorsal closure in *Drosophila* [20]. Filopodia also mediate the joining of single epithelial cells in the end-to-end connection of branches that occurs during tracheal development [21,22]. In dorsal closure in *Drosophila*, the filopodia act to initiate junction formation [20] but also to facilitate the matching of segments across the embryo [23] — i.e. these filopodia display both mechanical and sensory roles.

The properties of filopodia allow them to act as highly adaptable sensory organelles. Their dynamic nature suits them to the role of exploration of the cell periphery. Their mechanical properties allow them to both probe the physical environment and also to apply traction force to surrounding objects. Critically, they are highly sensitive — the clustering of specific cellular receptors allows them to respond to a variety of signals; however, their morphology is itself adapted to perception. The high surface area to volume ratio of a filopodium means that relatively few activated signalling molecules

* Tel.: +44 117 33 12168; fax: +44 117 33 12174.
E-mail address: h.mellor@bristol.ac.uk.

0167-4889/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.12.018

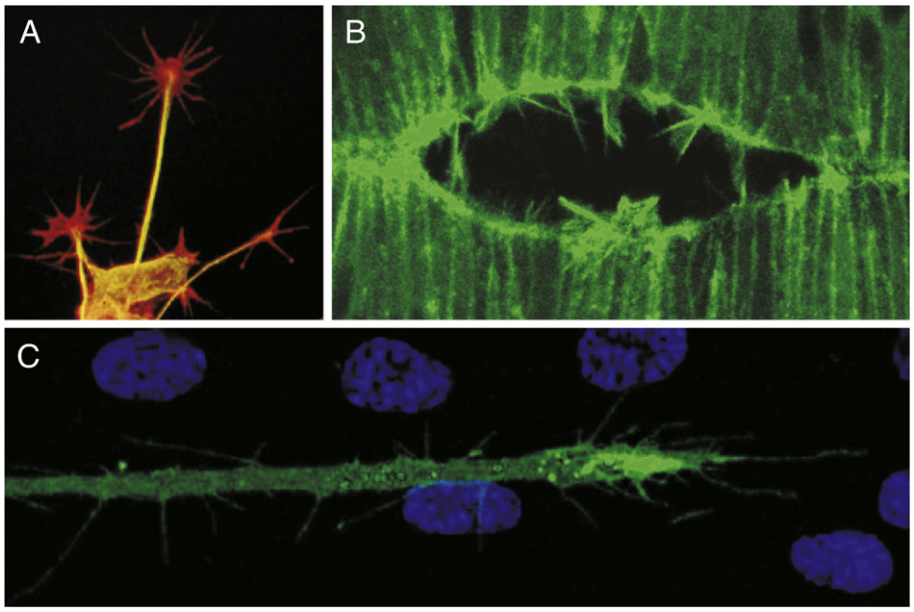


Fig. 1. Filopodia in context. (A) Filopodia projecting from a neuronal growth cone (Kate Nobes). (B) Filopodia projecting from the two epithelial sheets during dorsal closure in *Drosophia* (Sarah Woolner and Paul Martin). (C) Filopodia projecting from the tip cell at the front of an endothelial sprout during angiogenesis (Harry Mellor).

are required to achieve high local concentrations of signal. Kater et al. have proposed that this direct consequence of filopodial shape makes them inherently hypersensitive detectors of external signals [24].

2. The structure of filopodia

Filopodia are cylindrical protrusions approximately 100–200 nm in diameter and up to 10 μm or more in length. The shortest of these structures may barely protrude from the cell surface and these are sometimes referred to as 'microspikes' to discriminate them from longer filopodia. All filopodia contain a central core of around 10–30 actin filaments packed tightly together in a parallel array [25–27], forming the shaft of the filopodium (Fig. 2). Detailed electron tomography studies of *Dictyostelium* filopodia have shown that the average length of these shaft filaments is approximately 200 nm, meaning that overlapping filaments must be used to span the length of longer structures [26]. Filaments in the shaft have a uniform polarity, with the growing or 'barbed' end orientated towards the filopodial tip [25,28] These barbed ends terminate in a region called the tip complex — a collection of actin-binding proteins and filaments that can be seen as an electron-dense structure by EM [26,29]. At the base of the filopodium, the actin filaments are often routed deep into the web of actin that lies beneath the plasma membrane [25,29,30] (Fig. 2). The filopodia tip is the site of actin monomer addition to the actin filaments [31]. Filaments in the filopodium constantly cycle backwards toward the base through

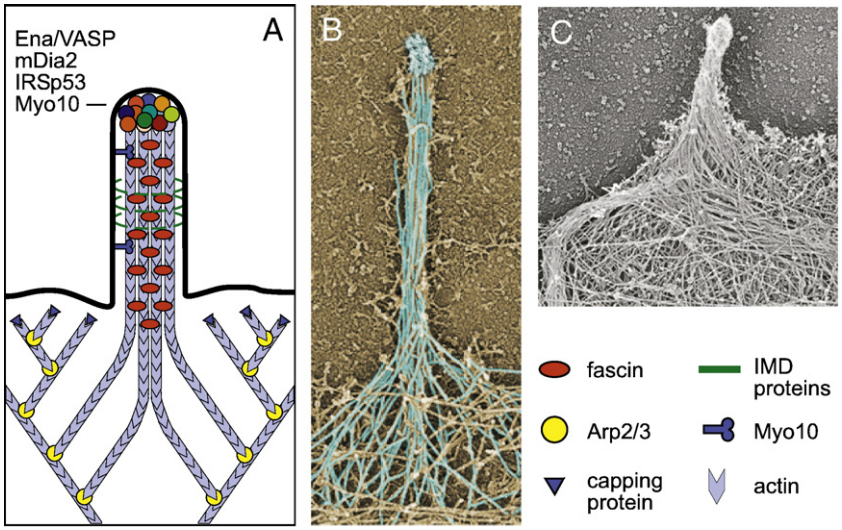


Fig. 2. Filopodial structure. (A) Schematic diagram of the structure of a filopodium. The central core is composed of a parallel bundle of actin filaments orientated with their plus or barbed ends towards the tip. The tip region contains a cluster of proteins including mDia2, Myo10, Ena/VASP proteins and IRSp53. The filaments in the stem are held together by the actin cross-linking protein fascin, and by links to the plasma membrane mediated by ERM proteins. IMD proteins line the interface between the actin bundle and the plasma membrane and help stabilise membrane curvature. At the base of the filopodium, the actin filaments splay out and are integrated into the dendritic network of actin below the plasma membrane. (B) An electron micrograph showing the organisation of actin filaments in a filopodium (Tanya Svitkina). The filaments in the filopodium are pseudocoloured blue to allow them to be traced against the background array of actin beneath the plasma membrane. The tip complex can be clearly seen as a density at the end of the filopodium. (C) An electron micrograph showing the arrangement of actin filaments in a filopodium from a cell with reduced fascin expression. In the absence of fascin, the filopodium buckles (Danijela Vignjevic; [38]).

myosin-dependent retrograde flow [31,32]. The rate of growth or retraction of a filopodium is governed by the balance between this rate of retrograde flow and the rate of polymerisation at the tip [33]. Typical filopodia will grow at a rate of approximately 0.2 μm/s until reaching critical length [33]. At this point, filopodia will frequently enter a rapid cycle of retraction; however, recent studies have shown that they can also give rise to other actin structures. Work from Small et al. has shown that filopodia can merge with the lamellipodium, either by folding and fusing or by being subsumed by the protrusive front [34]. Filopodia can fold sideways and fuse with the plasma membrane to give rise to contractile actin bundles along the cell edge. Filopodia can also fold backwards and fuse, seeding the formation of actin stress fibers [34].

2.1. Cross-linking proteins

The bundle of actin filaments in a filopodium is given mechanical cohesion by cross-links between neighbouring filaments, as well as cross-links between filaments and the plasma membrane. These bridges can be observed in high-resolution electron tomography images [26]. The best-characterised actin cross-linking protein in filopodia is fascin, which was first shown to be highly enriched in the filopodia of echinoderm coelomocytes [35,36], and which has subsequently been shown to be a component of filopodia from a wide range of sources [37]. Silencing of fascin expression by RNA interference leads to a reduction in filopodia number, suggesting that the protein also plays an important role in the formation of filopodia in addition to conferring mechanical stability [38]. Fascin binds at a relatively high density on filopodial filaments — once every 25–60 actin monomers; however, this binding is highly dynamic, with an off-rate of 0.12 s^{-1} [39]. This high off-rate is thought to allow fascin to diffuse towards the filopodia tip, fighting the tide of retrograde flow of filaments that has the effect of transporting filament-bound proteins in the opposite direction. Other filament cross-linking proteins that have been shown to localise to filopodia include α-actinin [40], fimbrin [41], and filamin [42,43]. In experiments that directly compared the localisation of exogenously expressed fascin, α-actinin, fimbrin, espin and filamin, only fascin and espin showed clear localisation to filopodia [38]. Despite this, it seems probably that, while fascin may be the main actin cross-linking protein in filopodia, other cross-linkers are used in specialised cell types and/or conditions — for example, filamin is recruited to the filopodia induced by Wnt5a signalling during development [42]. In all cases, filament cross-linking is critical to filopodia function as it imparts stiffness and cohesion to the structure, allowing an emerging filopodium to resist buckling forces as it deforms the plasma membrane [44]. This role is clearly illustrated in studies where fascin is depleted from cells and filopodia become severely deformed ([38]; Fig. 2).

As well as the cross-links that hold filaments in the filopodium together, the filament bundle must be tethered to the plasma membrane. The ezrin/radixin/moesin (ERM) family of proteins plays an important role in this. These proteins can bind both to actin filaments and to the plasma membrane [45]. Ezrin is a major component of brush border microvilli [46] — structures that bear some similarity to filopodia. Intestinal microvilli from ezrin$^{-/-}$ mice are shorter and thicker than normal [47]. Ezrin is less obviously localised to filopodia, although it is concentrated in the long filopodia induced by the Rif GTPase (HM, unpublished observations). Instead, the related protein moesin can be observed in filopodia from several sources, including growth cone filopodia [48,49].

