Filopodia: molecular architecture and cellular functions

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Abstract | Filopodia are thin, actin-rich plasma-membrane protrusions that function as antennae for cells to probe their environment. Consequently, filopodia have an important role in cell migration, neurite outgrowth and wound healing and serve as precursors for dendritic spines in neurons. The initiation and elongation of filopodia depend on the precisely regulated polymerization, convergence and crosslinking of actin filaments. The increased understanding of the functions of various actin-associated proteins during the initiation and elongation of filopodia has provided new information on the mechanisms of filopodia formation in distinct cell types.

Lamellipodium
A cellular protrusion that is composed of a branched F-actin meshwork with, typically, 70° angles between the filaments. It results from ARP2/3 complex-mediated filament nucleation and branching. Branching frequency is highest when in close proximity to the plasma membrane, resulting in short filaments pushing against the membrane.

Barbed end
Actin filaments are polar structures. Based on the arrowhead pattern created when myosin binds actin filaments, the rapidly growing filament end is called the barbed end and the slowly growing end is called the pointed end.

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The coordinated polymerization of actin filaments against cellular membranes provides the force for a number of processes, such as cell migration, morphogenesis, endocytosis and phagocytosis. During these processes, actin filaments are organized into three-dimensional (3D) assemblies that are constantly adjusted to fulfil varying needs of the cell. Cell migration is an extensively studied process that depends on several dynamic actin assemblies (FIG. 1). The protrusive structures at the leading edge of a motile cell are called lamellipodia and filopodia. A lamellipodium is a thin (0.1–0.2 μm) sheet-like protrusion that is filled with a branched network of actin. By contrast, filopodia are thin (0.1–0.3 μm), finger-like structures that are filled with tight parallel bundles of filamentous (F)-actin. In both cases, the fast-growing barbed ends of actin filaments are orientated towards the plasma membrane. The elongation of these filaments pushes the leading edge forward, and thus promotes cell migration or extension[1,2]. Non-muscle cells also contain contractile structures called stress fibres, which are composed of actin and myosin bundles. These actomyosin structures, which are generated by formin-mediated actin polymerization and from the actin-related protein-2/3 (ARP2/3) complex-nucleated lamellipodial actin network (BOX 1), provide contractile forces for cell migration and morphogenesis[3,4].

Filopodia are often found embedded in or protruding from the lamellipodial actin network[5–7]. In addition to cell migration, filopodia are involved in a number of cellular processes, including wound healing, adhesion to the extracellular matrix, guidance towards chemoattractants, neuronal growth-cone pathfinding and embryonic development[8,9]. Filopodial protrusions and retractions are promoted by a dynamic balance between actin polymerization at the barbed ends of a filament and the retrograde flow of the actin filament bundle[10]. Other morphologically related but more specialized and stable cellular protrusions exist, such as microvilli, stereocilia and the bristles of *Drosophila melanogaster*, but will not be described in this review.

Studies on filopodia have revealed variations in the dynamics, length and positioning of these protrusions in different cells, indicating that distinct or differently regulated molecules generate discrete sets of filopodia. For example, fibroblast filopodia and nerve growth-cone filopodia rarely exceed 10 μm in length, but in sea-urchin embryos filopodia extend up to 40 μm[11]. Very short filopodia that are almost completely embedded in the cell cortex or leading edge are often termed microspikes.

Despite extensive studies, the biological functions of filopodia and the mechanisms of filopodia assembly are still incompletely understood. Many seemingly contradictory findings have been reported — especially concerning the mechanisms of actin assembly in filopodia[7,12,13] — and information concerning possible new players in filopodia formation is constantly being uncovered[14,15]. Here, we review the current knowledge concerning the functions of filopodia and we discuss the architecture of filopodia and the mechanisms of their formation.

Cellular roles of filopodia

Generally, filopodia have been described as 'antennae' or 'tentacles' that cells use to probe their microenvironment, thus serving as pioneers during protrusion. However, the roles of filopodia seem diverse and in many cases remain vague. Filopodia have been implicated in several fundamental physiological processes, of which cell migration is perhaps the best characterized (FIG. 1).