2.2. The tip complex

At the filopodial tip is a complex containing many actin regulatory proteins. Amongst the first components of the tip complex to be identified were the Ena/VASP proteins. Mammals have three members of this family — Mena, VASP and Evl [50], and all three are concentrated at the tips of filopodia [50–53]. Loss of Ena/VASP function leads to a profound inhibition of filopodia formation in *Dictyostelium* [54] and in mammalian cells [55]. The roles of Ena/VASP proteins in filopodia formation are multiple and controversial. Work from several studies supports a role for Ena/VASP proteins in protecting filopodial actin filaments from capping [56–58]. In order for a filopodium to grow, the filament barbed ends at the tip must remain uncapped to allow the addition of actin monomers. In keeping with this, silencing of capping protein (CP) leads to a dramatic increase in filopodia formation [59]. Biochemical studies support a model where Ena/VASP proteins would physically block interaction with capping proteins, while being flexible enough to allow addition of actin monomers [56–58]. Other studies have presented evidence arguing against an anti-capping function [60,61], however, and the discussion remains open. In addition to their potential anti-capping activity, work in cells depleted of capping protein supports additional roles for Ena/VASP proteins in filopodia formation [62]. Ena/VASP proteins can bundle actin filaments [63–65] and this bundling activity has been shown to be important to filopodia formation [61]. Ena/VASP proteins concentrate at filopodia tip as it emerges [29]; however, unlike fascin, they localise only at the tip [61] where they are stably bound [62]. In this respect, it seems likely that fascin is required for the integrity of the filopodia shaft, whereas, Ena/VASP proteins hold together filaments at the tip [29]. Finally, Ena/VASP proteins also serve as a scaffold for many other actin-regulatory proteins [50]. The best-studied interaction is with profilin [66,67], an actin regulator that can lower the critical concentration of actin required for polymerisation [68] — a function that may help maintain rapid polymerisation at the tip.

2.3. IMD proteins

The plasma membrane at the filopodial tip is dramatically curved, as is the radius of the filopodial shaft. Recent studies have suggested a role for IMD-domain (IRSp53/missing-in-metastasis domain) containing proteins in supporting this curvature. Overexpression of the best-characterised IMD protein, IRSp53, causes filopodia formation [69,70] and IRSp53 is localised along the filopodium, with some concentration in the tip region [71]. The IMD domain of IRSp53 is structurally related to the BAR/F-BAR domains, which bind to membranes and induce curvature [72]. These domains are formed of a bundle of 6 α-helices that assemble into curved dimers with an asymmetrical distribution of positively-charged residues on the surface. The BAR/F-BAR domains are 'banana-shaped' with a concentration of positive charge on their concave surface. When they bind membranes along this surface they induce positive membrane curvature and the formation of membrane invaginations [72]. The IMD domains from IRSp53 and the related protein missing-in-metastasis (MIM) are 'zeppelin-shaped' [73,74] and the distribution of surface charge means that they induced negative curvature and the formation of membrane protrusions. Expression of the isolated IMD domain will induce filopodia in cells [75,76] and causes the formation of tube-like membrane protrusions *in vitro* [77,78]. The diameter of these protrusions (80 nm; [78]) is very similar to the diameter of a filopodium, suggesting that the curvature of the inside of a filopodial projection is a good match to that of the convex face of the IMD dimer. Previous studies have also identified an actin-bundling activity for the IMD domain, which as been suggested to underpin the stimulation of filopodia formation [74,75,79]. High-resolution imaging of the localisation of the isolated IMD domain of MIM shows that it surrounds the central F-actin core of the filament, but does not stain the filaments themselves [78]. Whether or not this is also true for the full-length IMD proteins is unclear. Taken together, one can imagine a model where bands of IMD proteins running beneath the plasma membrane,

perpendicular to the long axis, would support and/or induce the membrane curvature required for filopodia formation — rather like the circular ribs that support a tunnel (Fig. 2). These proteins may also further strengthen the filament by interacting with the actin filaments at the interface.

2.4. Motors and cargoes

The filopodium is such a confined space that it is hard to imagine free movement of proteins within it. Surprisingly, there is clear evidence for significant motor-based trafficking of receptors and integrins within the filopodium, with actin filaments being used as tracks. The best-characterised filopodial motor protein is myosin-X (Myo10), which is highly-concentrated at the filopodial tip [80]. Myo10 is a plus-end directed motor and exhibits bidirectional movement along filopodia. The fast (100 nm s^{-1}) forward movement of Myo10 towards the tip is presumed to be a function of its motor activity, whereas its slower (10–20 nm s^{-1}) backward movement is consistent with the motor being carried by retrograde flow [81]. Myo10 has a C-terminal FERM (Band 4.1/ezrin/radixin/moesin) domain that allows it to interact with cargo [82]. This domain binds to β-integrins and Myo10 transports β-integrins to the tips of filopodia [83]. In neurons this domain binds to the netrin-1 receptor; deleted-in-colorectal-cancer (DCC), and transports it into neurite filopodia [84]. In endothelial cells undergoing sprouting angiogenesis, the Myo10 cargo is the BMP6 (bone morphogenic protein 6) receptor, ALK6 [85]. In all of these cases, one can imagine that motor-based transport allows for the concentration of receptors in filopodia, increasing their sensitivity to external signals. In keeping with this, endothelial cells lacking Myo10 are no longer able to sense a gradient of BMP6 [85]. Myo10 also plays a direct role in the formation of filopodia. Overexpression of Myo10 will stimulate filopodia formation [81] and silencing of Myo10 using RNA interference leads to a dramatic reduction of filopodia emanating from the dorsal cell surface [86]. The Myo10 FERM domain is not required for the stimulation of filopodia formation by Myo10, suggesting that this is not a function of cargo transport [86,87].

3. Arp2/3 and the convergence model of filopodia formation

Filopodia formation requires *de novo* actin polymerisation [88]. An early assumption was that this would involve actin nucleation and interested settled first on the Cdc42-WASP-Arp2/3 axis. The small GTPase Cdc42 is an important regulator of filopodia and its activation leads to filopodia formation [89,90]. Cdc42 binds to many actin regulatory proteins, including the Wiskott–Aldrich syndrome protein (WASP) and the related protein N-WASP [91–93]. Early studies showed that overexpression of N-WASP enhanced the ability of Cdc42 to stimulate filopodia formation [94]. At the same time WASP was shown to be an activator of the Arp2/3 complex [95]. Arp2/3 is a highly-conserved complex of seven proteins that is able to nucleate the polymerisation of new actin filaments from the minus or 'pointed' end [96]. Arp2/3 binds to the side of pre-existing actin filaments, with new filaments growing from this junction at a characteristic angle of 70° [97]. The resulting branches can then also bind Arp2/3, allowing Arp2/3 to generate highly branched, 'dendritic' arrays of filaments [96]. Arp2/3 was shown to be required for Cdc42-induced actin polymerisation *in vitro* [98] and the final piece of the puzzle was put in place with the finding that Cdc42 activates Arp2/3 through N-WASP [99].

These studies explained how Cdc42 activation could lead to actin polymerisation; however, the highly-branched arrays of actin filaments produced by Arp2/3 are very different from the parallel bundles of actin seen in filopodia. The convergent elongation model of filopodia formation, originally proposed by Borisy and Svitkina, seeks to explain how these dendritic arrays could contribute to filopodia formation. Filopodia often arise from areas of flattened membrane protrusion called lamellipodia. These lamellipodia are supported by a highly-branched actin meshwork that is generated by Arp2/3 [100]. Short, nascent filopodia (microspikes) can be observed to form within the body of the lamellipodium, often as fishtail-shaped actin densities termed Λ-precursors ([29]; Fig. 3). Microspikes can move laterally within the lamellipodium and fuse together [101,102] — an event that often precedes elongation of the structure to a filopodium [29]. VASP and fascin appear at the tip of the Λ-precursor prior to elongation,

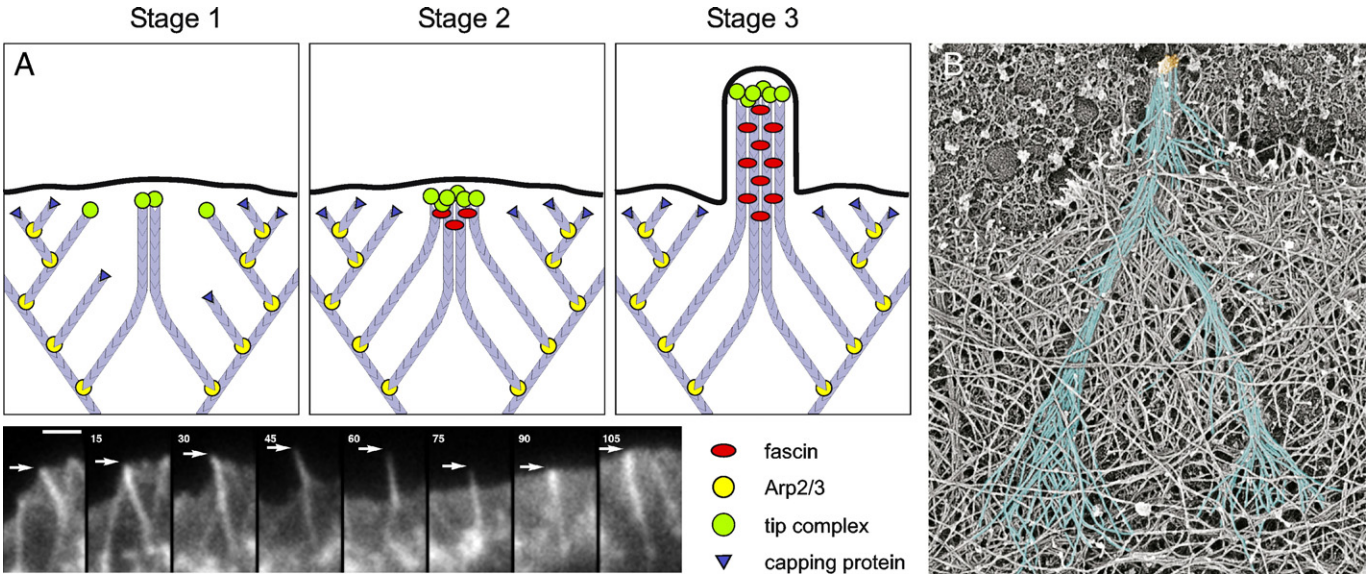


Fig. 3. The convergent elongation model of filopodia initiation. (A) Schematic model of convergent elongation. Stage 1 — filaments in the dendritic array of the lamellipodium may bind tip complex components that displace capping protein and bundle barbed ends together. Stage 2 — additional filaments are bundled together and may join the convergent structure through lateral movement of microspike bundles through the lamellipodium. The resultant deeply-rooted, fishtail-shaped structure is called a Λ-precursor. Stage 3 — in the absence of capping protein, the privileged barbed ends are able to elongate into a filopodium. The assembly of a critical number of filaments and the association of cross-linking proteins like fascin give the required rigidity to deform the plasma membrane. Below the diagram are frames from a time-lapse movie of the filopodial life-cycle. Cells transfected with GFP-actin were imaged over a time period of 105 s. The formation of a Λ-precursor is indicated at 15 s (arrow). The structure elongates to form a filopodium (60 s), which then retracts over 45 s. Bar = 2 μm. Images taken by Tanya Svitkina [29]. (B) An electron micrograph showing the arrangement of actin filaments in a nascent filopodium. The structure is deeply rooted into the lamellipodial actin network, giving rise to the characteristic Λ-precursor (Tanya Svitkina; [29]).

with some suggestion that VASP association precedes fascin [29]. High-resolution EM of the Λ-precursor structure shows how the nascent filopodium is routed deep within the lamellipodial actin network ([25,29]; Fig. 3). Early electron microscopy studies of the base of filopodia prompted Small to suggest that actin filaments in the lamellae might become bundled together to form filopodia [30]. The convergent elongation model of filopodia formation extends this idea provide a model for filopodial initiation [29]. The bundling activities of Ena/VASP proteins would cluster barbed filaments ends together and allow them to elongate preferentially by inhibiting capping. Fascin recruitment would then give rigidity to these elongating filaments, allowing them to push against the plasma membrane and emerge as a protrusive filopodium ([29]; Fig. 3). This model explains the structure of the Λ-precursor, which would correspond to Stage 2 in Fig. 3A. It would also explain why lateral fusion of microspikes in the lamellipodium often precedes filopodial extension — this would be a mechanism whereby pre-filopodial bundles could gain the requisite number of filaments to push effectively against the plasma membrane.

4. Formins and the tip nucleation model of filopodia formation

The ability to stimulate the nucleation of long, parallel actin filaments is a fundamental property of the formin family of actin regulatory proteins [96,103]. While the convergence model explains how a highly-branched network of filaments nucleated by Arp2/3 could be transformed into a linear filopodial bundle, formins seem to offer a simpler alternative — the direct formation of linear filaments at the extending filopodial tip. The breakthrough observation was made by Alberts et al., who showed that the formin mDia2 is localised at the tips of Cdc42-induced filopodia ([104]; Fig. 4). mDia2 (Drf3) is a member of the family of diaphanous-related formins (Drfs). These proteins all have a conserved N-terminal GBD (G-protein binding) domain through which they can bind Rho family GTPases [105,106]. Binding of the Rho GTPase to the GBD domain is thought to activate diaphanous-related formins by relieving an autoinhibitory interaction with the C-terminal DAD (diaphanous autoregulatory domain) [107–109]. mDia2 also contains a CRIB motif within the GBD domain, allowing it to interact specifically with the activated form of the Rho family member Cdc42 [104,106]. The localisation of mDia2 to filopodial tips does not depend on Cdc42 binding, however, as a truncation mutant of the protein lacking the GBD domain also localises there [104]. Instead, the hypothesis is that Cdc42 activates mDia2 in filopodia formation, allowing it to nucleate actin polymerisation from the filopodial tip. In keeping with this, inhibition of mDia2 function by interfering antibodies significantly inhibits filopodia formation by Cdc42 [104]. Subsequent work has broadened the role for mDia2 in the formation of filopodia. Pellegrin and Mellor have shown that mDia2 is also required for the formation of the long filopodia stimulated by the novel Rho family member, Rif [110]. Faix et al. have shown that the *Dictyostelium* orthologue of mDia2 is required for the formation of filopodia in this organism, where it signals downstream of the Rac1 GTPase [111]. In both cases, and in other studies [112,113], mDia2 is highly-concentrated at the filopodial tip (Fig. 4). Other Drfs may also play important roles in filopodia formation and we return to this later.

In a tip nucleation model of filopodia formation, mDia2 would drive the process from the top down, rather than from the base of the filopodium, as with the convergence model [114,115]. One can imagine how nucleation of linear actin filaments at the plasma membrane by mDia2 could lead to the formation of a filopodial protrusion. Formins like mDia2 nucleate actin filament formation by association with the barbed end [116,117]. They remain continuously associated with the barbed end as the filament elongates by moving processively along the filament [118–120]. This allows the continued stimulation of actin monomer addition and also physically protects the barbed end from capping [121–124] — an important property in filopodia formation. The processive movement of formins along the filament generates a force in the range of around 1.3 pN per actin filament [119]. The force required for an emerging filopodium to deform the membrane is estimated at approximately 10–20 pN for a bundle of 10–20 filaments [125]. Faix has proposed that the sum of the forces generated by mDia2 at the filopodial tip could make a significant contribution to membrane deformation [61].

4.1. Drfs are a nexus for Rho GTPase signalling

The Drf proteins contain an N-terminal GBD domain, which allows them to interact with Rho family GTPases. This domain was originally mapped as the minimal binding site for RhoA on mDia1 [126] and is a 198-residue footprint at the N-terminus of the protein (Fig. 5A). The GBD overlaps with the DID (diaphanous inhibitory domain) [108,127]. The DID domain forms an autoinhibitory interaction with the C-terminal DAD region that holds the protein in a closed inactive state where it cannot nucleate actin polymerisation [105,108]. The crystal structure of the complex between the mDia1 N-terminus and RhoC has been solved to a resolution of 3 Å [109]. In this complex, the Rho GTPase makes two sets of contacts: contacts with the G region N-terminal to the DID domain and contacts with the DID domain itself. Although the binding sites for Rho and DAD are not overlapping [109,127], the binding of RhoA and DAD to the DID domain is mutually exclusive [108,109]. This is thought to be due to electrostatic repulsion and steric clashes induced by Rho binding [128], allowing RhoA to

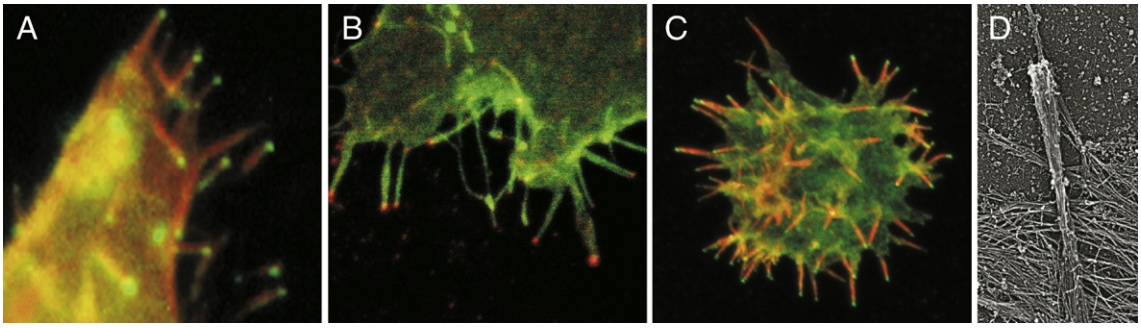
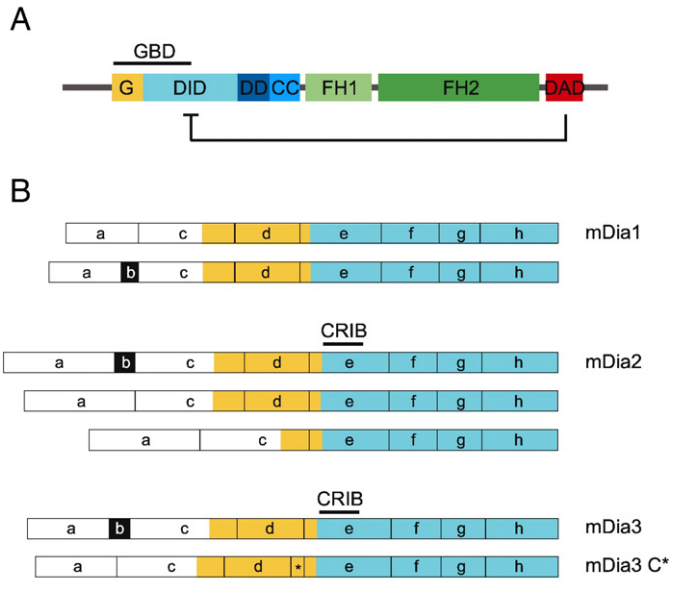


Fig. 4. Filopodia formation and mDia2. The figure shows the localisation of mDia2 in filopodia generated under different conditions. (A) Cells expressing activated Cdc42 (red) and GFP-mDia2 (green; Art Alberts). (B) NIH3T3 fibroblasts expressing activated Rif GTPase (green) and mDia2 (red). Activated Rif stimulates the formation of long filopodia that emanate mainly from the dorsal surface (Harry Mellor; [110]). (C) *Dictyostelium* expressing GFP-tagged dDia2 (green) and co-stained (red) for F-actin (Jan Faix; [114]). In all three cases, mDia2 can be seen concentrated to the filopodia tip. (D) An electron micrograph showing the arrangement of actin filaments in a filopodium driven by transfection of activated mDia2. The resultant structure is long, unusually club-shaped and contains free actin filament ends. It should be noted that this structure is likely to represent an exaggerated version of the mDia2-induced filopodia, as a result of the process being driven by an activated mDia2 mutant (Tanya Svitkina; [112]).

activate mDia1 by disrupting the autoinhibitory DID–DAD interaction [105].