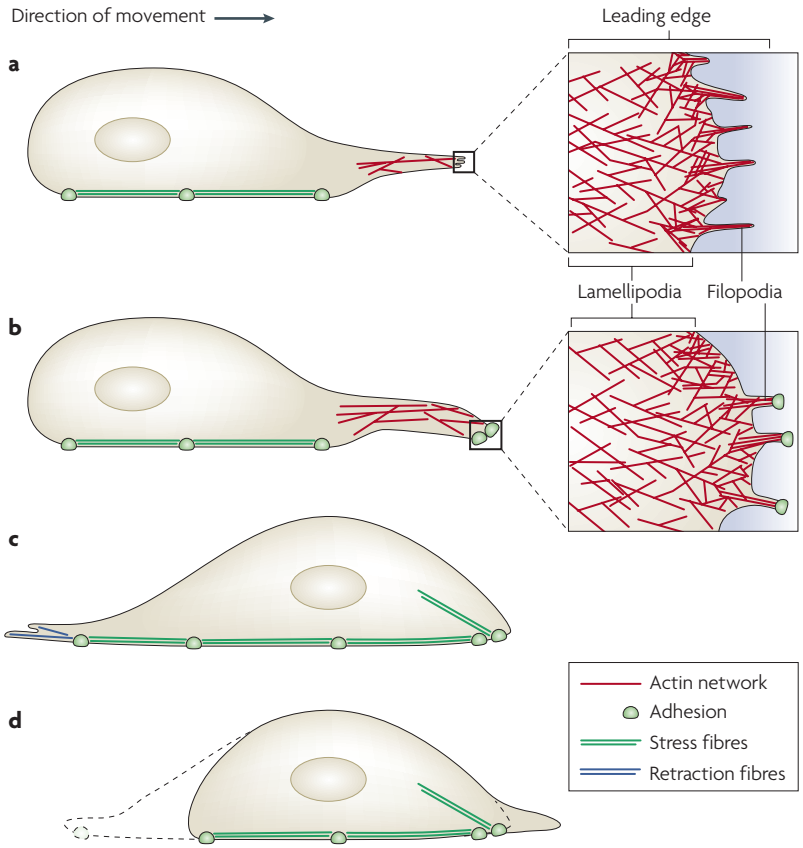


Figure 1 | **Cell migration is dependent on different actin filament structures.** **a** | Motility is initiated by an actin-dependent protrusion of the leading edge, which is composed of lamellipodia and filopodia. These protrusive structures contain actin filaments, with elongating barbed ends orientated towards the plasma membrane. **b** | During cellular extension, new adhesions with the substratum are formed under the leading edge. **c** | Next, the nucleus and the cell body are translocated forward through actomyosin-based contraction forces that might be mediated by focal adhesion-linked stress fibres, which also mediate the attachment to the substratum. **d** | Then, retraction fibres pull the rear of the cell forward, adhesions at the rear of the cell disassemble and the trailing edge retracts.

Stress fibre
A contractile structure that is composed of antiparallel arrays of actin filaments associated with myosin II bundles. Stress fibres provide the contractile force that contributes to cell morphogenesis and migration.

Retrograde flow
The phenomenon whereby the speed of actin polymerization is typically faster than the velocity of cell protrusions, which leads to the sliding of actin filaments backwards with respect to the substratum.

Microspike
A short filopodium that is almost completely embedded in the cell cortex or leading edge.

Probing the environment. Filopodia are linked to the enhancement of directed cell migration (FIG. 2a), because key components of filopodia promote cell motility. Abundant filopodia are also considered a characteristic of invasive cancer cells[16]. Filopodia contain receptors for diverse signalling molecules and extracellular matrix molecules. This correlates with the role of filopodia in sensing the cell's surroundings and acting as sites for signal transduction. Integrins and cadherins, which are cell adhesion molecules, are often found in the tips of filopodia or along the shafts[17–20]. Integrins were reported to accumulate in filopodia in an unligated but activated state. Thus, integrins in filopodia are primed to probe the matrix, creating 'sticky fingers' along the leading edge that promote cell adhesion and migration[17].

During cell spreading, a process that is at least partially analogous to cell migration, integrin-containing filopodia form the initial adhesion sites. Subsequently, other focal adhesion complex components, such as talin, focal adhesion kinase (FAK) and paxillin, are recruited to these sites to form mature focal adhesions. Finally, the focal adhesions and associated F-actin structures reorganize, leading to the formation of focal adhesion-anchored actin stress fibres[18]. A study carried out on spreading fibroblasts on micropatterned surfaces revealed that filopodia, which contain adhesion structures, are converted into lamellipodia-like protrusions. Furthermore, inhibition of filopodia formation by the expression of a dominant-negative form of the small GTPase CDC42 impaired cell spreading[21].

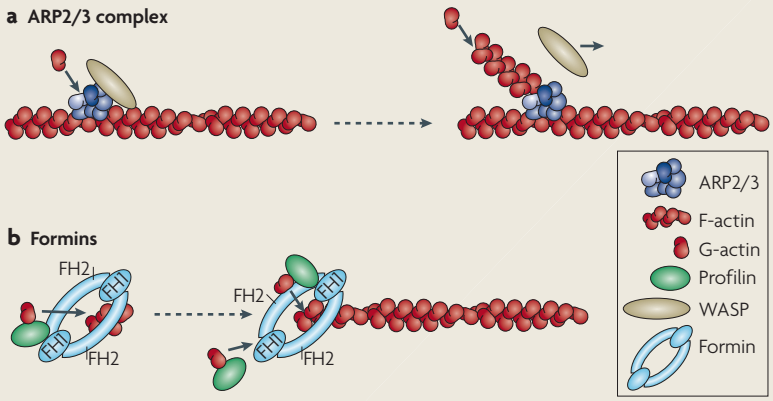
In macrophages, several filopodia per cell explore the environment. After finding a pathogen, the filopodia will bind to it and then retract towards the macrophage cell body. The filopodia and underlying lamellipodia then transform into an actin-based membrane structure called the phagocytic cup[22]. Recent studies revealed that macrophage filopodia act as cellular tentacles: a few seconds after binding to a particle, filopodia retract and pull the bound particle toward the cell. This intracellular stepping motion, which produced forces of up to 19 pN, was transmitted to the extracellular tracked particle through the filopodial F-actin bundle[23,24]. It was also shown that the inhibition of filopodia formation by the depletion of myosin VII from *Dictyostelium discoideum* amoebae decreases phagocytosis rates and inhibits migration[25].