The mammalian Drf protein family includes mDia1-3, DAAM1 (dishevelled-associated activator of morphogenesis-1), DAAM2 and the formin-related (FRL/FMNL) proteins FMLN1-3. The mDia1 protein binds RhoA and the two highly-related proteins RhoB and RhoC ([105,126]; Fig. 5C). Both mDia2 and mDia3 also bind RhoA [106,129], and mDia2 has been shown to bind RhoB [130]. It is highly-likely that all three mDia proteins bind RhoA, B and C. Similarly, DAAM1 binds to all three of these closely-related Rho GTPases [131,132]. Effector-binding domains for Rho GTPases tend to show specificity — for example, binding domains for RhoA usually will not bind to Cdc42. Early studies showed that mDia2 was unusually in this respect, in that it could bind to both RhoA and Cdc42 [106]. This property was made clearer by the identification of a CRIB motif within the DID domain of mDia2 [104] — a conserved binding motif for Cdc42 [133]. The CRIB domain also present in mDia3, which binds Cdc42 [129]; however, it is absent from mDia1, which does not [105]. Clearly, binding of Cdc42 to the DID domain has the potential to activate mDia proteins in the same way that RhoA binding does, although no work has been done on the structural basis of this interaction as yet. Wittinghofer et al. have proposed a two-step mechanism for mDia activation by RhoA, whereby RhoA first makes a low-affinity interaction with the GBD and then a second interaction with the DID domain that would lead to displacement of the DAD region [128]. It is possible that RhoA and Cdc42 could act co-operatively to activate mDia2 by making separate, concerted interactions with the GBD.



C

Drf	Gene	Chr.	Known partners	Refs
mDia1	DIAPH1	5	RhoA,B,C	[106,127]
mDia2	DIAPH3	13	RhoA,B, Cdc42, Rif	[107,108,111]
mDia3	DIAPH2	X	RhoA, Cdc42	[130]
mDia3 C*	DIAPH2	X	RhoD	[135]
DAAM1	DAAM1	14	RhoA,B,C, Cdc42	[153]
FRLα	FMNL1	17	Rac1, Cdc42	[152]
FHOD1	FHOD1	16	Rac1	[154,155]

Fig. 5. Drf protein structure and the GBD domain. (A) Cartoon of mDia protein structure. Each protein contains an N-terminal GBD (GTPase binding domain). The overlaps the G-protein domain and the DID domain. The DID domain forms an autoinhibitory interaction with the C-terminal DAD domain that is broken by Rho GTPase binding to the GBD. When the autoinhibition is released, the FH2 domain is free to nucleate the polymerisation of actin filaments. The FH1 domain binds profilin and can deliver actin monomers to the FH2 domain. mDia proteins dimerise through the DD domain. (B) Alternative splicing of the GBD domain. The block diagrams show the exon structure of the GBD for mDia1-3. Each GBD is coded by 7 exons (a–h) with highly-conserved splice boundaries. The colouring shows the boundaries of the G-protein and DID domains (same as panel A). The splice form commonly used experimentally is indicated for each mDia protein. Examination of the public sequence databases shows that mDia1 is present as at least 2 splice forms, mDia2 as 3 and mDia3 as 2. A common event is inclusion/exclusion of exon b, which codes for 9–11 residues immediately preceding the GBD. mDia3C (*confusingly, also called hDia2C) has an additional exon between exons d and e (asterisk). This isoform shows specific binding to RhoD. (C) Members of the mammalian Drf protein family and their known Rho GTPase binding partners [151–154]. Gene names and chromosome numbers are for reference and correspond to the human sequences.

The interactions between Rho GTPases and Drfs appear to be further regulated by alternative splicing. The GBD and upstream N-terminal region of mDia proteins is coded by 8–9 exons with highly conserved splice boundaries (Fig. 5B). All three proteins show alternate splicing within this N-terminal region. Examination of the public sequence databases shows that alternate splicing of the second exon is a frequent event. The exon encodes a short peptide sequence of 9–11 residues immediately before the GBD. All three mDias can be expressed as proteins with or without this insert. mDia2 can also be expressed as a splice form lacking the forth exon. This results in loss of most of the low-affinity Rho binding region; however, the Cdc42-binding CRIB motif is retained (Fig. 5B). These multiple splicing events are intriguing as they suggest ways of adapting the specificity of the mDia GBD. So far, the only information we have on the role of alternative splicing of the GBD comes from work done with splice forms of mDia3. Zerial et al. showed that mDia3 is a binding partner for the RhoD GTPase — but this is dependent on alternative splicing. They identified two isoforms of mDia3; confusingly, they termed these hDia2B and hDia2C. hDia2C lacks exon b, but instead has an additional short exon immediately after exon d (Fig. 5B). Only the hDia2C isoform binds to RhoD [134], suggesting that specificity comes from gain of the additional exon in the G domain and/or loss of exon b. The most commonly studied isoform of mDia1 lacks exon b; whereas the most commonly studied isoform of mDia2 contains this insertion. As both proteins bind RhoA, it would seem that exon b is not required for RhoA binding *per se*. The conservation of this exon and the splicing event between all three mDia proteins strongly suggests that it plays a role in protein–protein interaction in some way — either in modulating Rho binding or interacting with another binding partner.

4.2. Why don't all Drfs make filopodia?

Cdc42 binds to mDia2 to induce filopodia [104]. RhoA binds to mDia1 to stimulate the nucleation of actin filaments used to construct actin stress fibers [126,135,136]. In both cases, a membrane-anchored Rho GTPase is binding to the GBD of Drf protein to relieve autoinhibition and allow nucleation of linear actin filaments — so why doesn't RhoA/mDia1 produce filopodia? The answer is that it does – or at least it can, depending on context. In epithelial cells, RhoA signalling through mDia1 stabilises cell–cell junctions [137]. In cells treated with blebbistatin to inhibit myosin II, actin stress fibers are lost and mDia1 is seen in filopodia localised at the cell–cell interface [138]. Filopodia formation precedes adherens junction formation between epithelial sheets and these filopodia zipper together to initiate junction formation [18–20]. The filopodia seen on blebbistatin treatment are enriched in the adherens junction component E-cadherin and localise mDia1 to their tips [138]. This suggests that they might correspond to the filopodia involved in junction formation. In MTLn3 carcinoma cells, siRNA silencing of a combination of WAVE2 and N-WASP causes the cells to form numerous filopodia. Formation of these filopodia is dependent on mDia1 (but not mDia2) and also requires the activity of RhoA [139]. In both studies, cells are highly perturbed by the experimental conditions and it is possible that mDia1 does not play an active role in filopodia formation normally — but both studies clearly demonstrate that RhoA/mDia1 can stimulate filopodia formation. Both RhoA and Cdc42 recruit mDia proteins to the plasma membrane; however, the signalling environment will be

different in each case. Local concentrations of activated RhoA would be expected to recruit other RhoA effectors involved in stress fiber formation like ROCK [140,141], whereas patches of active Cdc42 would recruit other filopodia regulators like IRSp53 [142]. The loss of stress fibers caused by blebbistatin treatment may free mDia from this pathway, allowing it to participate in filopodia formation. So, the outcome of mDia activation may depend on the community of signalling proteins around the formin — a factor determined by the activating signal.

5. Towards an integrated model of filopodia formation

Currently we have two models describing the initiation of filopodia formation — is this one model too many, or can both be integrated into a more complete description of the process? For each model, there is experimental work that suggests the need for some modification to the initial hypothesis:

Cells where the Arp2/3 regulator N-WASP has been genetically deleted can still make filopodia, although the number of filopodia induced in response to activated Cdc42 is reduced by at least 50% [143,144]. Targeting of Arp2/3 using RNA interference appears to have different effects on filopodia formation in different cell types. In mouse embryonic fibroblasts [145] and in the B16-F1 mouse melanoma cell line [146] siRNA silencing of the Arp2/3 complex has little or no effect on the formation of filopodia — either in unperturbed cells [145] or in cells expressing activated Cdc42 [146]. In nerve growth cones, however, siRNA silencing of Arp2/3 significantly (but not completely) suppresses filopodia formation [147]. The overall message seems to be that Arp2/3 is not absolutely required for filopodia formation, but that it has a significant role — possibly dependent on the cell type and conditions. Borisy et al. have recently refined their convergence model of filopodia into the 'cascade pathway model'. In this model, the dendritic network of filaments in the lamellipodium acts as a seedbed for filopodia formation — i.e. the role of Arp2/3 is to provide filaments that may then be used to make filopodia, rather than to drive filopodia formation directly. This is supported by work in insect cells that shows that inhibiting lamellipodia formation by targeting the Arp2/3 activator WAVE/SCAR also leads to an inhibition of filopodia formation [148]. Two recent studies using electron microscopy to study the arrangement of actin filaments in filopodia shed further light on the role of lamellipodial actin filaments. Actin filaments emerging from the base of filopodia can be seen to originate from branch points that are decorated with antibodies to the Arp2/3 complex, proving that the dendritic array *can* provide filaments used to make filopodia [147]. In contrast, examination of the base of filopodia generated in the absence of Arp2/3 shows that these structures are not rooted in the subplasmalemal actin array — i.e. convergent elongation is not *necessary* for filopodia formation [146].

In a tip nucleation model of filopodia formation, mDia2 sitting in the tip complex would stimulate the nucleation and elongation of filaments as the filopodium grew. Electron micrographs of filopodia formed in cells expressing activated mutants of mDia2 show long, club-like structures. The stems of these filopodia frequently contain free filament ends that do not reach to the base ([112]; Fig. 4D). Both observations are consistent with continued nucleation of actin filaments from the tip as the filopodium elongates, with the activated mDia2 leading to filopodia with additional filaments at the distal end (club-shaped) that do not extend all of the way to the base (free ends). Filopodia induced by the Rif GTPase are very long and frequently emerge from the dorsal surface — i.e. not from the lamellipodium [110]. Similarly, RhoD, which is a binding partner for mDia3, produces long filopodia when expressed in cells [149]. These two Rho GTPases would seem to produce filopodia with 'pure' mDia2 characteristics. In the long filopodia produced by mDia2 in *Dictyostelium*, the stem is composed of filaments of approximately 200 nm in length [26]. In these longer filopodia, continued filament production from the tip would give rise to the overlapping filaments needed to span these structures.

An interesting recent twist is the finding that mDia2 is also required for efficient formation of lamellipodia [112]. Live-cell imaging shows that mDia2 is present along the edge of the lamellipodium and that this staining condenses into localised spots prior to the emergence of filopodia [112] — similar to the behaviour of Ena/VASP proteins during convergent elongation [29]. Interestingly, loss of mDia2 also leads to a loss of Ena/VASP recruitment to the lamellipodium [29]. VASP is a known binding partner of mDia2 and Faix et al. have shown that the bundling activity of VASP is critical to filopodia formation by mDia2 in *Dictyostelium* [61]. It would seem that mDia2 might also be wired into the convergent elongation model, with a role in bundling and protecting barbed ends at the initiation of filopodial protrusion.

How might we combine these observations into a unified model? The simplest explanation is that both Arp2/3 and mDia2 play important roles in filopodia formation. It is possible that the contribution of Arp2/3 is more strongly felt in the formation of short filopodia or microspikes, whereas the actions of mDia2 might predominate in the extension of longer structures, where continued nucleation of filaments is required. The balance of the two activities may alter in different cell types and different conditions; however, it seems unlikely that the two activities are truly separable. This ability to change the properties of filopodia is likely to have important biological consequences — filopodia vary in length and form, allowing them to fulfil their specialised roles [150]. Clearly, control over filopodial structure and dynamics must underpin this.

6. Conclusions and perspectives

When trying to understand how filopodia formation is regulated, one important conceptual point is that overexpression of any of the individual components (WASP, mDia2, fascin, IMD proteins, Myo10) drives the process. It appears that the cell is poised to make filopodia and that changing the balance of any of the mechanistic components is enough to cause these structures to form. In this respect, it is probably unproductive to look for primary initiators of the process, or to attempt to rank components as being more or less important. Co-operative models like the convergence/cascade model of filopodia formation would seem to fit with the stochastic nature of filopodia formation, and to have a sense of similarity to other complex cytoskeletal behaviours — like the turning of lamellipodia or the breaking of symmetry in migrating cells. Such models also allow us to imagine how cells could blend together components to alter the properties of filopodia — for example, adding more active Drfs for extra length, or more cross-linking proteins for extra strength. Experiments designed to look at the contributions of individual filopodial components within the context of the ensemble would seem to be important at this stage. Comparing differences between filopodia that are involved in physiological processes (growth cones, dorsal closure, etc) would also seem important, and to be the route to a deeper understanding of these fascinating structures.

Acknowledgements

The author would like to thank Art Alberts, Gary Borisy, Jan Faix, Paul Martin, Kate Nobes, Tanya Svitkina, Danijela Vignjevic and Sarah Woolner for permission to reproduce the micrographs of filopodia shown in this review.

References

[1] R.G. Harrison, The outgrowth of the nerve fiber as a mode of protoplasmic movement, J. Exp. Zool. 9 (1910) 787–848.
[2] K.M. Wilbur, R. Chambers, Cell movements in the healing of microwounds in vitro, J. Exp. Zool. 91 (1942) 287–302.
[3] K.R. Porter, A. Claude, E.F. Fullam, A study of tissue culture cells by electron microscopy, J. Exp. Med. 81 (1945) 233–246.