Role in cell–cell adhesion. The fusion of sheets of epithelial cells is a common event during embryonic development and also occurs during wound healing. Live-cell imaging studies revealed that dynamic filopodia project at the edges of epithelial cells during these processes. Furthermore, filopodia have an important role during the formation of adherens junctions between epithelial cells, as well as a role during wound healing, and dorsal closure and epithelial-sheet sealing in *D. melanogaster* and *Caenorhabditis elegans* embryos[20,26–28]. What these processes have in common is that interdigitated filopodia, which protrude from opposing cells, help the sheets of cells to align and adhere together (FIG. 2b). For example, this 'adhesion zippering' of filopodia results in the formation of mature adherens junctions between keratinocytes in a calcium-dependent manner[20].

Embryonic epithelial fusions must occur in a precise fashion when the fusing sheets are patterned, as in the case of dorsal closure in flies. Importantly, filopodia were shown to have an important role in the cell–cell matching that occurs during epithelial fusion when the fusing sheets are patterned. Filopodia facilitate cell–cell matching by allowing a cell to search for its match and also to pull misaligned sheets into alignment[29].

Filopodia in neurons. Neuronal growth cones are motile actin-rich and microtubule-rich structures at the ends of neurites that guide axons and dendrites to their proper targets. Growth cones contain a large amount of filopodia, which sense gradients of guidance cues and consequently turn the neurite towards chemoattractants[30] (FIG. 2c). Although it was proposed that chemoattractant sensing and subsequent downstream signalling are the major functions of filopodia

Box 1 | **The two major actin-nucleating machineries of motile cells**



The actin-related protein-2/3 (ARP2/3) complex (see figure, panel **a**) is a stable complex of seven subunits (two actin-related proteins and five other proteins). The ARP2/3 complex binds to the side of a pre-existing actin filament and the actin-related subunits form a 'seed' that nucleates actin polymerization. The new filament is orientated with its rapidly growing barbed end extending away from the mother filament. The activation of the ARP2/3 complex is induced by binding to nucleation-promoting factors, such as WASP/WAVE-family proteins, which dissociate from the ARP2/3 complex after filament nucleation. Formins are large multidomain proteins that directly nucleate polymerization of unbranched actin filaments (see figure, panel **b**). The formin homology-2 (FH2) domain initiates filament assembly and remains persistently associated with the fast-growing barbed end, enabling rapid insertion of actin subunits while protecting the end from capping proteins. Most formins also contain a profilin-binding formin homology-1 (FH1) domain, which recruits profilin–G-actin complexes to the barbed ends of the growing filament.

Focal adhesion
A flat, elongated structure that forms cell–substrate adhesions. Focal adhesions are composed of a large number of signalling and adhesion molecules and they are associated with the ends of actin stress fibres in a wide variety of cultured adherent cells.

Adherens junction
A specialized intercellular junction of the plasma membrane, in which cadherin molecules of adjacent cells interact in a Ca^{2+}-dependent manner. Actin filaments are linked to this structure through catenins, which are located underneath the junction.

Tectum
The midbrain roof is a retinorecipient region, referred to as the optic tectum in lower vertebrates and the superior colliculus in mammals.

I-BAR domain
A lipid-binding and deforming protein domain, which is also known as an insulin-receptor substrate p53 (IRSp53)/missing-in-metastasis (MIM) (IM)-domain.

in growth cones[31,32], other studies provided evidence that filopodia are not essential for all types of neurite guidance. Retinal ganglion cells that are depleted of filopodia can migrate slowly along the optic tract but fail to establish axon terminal branching inside the tectum[33].

In addition to neurite outgrowth, filopodia are necessary for the initial neurite formation of cortical neurons[34,35]. Furthermore, the development of dendritic spines involves filopodia-like precursors[36]. Dendritic spines are the postsynaptic regions of most excitatory neuronal synapses and have an important role in higher brain functions, such as learning and memory. Constitutively active Rac1, which is a small GTPase that regulates actin dynamics, induces the formation of dendritic spines. This study provided the first evidence that the actin cytoskeleton is important for spine formation[37]. Spines continuously change morphology by modulating their underlying actin machinery, which has a pivotal role in the plasticity and integrity of the spine. Filopodial precursors of spines have been suggested to dynamically grow, reach the presynaptic partner and either stabilize and mature to a spine or, without a proper signal, shrink back to the dendrite backbone[36] (FIG. 2c). However, only limited data are available on the mechanisms of actin dynamics in dendritic filopodia and spine formation, and further studies are thus required to elucidate whether these structures are generated through a mechanism similar to that of filopodia in motile cells.

Small GTPases and filopodia formation

Small GTPases of the Rho superfamily are linked to the regulation of cell morphology and, particularly, the actin cytoskeleton. The best-studied mammalian Rho GTPases are Rac1, CDC42 and RhoA[38]. RhoA is implicated in the formation of stress fibres and focal adhesions, Rac1 promotes lamellipodium formation and CDC42 functions in the formation of filopodia[39].

Role of CDC42. CDC42 functions by interacting with multiple proteins. Perhaps the best-studied signalling pathway regulated by CDC42 is the induction of the ARP2/3 complex-dependent actin filament nucleation through activation of WASP and N-WASP[40–42]. The ARP2/3 complex is considered a major nucleator of the branched lamellipodial actin network (BOX 1). The interaction of CDC42 with WASP and N-WASP, together with binding to phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$), relieves the autoinhibited conformation of WASP proteins and subsequently leads to activation of the ARP2/3 complex[43]. Expression of the CDC42/Rac-interactive binding (CRIB) domain of WASP blocks the induction of filopodia by CDC42, which suggests that CDC42 might exert its function through the WASP–ARP2/3 signalling pathway[44]. However, other studies showed that fibroblasts devoid of N-WASP and WASP can produce filopodia following CDC42 stimulation, suggesting that multiple signalling pathways must regulate this process[45,46]. Furthermore, N-WASP is typically not enriched in filopodia[38,43].

Another way for CDC42 to function, alternative to the WASP–ARP2/3 pathway, involves the insulin-receptor substrate p53 (IRSp53), which is an I-BAR-domain/IM-domain-containing protein (see below). This relatively large scaffolding protein binds the small GTPase CDC42, its effector WAVE2 and the ENA/VASP (enabled/vasodilator-stimulated phosphoprotein) family protein MENA (see TABLE 1), and induces filopodia and lamellipodia formation when overexpressed in cells[47]. IRSp53 was proposed to induce filopodia formation together with MENA under the control of CDC42 (REF. 48). However, IRSp53 was also shown to co-localize with WAVE2 in filopodial tips independently of MENA[49], although WAVE2 has been mainly linked to lamellipodial formation[43]. It is possible that IRSp53 recruits MENA downstream of the CDC42 signal to filopodial tips, and that it activates WAVE2 downstream of Rac1 in order to induce ARP2/3-promoted actin filament nucleation, which is essential for lamellipodium formation and might also contribute to initiating filopodia[48–50]. In addition to its possible role in recruiting actin-regulatory proteins to the site of protrusion, IRSp53 can directly facilitate filopodia formation by inducing membrane tubulation and/or F-actin bundling[14,51], as described below.

Role of other small GTPases. Despite its importance in many cellular systems, filopodia formation also occurs in cells that are depleted of CDC42 (REFS 44,52). In line with these data, another small GTPase, called Rho in filopodia (RIF), stimulates filopodia formation following overexpression[53]. However, as compared with

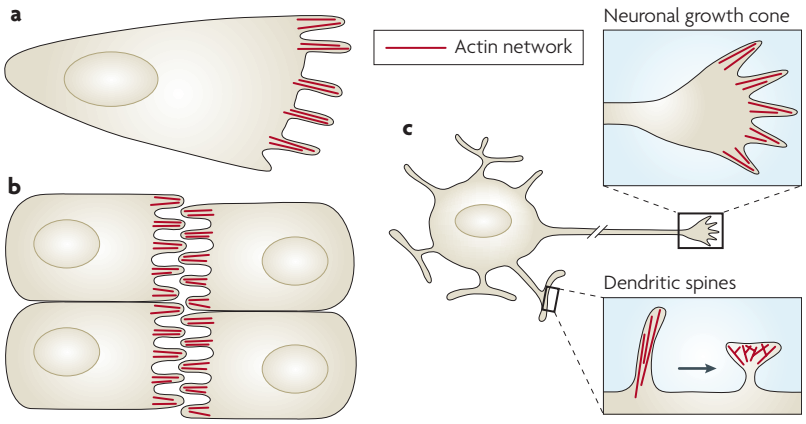


Figure 2 | **Examples of different types of filopodia. a** | In migrating cells, filopodia that are localized at the leading edge probe the microenvironment and serve as pioneers in cell protrusion. **b** | Filopodia of epithelial sheets, which protrude from opposing cells, help the sheets of cells to align and adhere together. **c** | Filopodia participate in the guidance and migration of neuronal growth cones (top inset) and are precursors of the dendritic spine (bottom inset), which are the postsynaptic regions of most excitatory neuronal synapses. As a dendritic spine matures, its morphology changes from a filopodia-like protrusion to a mushroom-shaped structure.

CDC42-induced filopodia, the filopodia induced by overexpression of RIF are longer and also project from the apical surface of the cell. RIF seems to exert its function mainly through the activation of the formin Dia2 (diaphanous-related formin-2) (REF. 44), which promotes the nucleation and elongation of linear F-actin arrays that are required for filopodia formation. Additionally, other Rho GTPases, such as TC10, RhoT and the CDC42-like protein WRCH1, were also demonstrated to induce filopodia formation[54–56]. So, it is clear that multiple Rho GTPases can induce cellular protrusions when overexpressed, but their roles under physiological conditions remain to be elucidated.