[4] J.Q. Zheng, J.J. Wan, M.M. Poo, Essential role of filopodia in chemotropic turning of nerve growth cone induced by a glutamate gradient, J. Neurosci. 16 (1996) 1140–1149.
[5] D. Bentley, A. Toroian-Raymond, Disoriented pathfinding by pioneer neurone growth cones deprived of filopodia by cytochalasin treatment, Nature 323 (1986) 712–715.
[6] W.E. Allen, D. Zicha, A.J. Ridley, G.E. Jones, A role for Cdc42 in macrophage chemotaxis, J. Cell. Biol. 141 (1998) 1147–1157.
[7] H. Gerhardt, M. Golding, M. Fruttiger, C. Ruhrberg, A. Lundkvist, A. Abramsson, M. Jeltsch, C. Mitchell, K. Alitalo, D. Shima, C. Betsholtz, VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia, J. Cell. Biol. 161 (2003) 1163–1177.
[8] A.M. Rajnicek, L.E. Foubister, C.D. McCaig, Growth cone steering by a physiological electric field requires dynamic microtubules, microfilaments and Rac-mediated filopodial asymmetry, J. Cell. Sci. 119 (2006) 1736–1745.
[9] C.B. Chien, D.E. Rosenthal, W.A. Harris, C.E. Holt, Navigational errors made by growth cones without filopodia in the embryonic *Xenopus* brain, Neuron 11 (1993) 237–251.
[10] R.W. Gundersen, J.N. Barrett, Characterization of the turning response of dorsal root neurites toward nerve growth factor, J. Cell. Biol. 87 (1980) 546–554.
[11] P.C. Bridgman, S. Dave, C.F. Asnes, A.N. Tullio, R.S. Adelstein, Myosin IIB is required for growth cone motility, J. Neurosci. 21 (2001) 6159–6169.
[12] S.R. Heidemann, P. Lamoureux, R.E. Buxbaum, Growth cone behavior and production of traction force, J. Cell. Biol. 111 (1990) 1949–1957.
[13] P.C. Letourneau, T.A. Shattuck, Distribution and possible interactions of actin-associated proteins and cell adhesion molecules of nerve growth cones, Development 105 (1989) 505–519.
[14] C.G. Galbraith, K.M. Yamada, J.A. Galbraith, Polymerizing actin fibers position integrins primed to probe for adhesion sites, Science 315 (2007) 992–995.
[15] H.K. Koerten, J.S. Ploem, W.T. Daems, Ingestion of latex beads by filopodia of adherent mouse peritoneal macrophages. A scanning electron microscopical and reflection contrast microscopical study, Exp. Cell. Res. 128 (1980) 470–475.
[16] H. Kress, E.H. Stelzer, D. Holzer, F. Buss, G. Griffiths, A. Rohrbach, Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 11633–11638.
[17] L. Vonna, A. Wiedemann, M. Aepfelbacher, E. Sackmann, Micromechanics of filopodia mediated capture of pathogens by macrophages, Eur. Biophys. J. 36 (2007) 145–151.
[18] W.B. Raich, C. Agbunag, J. Hardin, Rapid epithelial-sheet sealing in the *Caenorhabditis elegans* embryo requires cadherin-dependent filopodial priming, Curr. Biol. 9 (1999) 1139–1146.
[19] V. Vasioukhin, C. Bauer, M. Yin, E. Fuchs, Directed actin polymerization is the driving force for epithelial cell–cell adhesion, Cell 100 (2000) 209–219.
[20] A. Jacinto, W. Wood, T. Balayo, M. Turmaine, A. Martinez-Arias, P. Martin, Dynamic actin-based epithelial adhesion and cell matching during *Drosophila* dorsal closure, Curr. Biol. 10 (2000) 1420–1426.
[21] C. Samakovlis, G. Manning, P. Steneberg, N. Hacohen, R. Cantera, M.A. Krasnow, Genetic control of epithelial tube fusion during *Drosophila* tracheal development, Development 122 (1996) 3531–3536.
[22] M. Tanaka-Matakatsu, T. Uemura, H. Oda, M. Takeichi, S. Hayashi, Cadherin-mediated cell adhesion and cell motility in *Drosophila* trachea regulated by the transcription factor Escargot, Development 122 (1996) 3697–3705.
[23] T.H. Millard, P. Martin, Dynamic analysis of filopodial interactions during the zippering phase of *Drosophila* dorsal closure, Development 135 (2008) 621–626.
[24] R.W. Davenport, P. Dou, V. Rehder, S.B. Kater, A sensory role for neuronal growth cone filopodia, Nature 361 (1993) 721–724.
[25] A.K. Lewis, P.C. Bridgman, Nerve growth cone lamellipodia contain two populations of actin filaments that differ in organization and polarity, J. Cell. Biol. 119 (1992) 1219–1243.
[26] O. Medalia, M. Beck, M. Ecke, I. Weber, R. Neujahr, W. Baumeister, G. Gerisch, Organization of actin networks in intact filopodia, Curr. Biol. 17 (2007) 79–84.
[27] K. Katoh, K. Hammar, P.J. Smith, R. Oldenbourg, Arrangement of radial actin bundles in the growth cone of *Aplysia* bag cell neurons shows the immediate past history of filopodial behavior, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 7928–7931.
[28] J.V. Small, G. Isenberg, J.E. Celis, Polarity of actin at the leading edge of cultured cells, Nature 272 (1978) 638–639.
[29] T.M. Svitkina, E.A. Bulanova, O.Y. Chaga, D.M. Vignjevic, S. Kojima, J.M. Vasiliev, G.G. Borisy, Mechanism of filopodia initiation by reorganization of a dendritic network, J. Cell. Biol. 160 (2003) 409–421.
[30] J.V. Small, Organization of actin in the leading edge of cultured cells: influence of osmium tetroxide and dehydration on the ultrastructure of actin meshworks, J. Cell. Biol. 91 (1981) 695–705.
[31] S. Okabe, N. Hirokawa, Actin dynamics in growth cones, J. Neurosci. 11 (1991) 1918–1929.
[32] C.H. Lin, E.M. Espreafico, M.S. Mooseker, P. Forscher, Myosin drives retrograde F-actin flow in neuronal growth cones, Neuron 16 (1996) 769–782.
[33] A. Mallavarapu, T. Mitchison, Regulated actin cytoskeleton assembly at filopodium tips controls their extension and retraction, J. Cell. Biol. 146 (1999) 1097–1106.
[34] M. Nemethova, S. Auinger, J.V. Small, Building the actin cytoskeleton: filopodia contribute to the construction of contractile bundles in the lamella, J. Cell. Biol. 180 (2008) 1233–1244.
[35] D.J. DeRosier, K.T. Edds, Evidence for fascin cross-links between the actin filaments in coelomocyte filopodia, Exp. Cell. Res. 126 (1980) 490–494.
[36] J.J. Otto, R.E. Kane, J. Bryan, Formation of filopodia in coelomocytes: localization of fascin, a 58,000 Dalton actin cross-linking protein, Cell 17 (1979) 285–293.
[37] N. Kureishy, V. Sapountzi, S. Prag, N. Anilkumar, J.C. Adams, Fascins, and their roles in cell structure and function, Bioessays 24 (2002) 350–361.
[38] D. Vignjevic, S. Kojima, Y. Aratyn, O. Danciu, T. Svitkina, G.G. Borisy, Role of fascin in filopodial protrusion, J. Cell. Biol. 174 (2006) 863–875.
[39] Y.S. Aratyn, T.E. Schaus, E.W. Taylor, G.G. Borisy, Intrinsic dynamic behavior of fascin in filopodia, Mol. Biol. Cell 18 (2007) 3928–3940.
[40] K. Sobue, K. Kanda, Alpha-actinins, calspectin (brain spectrin or fodrin), and actin participate in adhesion and movement of growth cones, Neuron 3 (1989) 311–319.
[41] A. Bretscher, K. Weber, Fimbrin, a new microfilament-associated protein present in microvilli and other cell surface structures, J. Cell. Biol. 86 (1980) 335–340.
[42] M. Nishita, S.K. Yoo, A. Nomachi, S. Kani, N. Sougawa, Y. Ohta, S. Takada, A. Kikuchi, Y. Minami, Filopodia formation mediated by receptor tyrosine kinase Ror2 is required for Wnt5a-induced cell migration, J. Cell. Biol. 175 (2006) 555–562.
[43] Y. Ohta, N. Suzuki, S. Nakamura, J.H. Hartwig, T.P. Stossel, The small GTPase RalA targets filamin to induce filopodia, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 2122–2128.
[44] A. Mogilner, B. Rubinstein, The physics of filopodial protrusion, Biophys. J. 89 (2005) 782–795.
[45] B. Fievet, D. Louvard, M. Arpin, ERM proteins in epithelial cell organization and functions, Biochim. Biophys. Acta 1773 (2007) 653–660.
[46] A. Bretscher, Purification of an 80,000-dalton protein that is a component of the isolated microvillus cytoskeleton, and its localization in nonmuscle cells, J. Cell. Biol. 97 (1983) 425–432.
[47] I. Saotome, M. Curto, A.I. McClatchey, Ezrin is essential for epithelial organization and villus morphogenesis in the developing intestine, Dev. Cell. 6 (2004) 855–864.
[48] M.R. Amieva, H. Furthmayr, Subcellular localization of moesin in dynamic filopodia, retraction fibers, and other structures involved in substrate exploration, attachment, and cell–cell contacts, Exp. Cell. Res. 219 (1995) 180–196.
[49] H. Furthmayr, W. Lankes, M. Amieva, Moesin, a new cytoskeletal protein and constituent of filopodia: its role in cellular functions, Kidney. Int. 41 (1992) 665–670.
[50] M. Krause, E.W. Dent, J.E. Bear, J.J. Loureiro, F.B. Gertler, Ena/VASP proteins: regulators of the actin cytoskeleton and cell migration, Annu. Rev. Cell. Dev. Biol. 19 (2003) 541–564.
[51] L.M. Lanier, M.A. Gates, W. Witke, A.S. Menzies, A.M. Wehman, J.D. Macklis, D. Kwiatkowski, P. Soriano, F.B. Gertler, Mena is required for neurulation and commissure formation, Neuron 22 (1999) 313–325.
[52] K. Rottner, B. Behrendt, J.V. Small, J. Wehland, VASP dynamics during lamellipodia protrusion, Nat. Cell. Biol. 1 (1999) 321–322.
[53] F. Castellano, P. Montcourrier, J.C. Guillemot, E. Gouin, L. Machesky, P. Cossart, P. Chavrier, Inducible recruitment of Cdc42 or WASP to a cell-surface receptor triggers actin polymerization and filopodium formation, Curr. Biol. 9 (1999) 351–360.
[54] Y.H. Han, C.Y. Chung, D. Wessels, S. Stephens, M.A. Titus, D.R. Soll, R.A. Firtel, Requirement of a vasodilator-stimulated phosphoprotein family member for cell adhesion, the formation of filopodia, and chemotaxis in *Dictyostelium*, J. Biol. Chem. 277 (2002) 49877–49887.
[55] C. Lebrand, E.W. Dent, G.A. Strasser, L.M. Lanier, M. Krause, T.M. Svitkina, G.G. Borisy, F.B. Gertler, Critical role of Ena/VASP proteins for filopodia formation in neurons and in function downstream of netrin-1, Neuron 42 (2004) 37–49.
[56] M. Barzik, T.I. Kotova, H.N. Higgs, L. Hazelwood, D. Hanein, F.B. Gertler, D.A. Schafer, Ena/VASP proteins enhance actin polymerization in the presence of barbed end capping proteins, J. Biol. Chem. 280 (2005) 28653–28662.
[57] J.E. Bear, T.M. Svitkina, M. Krause, D.A. Schafer, J.J. Loureiro, G.A. Strasser, I.V. Maly, O.Y. Chaga, J.A. Cooper, G.G. Borisy, F.B. Gertler, Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility, Cell 109 (2002) 509–521.
[58] L. Pasic, T.I. Kotova, D.A. Schafer, Ena/VASP proteins capture actin filament barbed ends, J. Biol. Chem. 11 (2008) 9814–9819.
[59] M.R. Mejillano, S. Kojima, D.A. Applewhite, F.B. Gertler, T.M. Svitkina, G.G. Borisy, Lamellipodial versus filopodial mode of the actin nanomachinery: pivotal role of the filament barbed end, Cell 118 (2004) 363–373.
[60] S. Samarin, S. Romero, C. Kocks, D. Didry, D. Pantaloni, M.F. Carlier, How VASP enhances actin-based motility, J. Cell. Biol. 163 (2003) 131–142.
[61] A. Schirenbeck, R. Arasada, T. Bretschneider, T.E. Stradal, M. Schleicher, J. Faix, The bundling activity of vasodilator-stimulated phosphoprotein is required for filopodium formation, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 7694–7699.
[62] D.A. Applewhite, M. Barzik, S. Kojima, T.M. Svitkina, F.B. Gertler, G.G. Borisy, Ena/VASP proteins have an anti-capping independent function in filopodia formation, Mol. Biol. Cell 18 (2007) 2579–2591.
[63] V. Laurent, T.P. Loisel, B. Harbeck, A. Wehman, L. Grobe, B.M. Jockusch, J. Wehland, F.B. Gertler, M.F. Carlier, Role of proteins of the Ena/VASP family in actin-based motility of *Listeria* monocytogenes, J. Cell. Biol. 144 (1999) 1245–1258.
[64] S. Huttelmaier, O. Mayboroda, B. Harbeck, T. Jarchau, B.M. Jockusch, M. Rudiger, The interaction of the cell-contact proteins VASP and vinculin is regulated by phosphatidylinositol-4,5-bisphosphate, Curr. Biol. 8 (1998) 479–488.
[65] C. Bachmann, L. Fischer, U. Walter, M. Reinhard, The EVH2 domain of the vasodilator-stimulated phosphoprotein mediates tetramerization, F-actin binding, and actin bundle formation, J. Biol. Chem. 274 (1999) 23549–23557.
[66] F.B. Gertler, K. Niebuhr, M. Reinhard, J. Wehland, P. Soriano, Mena, a relative of VASP and *Drosophila* Enabled, is implicated in the control of microfilament dynamics, Cell 87 (1996) 227–239.
[67] M. Reinhard, K. Giehl, K. Abel, C. Haffner, T. Jarchau, V. Hoppe, B.M. Jockusch, U. Walter, The proline-rich focal adhesion and microfilament protein VASP is a ligand for profilins, EMBO J. 14 (1995) 1583–1589.
[68] E.G. Yarmola, M.R. Bubb, Profilin: emerging concepts and lingering misconceptions, Trends. Biochem. Sci. 31 (2006) 197–205.