For controlled cell migration and extension, the actin-polymerization machinery has to be localized to the plasma membrane. Furthermore, elongating filaments have to be protected from proteins that inhibit barbed-end elongation by capping and from proteins that induce filament disassembly. The activities and localization of myriad regulators of the actin cytoskeleton are controlled by plasma-membrane phospholipids. The most potent signalling lipids include PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, which can activate filopodia formation by directly controlling the actin polymerization machinery (for example, PtdIns(4,5)P_2) and by spatially activating Rho GTPases (for example, PtdIns(3,4,5)P_3) (REF 57). The synthesis of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 is tightly regulated by phosphatidylinositol phosphate (PIP) kinases, which are localized to specific regions in cells[58]. In the future, it will be important to examine the possible specific roles of these phospholipids in filopodia formation.

Molecular composition of filopodia

A large number of proteins that regulate the actin cytoskeleton have been shown to localize to filopodia and/or to regulate filopodia formation. Although many of these proteins are specific to only certain cell types, a key set of proteins seems to contribute to the generation of filopodia in diverse cell types and organisms (TABLE 1). The best-characterized 'general' actin-regulatory proteins that are involved in filopodia formation and maintenance are discussed below.

ENA/VASP proteins. The ENA/VASP-family proteins are relatively large, multifunctional actin-binding proteins that contribute to filopodia formation in mammalian and insect cells as well as in *D. discoideum*[59]. In mammalian cells, ENA/VASP proteins typically localize to focal adhesions, cell–cell contacts, the leading edge and the tips of filopodia[60,61]. Initial analyses suggested that ENA/VASP proteins promote filopodia formation by preventing capping of actin filament barbed ends[62]. Subsequent biochemical and total internal reflection fluorescence (TIRF) microscopy analyses demonstrated that ENA/VASPs do not uncap filament barbed ends (that is, induce the removal of capping proteins), but protect filaments from capping proteins and thus display anti-capping activity[63,64]. ENA/VASPs also contain a profilin-binding poly-Pro sequence. Profilin enhances ENA/VASP-mediated barbed-end protection from capping proteins and was proposed to enhance ENA/VASP-induced filament polymerization[63,65]. However, more recent studies revealed that other functions, including the enhancement of filament elongation, anti-branching and F-actin bundling, in addition to the anti-capping activity, might be essential for ENA/VASP in filopodia formation[59,66–69]. Consequently, future studies are needed to establish the *in vivo* relevance of these activities.

Recently, a triple-knockout mouse for the three mammalian ENA/VASP proteins (MENA, VASP and enabled) was reported. Surprisingly, these animals developed relatively normally until embryonic day 16, which demonstrates that ENA/VASP proteins are not needed in the early developmental stages. Most defects seen in these mice were in neuronal and endothelial cells[35,70]. The ENA/VASP-null cortical neurons failed to initiate neurite formation owing to the lack of filopodia. However, this phenotype could be rescued in cell culture by the expression of other filopodia-inducing proteins, myosin-X and mammalian Dia2 (REF. 34). Together, these data suggest that ENA/VASP proteins are not essential for filopodial formation in mammalian cells. However, the VASP homologue of ENA/VASP proteins in *D. discoideum* is essential for filopodia formation[71].

Formins. Formins comprise a large protein family that induces the formation of unbranched actin filaments by processive barbed-end nucleation and elongation[72] (BOX1). Thus, they seem to be good candidates for proteins that nucleate the filopodial F-actin bundles. Indeed, overexpression of the formin Dia2 induces filopodia in mammalian cells and its depletion leads to defects in filopodia formation[13,73]. Furthermore, knockout studies in *D. discoideum* demonstrated an essential role of Dia2 in filopodia formation[74]. Interestingly, Dia2 cooperates with the single VASP orthologue of *D. discoideum* in filopodia formation, suggesting interdependency of these two proteins in filopodia formation[66]. It is also important to note

Capping activity
Many actin-binding proteins (for example, gelsolin, EPS8, twinfilin and tropomodulin) bind to filament ends where they inhibit actin-monomer association and/or dissociation and therefore display capping activity. Uncapping activity occurs when a protein is capable of removing a capping protein from the filament end, whereas anti-capping activity occurs if a protein protects filament ends from capping proteins.

Table 1 | **Key proteins involved in filopodia formation**

Protein	Proposed activities and functions	References
CDC42	Small GTPase that induces filopodia formation	39
RIF	Small GTPase that promotes the formation of long filopodia through the formin protein Dia2	44
ARP2/3 complex	Actin filament nucleator that generates the formation of a branched lamellipodial F-actin network	1
WASP/ WAVE	Proteins that activate the F-actin nucleation activity of the ARP2/3 downstream of Rho family GTPases	43
Dia2	Protein that induces the formation of unbranched actin filaments in filopodia	13, 74
ENA/VASP	Factors that promote actin filament elongation, anti-branching and/or bundling to induce filopodia formation	59
Myosin-X	Motor protein that promotes filopodia formation by converging filament barbed ends together and by transporting proteins to filopodial tips	83, 84
Fascin	Major F-actin-crosslinking protein of filopodia	88
IRSp53	Scaffolding protein that also deforms membranes to promote the formation of plasma-membrane protrusions	47
LPR1	Lipid phosphatase-related protein that induces filopodia formation through a currently unidentified mechanism	15

ARP2/3, actin-related protein-2/3; Dia2, diaphanous-related formin-2; ENA/VASP, enabled/ vasodilator-stimulated phosphoprotein; IRSp53, insulin receptor substrate p53; LPR1, lipid phosphatase-related protein-1; RIF, Rho in filopodia.

that mammals have 15 formin proteins and the functions of only a few of these have been analysed in detail so far[75]. Thus, it is possible that other currently uncharacterized formins have an important role in filopodia formation in certain mammalian cell types.

Myosin-X. Myosins form a large family of motor proteins that walk along actin filaments in a directed fashion. Certain myosins have been implicated in the formation of filopodia, although their exact role in this process is far from understood. Overexpression of the unconventional myosin-X (MYO10) induces filopodia formation in various cell-types and the protein localizes to filopodial tips[76]. Furthermore, related myosins VIIa, VIIb and XVa localize to and are crucial for the integrity of stereocilia and microvilli[77]. Myosin-X seems to move up and down along filopodia shafts by an ATP-dependent 'walking' mechanism towards filament barbed ends and then slides down with the retrograde actin flow[76,78]. In addition to F-actin, myosin-X interacts with VASP, phosphatidylinositol phosphates, integrins, netrin receptors and microtubules. Therefore, multiple roles for myosin-X in filopodia formation are plausible[79–82]. It was proposed that myosin-X transports other filopodial components, such as ENA/VASP proteins and integrins, to the dense tip of filopodia. Whereas the motor domain of myosin-X was shown to be essential for filopodia formation, the integrin-binding domain does not have a crucial role in filopodia formation[83].

A recent study elegantly demonstrated that the dimerization and motor activities of myosin-X are necessary and sufficient for the induction of filopodia[84]. Fibroblasts that lack myosin-X do not form filopodia. Moreover, myosin-X was shown to move laterally at the cell periphery. This was proposed to reflect the lateral movements of actin filament barbed ends and lead to the convergence of F-actin in order to initiate filopodia formation[84]. Although the motor domain alone was sufficient for the initiation of filopodia formation, it induced relatively short and unstable filopodia. This suggests that the myosin-X tail, and presumably the cargo linked to it, has an important role in the formation of long and more stable filopodia[84]. It was also demonstrated that binding to PtdIns(4,5)P_2 induces dimerization of myosin-IV, and that myosin-X contains pleckstrin homology domains (PH domains) that interact with PtdIns(3,4,5)P_3, at least *in vitro*[85,86]. It is therefore plausible that binding to phosphatidylinositol phosphates at the plasma membrane might contribute to myosin-X-induced filopodia formation.

Fascin. Actin filament-bundling proteins are essential for the generation and maintenance of tight F-actin bundles of filopodia. Among the actin filament-bundling proteins, fascin has been most directly linked to filopodia formation[87]. Biochemical characterization revealed that fascin is specialized for generating stiff and parallel filament bundles. Fascin is not an efficient bundler of preformed filaments, but specializes in bundling filaments as they polymerize or are already loosely linked together[88]. The interaction of fascin with actin bundles in filopodia is surprisingly dynamic. The reversible fascin–actin interaction, with a half-life of 6–9 seconds, enables dynamic crosslinking of F-actin, which might be required for the efficient bundling of the filaments reaching the tip of the filopodia, and for the adjustment of the actin core to bending of filopodia or for intrafilopodial traffic of vesicles and proteins. Conversely, fascin-mediated crosslinks are rigid and thus enable resistance against fast perturbations[89,90].

Depletion of fascin by RNA interference (RNAi) in mammalian cells resulted in the loss of filopodia and the appearance of actin bundles, reminiscent of those in filopodia, that were bent and buckling under the plasma membrane[89]. Increased fascin expression has been observed in several cancers[88] and fascin was recently demonstrated to be expressed in the invasive front of human colon cancer and to promote metastasis formation[16]. These studies provide an interesting link between filopodia, cell migration and invasiveness.

Also, other actin-crosslinking proteins, such as α-actinin, fimbrin, espin and filamin have been found in parallel actin bundle-containing structures. However, they are not specifically targeted to filopodia shafts. These proteins seem to be relatively flexible crosslinkers and are used mainly in more loosely packed F-actin structures, such as in proximal or lamellipodium-embedded parts of filopodia[89].

I-BAR domain proteins. Recent studies suggested that, in addition to the reorganization of the actin cytoskeleton, filopodia formation might also be linked to direct membrane deformation and tubulation[14,91]. This involves a conserved protein domain, called the IRSp53/missing-in-metastasis (MIM; also known as MTSS1) homology

PH domain
(Pleckstrin homology). A small signal transduction domain that binds phosphatidylinositol phosphates.