[69] S. Krugmann, I. Jordens, K. Gevaert, M. Driessens, J. Vandekerckhove, A. Hall, Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex, Curr. Biol. 11 (2001) 1645–1655.
[70] S. Govind, R. Kozma, C. Monfries, L. Lim, S. Ahmed, Cdc42Hs facilitates cytoskeletal reorganization and neurite outgrowth by localizing the 58-kD insulin receptor substrate to filamentous actin, J. Cell. Biol. 152 (2001) 579–594.
[71] H. Nakagawa, H. Miki, M. Nozumi, T. Takenawa, S. Miyamoto, J. Wehland, J.V. Small, IRSp53 is colocalised with WAVE2 at the tips of protruding lamellipodia and filopodia independently of Mena, J. Cell. Sci. 116 (2003) 2577–2583.
[72] G.O. Cory, P.J. Cullen, Membrane curvature: the power of bananas, zeppelins and boomerangs, Curr. Biol. 17 (2007) R455–R457.
[73] S.H. Lee, F. Kerff, D. Chereau, F. Ferron, A. Klug, R. Dominguez, Structural basis for the actin-binding function of missing-in-metastasis, Structure 15 (2007) 145–155.
[74] T.H. Millard, G. Bompard, M.Y. Heung, T.R. Dafforn, D.J. Scott, L.M. Machesky, K. Futterer, Structural basis of filopodia formation induced by the IRSp53/MIM homology domain of human IRSp53, EMBO J. 24 (2005) 240–250.
[75] A. Yamagishi, M. Masuda, T. Ohki, H. Onishi, N. Mochizuki, A novel actin bundling/filopodium-forming domain conserved in insulin receptor tyrosine kinase substrate p53 and missing in metastasis protein, J. Biol. Chem. 279 (2004) 14929–14936.
[76] G. Bompard, S.J. Sharp, G. Freiss, L.M. Machesky, Involvement of Rac in actin cytoskeleton rearrangements induced by MIM-B, J. Cell. Sci. 118 (2005) 5393–5403.
[77] S. Suetsugu, K. Murayama, A. Sakamoto, K. Hanawa-Suetsugu, A. Seto, T. Oikawa, C. Mishima, M. Shirouzu, T. Takenawa, S. Yokoyama, The RAC binding domain/IRSp53-MIM homology domain of IRSp53 induces RAC-dependent membrane deformation, J. Biol. Chem. 281 (2006) 35347–35358.
[78] P.K. Mattila, A. Pykalainen, J. Saarikangas, V.O. Paavilainen, H. Vihinen, E. Jokitalo, P. Lappalainen, Missing-in-metastasis and IRSp53 deform PI(4,5)P2-rich membranes by an inverse BAR domain-like mechanism, J. Cell. Biol. 176 (2007) 953–964.
[79] R. Gonzalez-Quevedo, M. Shoffer, L. Horng, A.E. Oro, Receptor tyrosine phosphatase-dependent cytoskeletal remodeling by the hedgehog-responsive gene MIM/BEG4, J. Cell. Biol. 168 (2005) 453–463.
[80] J.S. Berg, B.H. Derfler, C.M. Pennisi, D.P. Corey, R.E. Cheney, Myosin-X, a novel myosin with pleckstrin homology domains, associates with regions of dynamic actin, J. Cell. Sci. 113 (Pt 19) (2000) 3439–3451.
[81] J.S. Berg, R.E. Cheney, Myosin-X is an unconventional myosin that undergoes intrafilopodial motility, Nat. Cell. Biol. 4 (2002) 246–250.
[82] A.D. Sousa, R.E. Cheney, Myosin-X: a molecular motor at the cell's fingertips, Trends. Cell. Biol. 15 (2005) 533–539.
[83] H. Zhang, J.S. Berg, Z. Li, Y. Wang, P. Lang, A.D. Sousa, A. Bhaskar, R.E. Cheney, S. Stromblad, Myosin-X provides a motor-based link between integrins and the cytoskeleton, Nat. Cell. Biol. 6 (2004) 523–531.
[84] X.J. Zhu, C.Z. Wang, P.G. Dai, Y. Xie, N.N. Song, Y. Liu, Q.S. Du, L. Mei, Y.Q. Ding, W.C. Xiong, Myosin X regulates netrin receptors and functions in axonal path-finding, Nat. Cell. Biol. 9 (2007) 184–192.
[85] X. Pi, R. Ren, R. Kelley, C. Zhang, M. Moser, A.B. Bohil, M. Divito, R.E. Cheney, C. Patterson, Sequential roles for myosin-X in BMP6-dependent filopodial extension, migration, and activation of BMP receptors, J. Cell. Biol. 179 (2007) 1569–1582.
[86] A.B. Bohil, B.W. Robertson, R.E. Cheney, Myosin-X is a molecular motor that functions in filopodia formation, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12411–12416.
[87] H. Tokuo, K. Mabuchi, M. Ikebe, The motor activity of myosin-X promotes actin fiber convergence at the cell periphery to initiate filopodia formation, J. Cell. Biol. 179 (2007) 229–238.
[88] L.M. Machesky, A. Hall, Role of actin polymerization and adhesion to extracellular matrix in Rac- and Rho-induced cytoskeletal reorganization, J. Cell. Biol. 138 (1997) 913–926.
[89] C.D. Nobes, A. Hall, Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia, Cell 81 (1995) 53–62.
[90] R. Kozma, S. Ahmed, A. Best, L. Lim, The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts, Mol. Cell. Biol. 15 (1995) 1942–1952.
[91] P. Aspenstrom, U. Lindberg, A. Hall, Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott–Aldrich syndrome, Curr. Biol. 6 (1996) 70–75.
[92] M. Symons, J.M. Derry, B. Karlak, S. Jiang, V. Lemahieu, F. McCormick, U. Francke, A. Abo, Wiskott–Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization, Cell 84 (1996) 723–734.
[93] R. Kolluri, K.F. Tolias, C.L. Carpenter, F.S. Rosen, T. Kirchhausen, Direct interaction of the Wiskott–Aldrich syndrome protein with the GTPase Cdc42, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5615–5618.
[94] H. Miki, T. Sasaki, Y. Takai, T. Takenawa, Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP, Nature 391 (1998) 93–96.
[95] L.M. Machesky, R.H. Insall, Scar1 and the related Wiskott–Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex, Curr. Biol. 8 (1998) 1347–1356.
[96] T.D. Pollard, Regulation of actin filament assembly by Arp2/3 complex and formins, Annu. Rev. Biophys. Biomol. Struct. 36 (2007) 451–477.
[97] R.D. Mullins, J.A. Heuser, T.D. Pollard, The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of filaments, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6181–6186.
[98] L. Ma, R. Rohatgi, M.W. Kirschner, The Arp2/3 complex mediates actin polymerization induced by the small GTP-binding protein Cdc42, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 15362–15367.
[99] R. Rohatgi, L. Ma, H. Miki, M. Lopez, T. Kirchhausen, T. Takenawa, M.W. Kirschner, The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly, Cell 97 (1999) 221–231.
[100] T.M. Svitkina, G.G. Borisy, Progress in protrusion: the tell-tale scar, Trends. Biochem. Sci. 24 (1999) 432–436.
[101] K. Katoh, K. Hammar, P.J. Smith, R. Oldenbourg, Birefringence imaging directly reveals architectural dynamics of filamentous actin in living growth cones, Mol. Biol. Cell. 10 (1999) 197–210.
[102] J.V. Small, T. Stradal, E. Vignal, K. Rottner, The lamellipodium: where motility begins, Trends. Cell. Biol. 12 (2002) 112–120.
[103] H.N. Higgs, Formin proteins: a domain-based approach, Trends. Biochem. Sci. 30 (2005) 342–353.
[104] J. Peng, B.J. Wallar, A. Flanders, P.J. Swiatek, A.S. Alberts, Disruption of the Diaphanous-related formin Drf1 gene encoding mDia1 reveals a role for Drf3 as an effector for Cdc42, Curr. Biol. 13 (2003) 534–545.
[105] N. Watanabe, T. Kato, A. Fujita, T. Ishizaki, S. Narumiya, Cooperation between mDia1 and ROCK in Rho-induced actin reorganization, Nat. Cell. Biol. 1 (1999) 136–143.
[106] A.S. Alberts, N. Bouquin, L.H. Johnston, R. Treisman, Analysis of RhoA-binding proteins reveals an interaction domain conserved in heterotrimeric G protein beta subunits and the yeast response regulator protein Skn7, J. Biol. Chem. 273 (1998) 8616–8622.
[107] A.S. Alberts, Identification of a carboxyl-terminal diaphanous-related formin homology protein autoregulatory domain, J. Biol. Chem. 276 (2001) 2824–2830.
[108] F. Li, H.N. Higgs, Dissecting requirements for auto-inhibition of actin nucleation by the formin, mDia1, J. Biol. Chem. 280 (2005) 6986–6992.
[109] R. Rose, M. Weyand, M. Lammers, T. Ishizaki, M.R. Ahmadian, A. Wittinghofer, Structural and mechanistic insights into the interaction between Rho and mammalian Dia, Nature 435 (2005) 513–518.
[110] S. Pellegrin, H. Mellor, The Rho family GTPase Rif induces filopodia through mDia2, Curr. Biol. 15 (2005) 129–133.
[111] A. Schirenbeck, T. Bretschneider, R. Arasada, M. Schleicher, J. Faix, The Diaphanous-related formin dDia2 is required for the formation and maintenance of filopodia, Nat. Cell. Biol. 7 (2005) 619–625.
[112] C. Yang, L. Czech, S. Gerboth, S. Kojima, G. Scita, T. Svitkina, Novel roles of formin mDia2 in lamellipodia and filopodia formation in motile cells, PLoS. Biol. 5 (2007) e317.
[113] E.W. Dent, A.V. Kwiatkowski, L.M. Mebane, U. Philippar, M. Barzik, D.A. Rubinson, S. Gupton, J.E. Van Veen, C. Furman, J. Zhang, A.S. Alberts, S. Mori, F.B. Gertler, Filopodia are required for cortical neurite initiation, Nat. Cell. Biol. 9 (2007) 1347–1359.
[114] J. Faix, R. Grosse, Staying in shape with formins, Dev. Cell. 10 (2006) 693–706.
[115] J. Faix, K. Rottner, The making of filopodia, Curr. Opin. Cell. Biol. 18 (2006) 18–25.
[116] D. Pruyne, M. Evangelista, C. Yang, E. Bi, S. Zigmond, A. Bretscher, C. Boone, Role of formins in actin assembly: nucleation and barbed-end association, Science 297 (2002) 612–615.
[117] I. Sagot, A.A. Rodal, J. Moseley, B.L. Goode, D. Pellman, An actin nucleation mechanism mediated by Bni1 and profilin, Nat. Cell. Biol. 4 (2002) 626–631.
[118] C. Higashida, T. Miyoshi, A. Fujita, F. Oceguera-Yanez, J. Monypenny, Y. Andou, S. Narumiya, N. Watanabe, Actin polymerization-driven molecular movement of mDia1 in living cells, Science 303 (2004) 2007–2010.
[119] D.R. Kovar, T.D. Pollard, Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 14725–14730.
[120] S. Romero, C. Le Clainche, D. Didry, C. Egile, D. Pantaloni, M.F. Carlier, Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis, Cell 119 (2004) 419–429.
[121] S.H. Zigmond, M. Evangelista, C. Boone, C. Yang, A.C. Dar, F. Sicheri, J. Forkey, M. Pring, Formin leaky cap allows elongation in the presence of tight capping proteins, Curr. Biol. 13 (2003) 1820–1823.
[122] J.B. Moseley, I. Sagot, A.L. Manning, Y. Xu, M.J. Eck, D. Pellman, B.L. Goode, A conserved mechanism for Bni1- and mDia1-induced actin assembly and dual regulation of Bni1 by Bud6 and profilin, Mol. Biol. Cell. 15 (2004) 896–907.
[123] E.S. Harris, F. Li, H.N. Higgs, The mouse formin, FRLalpha, slows actin filament barbed end elongation, competes with capping protein, accelerates polymerization from monomers, and severs filaments, J. Biol. Chem. 279 (2004) 20076–20087.
[124] D.R. Kovar, J.Q. Wu, T.D. Pollard, Profilin-mediated competition between capping protein and formin Cdc12p during cytokinesis in fission yeast, Mol. Biol. Cell. 16 (2005) 2313–2324.
[125] C.S. Peskin, G.M. Odell, G.F. Oster, Cellular motions and thermal fluctuations: the Brownian ratchet, Biophys. J. 65 (1993) 316–324.
[126] N. Watanabe, P. Madaule, T. Reid, T. Ishizaki, G. Watanabe, A. Kakizuka, Y. Saito, K. Nakao, B.M. Jockusch, S. Narumiya, p140mDia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin, EMBO J. 16 (1997) 3044–3056.
[127] T. Otomo, C. Otomo, D.R. Tomchick, M. Machius, M.K. Rosen, Structural basis of Rho GTPase-mediated activation of the formin mDia1, Mol. Cell. 18 (2005) 273–281.
[128] M. Lammers, R. Rose, A. Scrima, A. Wittinghofer, The regulation of mDia1 by autoinhibition and its release by Rho*GTP, EMBO J. 24 (2005) 4176–4187.
[129] S. Yasuda, F. Oceguera-Yanez, T. Kato, M. Okamoto, S. Yonemura, Y. Terada, T. Ishizaki, S. Narumiya, Cdc42 and mDia3 regulate microtubule attachment to kinetochores, Nature 428 (2004) 767–771.