(IM) domain (IMD), also known as the inverse BAR (I-BAR) domain[92]. This domain, found in cytoskeletal regulatory and scaffolding proteins such as MIM and IRSp53 (see above), induces the drastic formation of filopodia on overexpression in cells[51]. Initial studies proposed that filopodia induction by IM/I-BAR domains is due to the F-actin-crosslinking activity of this domain[51], but this activity has been the subject of debate[14,93].

Intriguingly, the 3D structure of the IM/I-BAR domain shows remarkable similarity to BAR and F-BAR domains, which are membrane-deforming domains that induce plasma-membrane tubulation, for example, during endocytosis[92–95]. Subsequently, it was shown that the IM/I-BAR domains of MIM and IRSp53 bind phosphatidylinositol-rich membranes with high affinity and deform them into tubular structures. However, in contrast to BAR and F-BAR domains, the IM/I-BAR domain is 'zeppelin-shaped' rather than 'banana-shaped' and functions in an opposing direction, for example, to induce filopodia-like plasma-membrane protrusions rather than invaginations as shown for BAR and F-BAR domains[14,91].

A predominant proportion of IRSp53 was demonstrated to reside at cell membranes, supporting the hypothesis that this protein might promote filopodia formation through its membrane deformation activity[96]. In addition to the plasma-membrane lipids, IRSp53 interacts with the Rho GTPases CDC42 and Rac1 through its N-terminal region, and with WAVE2 and MENA through its central Src-homology-3 domain (SH3 domain)[48,50]. Moreover, EPS8, an actin-filament capping protein, was shown to bind to IRSp53, synergize its ability to induce filopodia and promote its F-actin-bundling activity[97,98]. IRSp53 has also been implicated in dendritic spine formation, but whether it contributes to the filopodial stage of spine formation or whether it is involved in the maturation of dendritic spines is not known[99,100]. The possible roles of other I-BAR proteins in filopodia formation have been less extensively characterized, although overexpression of the I-BAR proteins MIM, IRTKS and ABBA induce formation of filopodia-like protrusions in mammalian cells[101–103].

Other proteins that are involved in filopodia formation. Several new proteins that induce the formation of filopodia have also been identified. A recent study has shown that an integral membrane protein, the lipid phosphatase-related protein 1 (LPR1), induces filopodia formation in a manner that is independent of the ARP2/3 complex and ENA/VASP proteins. This protein is catalytically inactive compared with other members of the lipid phosphatase family, and has therefore evolved a distinct function that leads to filopodia formation by an as-yet-unknown mechanism[15]. A tempting hypothesis is that this membrane protein could function as a link between plasma membrane and filopodia core proteins or induce membrane curvature similarly to I-BAR-domain proteins.

The forces resulting from the polymerization of tightly packed filaments are traditionally thought to result in an extension of the plasma membrane, whereas Brownian motion of the filament ends creates space for the addition of new subunits between filament barbed ends and the plasma membrane. N-WASP, which binds actin monomers through its WASP-homology-2 domain (WH2 domain) and interacts with the plasma membrane through its lipid-binding polybasic region and through interactions with Rho GTPases, was shown to physically attach the growing filament barbed ends to the plasma membrane. It was shown that the actin-binding WH2 domain of the membrane-bound N-WASP feeds barbed ends with actin monomers and thus links the elongating filament ends to the membrane[104]. Future studies are required to reveal whether similar linkage between elongating filament barbed ends and the plasma membrane is necessary for filopodia formation.

Mechanisms of filopodia formation

Two alternative models for the mechanism of filopodia formation have been presented. In the so-called 'convergent elongation model' the filopodial actin filaments are derived from the lamellipodial actin network, whereas an alternative model proposes that actin filaments in filopodia are nucleated at filopodial tips by formins. Typically, filopodia are found at the leading edge of a migrating or an elongating cell, where they are connected to the lamellipodial actin network. However, in dendrites, for example, filopodia-like precursors of spines probably form without an underlying dendritic actin array. Therefore, divergence in the molecular mechanisms that generate these thin plasma-membrane protrusions is expected, and this might also explain the contradictory results concerning the mechanisms of filopodia formation[8,9].

Convergent elongation model. Platinum replica transmission electron microscopy analysis of the leading edge of melanoma cells suggested that filopodia arise from the lamellipodial F-actin network, and a continuous actin bundle extends from the root to the tip of filopodia[7]. This study led to a convergent elongation model, in which filopodial actin filaments are derived from the ARP2/3 complex-nucleated dendritic lamellipodial actin array. According to this model, for example, a subset of ARP2/3-nucleated actin filaments are protected from capping, and their barbed ends are clustered together by tip-complex proteins, which include ENA/VASP proteins, Dia2 and myosin-X. These rapidly elongating filaments would subsequently be crosslinked by fascin to generate the typical filopodial architecture of actin filaments[8].