[130] B.J. Wallar, A.D. Deward, J.H. Resau, A.S. Alberts, RhoB and the mammalian Diaphanous-related formin mDia2 in endosome trafficking, Exp. Cell. Res. 313 (2007) 560–571.
[131] P. Aspenstrom, N. Richnau, A.S. Johansson, The diaphanous-related formin DAAM1 collaborates with the Rho GTPases RhoA and Cdc42, CIP4 and Src in regulating cell morphogenesis and actin dynamics, Exp. Cell. Res. 312 (2006) 2180–2194.
[132] T. Higashi, T. Ikeda, R. Shirakawa, H. Kondo, M. Kawato, M. Horiguchi, T. Okuda, K. Okawa, S. Fukai, O. Nureki, T. Kita, H. Horiuchi, Biochemical characterization of the Rho GTPase-regulated actin assembly by diaphanous-related Formins, mDia1 and Daam1, in platelets, J. Biol. Chem. 283 (2008) 8746–8755.
[133] P.D. Burbelo, D. Drechsel, A. Hall, A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases, J. Biol. Chem. 270 (1995) 29071–29074.
[134] S. Gasman, Y. Kalaidzidis, M. Zerial, RhoD regulates endosome dynamics through Diaphanous-related Formin and Src tyrosine kinase, Nat. Cell. Biol. 5 (2003) 195–204.
[135] P. Hotulainen, P. Lappalainen, Stress fibers are generated by two distinct actin assembly mechanisms in motile cells, J. Cell. Biol. 173 (2006) 383–394.
[136] B. Butler, C. Gao, A.T. Mersich, S.D. Blystone, Purified integrin adhesion complexes exhibit actin-polymerization activity, Curr. Biol. 16 (2006) 242–251.
[137] E. Sahai, C.J. Marshall, ROCK and Dia have opposing effects on adherens junctions downstream of Rho, Nat. Cell. Biol. 4 (2002) 408–415.
[138] L. Carramusa, C. Ballestrem, Y. Zilberman, A.D. Bershadsky, Mammalian diaphanous-related formin Dia1 controls the organization of E-cadherin-mediated cell–cell junctions, J. Cell. Sci. 120 (2007) 3870–3882.
[139] C. Sarmiento, W. Wang, A. Dovas, H. Yamaguchi, M. Sidani, M. El-Sibai, V. Desmarais, H.A. Holman, S. Kitchen, J.M. Backer, A. Alberts, J. Condeelis, WASP family members and formin proteins coordinate regulation of cell protrusions in carcinoma cells, J. Cell. Biol. 180 (2008) 1245–1260.
[140] T. Ishizaki, M. Maekawa, K. Fujisawa, K. Okawa, A. Iwamatsu, A. Fujita, N. Watanabe, Y. Saito, A. Kakizuka, N. Morii, S. Narumiya, The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase, EMBO J. 15 (1996) 1885–1893.
[141] T. Leung, E. Manser, L. Tan, L. Lim, A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes, J. Biol. Chem. 270 (1995) 29051–29054.
[142] T. Fujiwara, A. Mammoto, Y. Kim, Y. Takai, Rho small G-protein-dependent binding of mDia to an Src homology 3 domain-containing IRSp53/BAIAP2, Biochem. Biophys. Res. Commun. 271 (2000) 626–629.
[143] S. Lommel, S. Benesch, K. Rottner, T. Franz, J. Wehland, R. Kuhn, Actin pedestal formation by enteropathogenic *Escherichia coli* and intracellular motility of *Shigella flexneri* are abolished in N-WASP-defective cells, EMBO Rep. 2 (2001) 850–857.
[144] S.B. Snapper, F. Takeshima, I. Anton, C.H. Liu, S.M. Thomas, D. Nguyen, D. Dudley, H. Fraser, D. Purich, M. Lopez-Ilasaca, C. Klein, L. Davidson, R. Bronson, R.C. Mulligan, F. Southwick, R. Geha, M.B. Goldberg, F.S. Rosen, J.H. Hartwig, F.W. Alt, N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility, Nat. Cell. Biol. 3 (2001) 897–904.
[145] A. Di Nardo, G. Cicchetti, H. Falet, J.H. Hartwig, T.P. Stossel, D.J. Kwiatkowski, Arp2/3 complex-deficient mouse fibroblasts are viable and have normal leading-edge actin structure and function, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 16263–16268.
[146] A. Steffen, J. Faix, G.P. Resch, J. Linkner, J. Wehland, J.V. Small, K. Rottner, T.E. Stradal, Filopodia formation in the absence of functional WAVE- and Arp2/3-complexes, Mol. Biol. Cell. 17 (2006) 2581–2591.
[147] F. Korobova, T. Svitkina, Arp2/3 complex is important for filopodia formation, growth cone motility and neuritogenesis in neuronal cells, Mol. Biol. Cell (2008).
[148] A. Biyasheva, T. Svitkina, P. Kunda, B. Baum, G. Borisy, Cascade pathway of filopodia formation downstream of SCAR, J. Cell. Sci. 117 (2004) 837–848.
[149] P. Aspenstrom, A. Fransson, J. Saras, Rho GTPases have diverse effects on the organization of the actin filament system, Biochem. J. 377 (2004) 327–337.
[150] S. Passey, S. Pellegrin, H. Mellor, What is in a filopodium? Starfish versus hedgehogs, Biochem. Soc. Trans. 32 (2004) 1115–1117.
[151] S. Yayoshi-Yamamoto, I. Taniuchi, T. Watanabe, FRL, a novel formin-related protein, binds to Rac and regulates cell motility and survival of macrophages, Mol. Cell. Biol. 20 (2000) 6872–6881.
[152] R. Habas, Y. Kato, X. He, Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1, Cell 107 (2001) 843–854.
[153] J.E. Gasteier, R. Madrid, E. Krautkramer, S. Schroder, W. Muranyi, S. Benichou, O.T. Fackler, Activation of the Rac-binding partner FHOD1 induces actin stress fibers via a ROCK-dependent mechanism, J. Biol. Chem. 278 (2003) 38902–38912.
[154] J.J. Westendorf, The formin/diaphanous-related protein, FHOS, interacts with Rac1 and activates transcription from the serum response element, J. Biol. Chem. 276 (2001) 46453–46459.