De novo *filament nucleation model.* Cryo-electron tomography analysis of *D. discoideum* filopodia revealed a discontinuous F-actin bundle in the filopodium core, and short individual filaments converging into the 'terminal cone'. Similar terminal cone structures have not been detected in mammalian cells and might therefore optimize the faster dynamics of the amoeba filopodia[105]. Furthermore, it was suggested that filopodia can also form in the absence of the ARP2/3 complex and/or its activators both in *D. discoideum* and in mammalian cells[12], although a recent study provided evidence that

SH3 domain
A small globular protein domain that interacts with Pro-rich peptides and is found in many signalling and cytoskeletal proteins.

WH2 domain
A small actin-monomer-binding protein domain that was originally identified from WASP-family proteins.

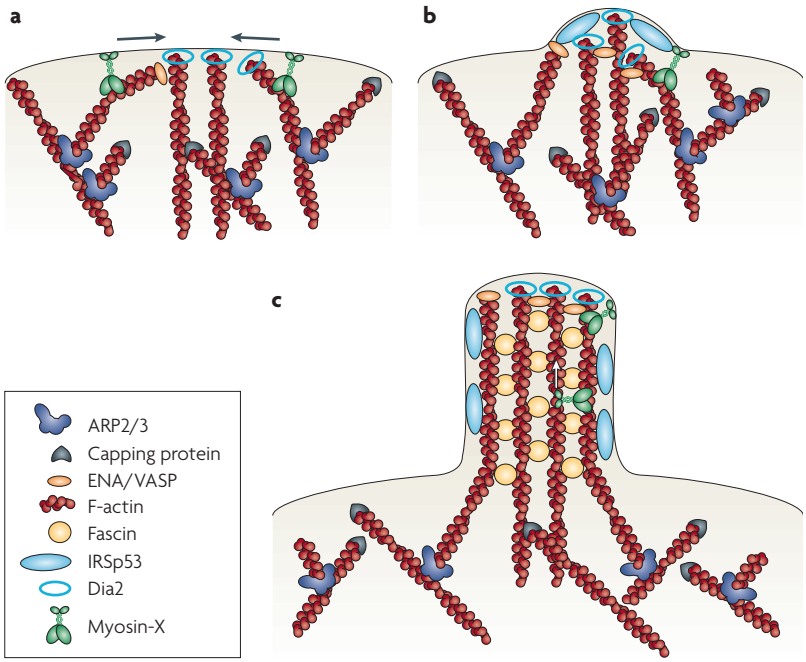


Figure 3 | **A working model for filopodia formation.** The model describes functions of key proteins at different stages during filopodia formation. However, it is important to note that some controversies exist concerning the activities of individual proteins, and it is likely that the relative importance of the filopodial components varies between different organisms and their cell types. **a** | A subset of uncapped actin filaments of the actin-related protein-2/3 (ARP2/3)-nucleated dendritic network are targeted for continued elongation by the formin Dia2 (diaphanous-related formin-2) and/or by ENA/VASP (enabled/vasodilator-stimulated phosphoprotein) proteins. Dia2 also nucleates the formation of new, unbranched actin filaments. The barbed ends of these elongating actin filaments are converged together through the motor activity of myosin-X, leading to the initiation of a filopodium. **b** | When the preliminary filopodium begins to push the plasma membrane, insulin-receptor substrate p53 (IRSp53) (or other inverse (I)-BAR domain-containing proteins) might further facilitate plasma membrane protrusion by directly deforming or tubulating the membrane. Alternatively, IRSp53 might sense the negative membrane curvature that is induced by pushing forces of elongating filaments and recruit other components to the site of filopodial initiation. ENA/VASP proteins can also function as initial F-actin-crosslinking proteins in the tip of an elongating filopodium. **c** | The incorporation of the actin crosslinking protein fascin in the shaft of the filopodium generates a stiff actin filament bundle. At this stage, myosin-X might localize adhesion molecules to the filopodium tip by processive barbed-end directed movement (arrow) and/or attach the elongating actin filament barbed ends to the plasma membrane through its interaction with phosphatidylinositol phosphates. Dia2 is localized in the 'tip complex' and controls the barbed-end elongation of the filaments.

the ARP2/3 complex participates in filopodia formation at least in neuronal cells[106]. These observations led to an alternative model for filopodia formation, in which actin filaments of filopodia do not derive from the underlying lamellipodial network, but are nucleated at filopodial tips by formins.

Indeed, both Dia2 formin and VASP are essential for filopodia formation in *D. discoideum*[66]. In the light of the initially suggested role of ENA/VASP proteins in filament anti-capping and barbed-end elongation, the synergistic role of ENA/VASP proteins with formins is rather difficult to interpret. Thus, Schirenbeck *et al.* postulated that ENA/VASP proteins promote filopodia formation through their F-actin-bundling activity[66]. However, overexpression of Dia2 was shown to rescue the lack of filopodia in mammalian ENA/VASP knockout cells, which suggests that these two proteins have at least partially overlapping roles in filopodia formation[34].

Current working model. Interestingly, a recent study showed that the depletion of mammalian Dia2 from melanoma cells severely inhibits the formation of both lamellipodial and filopodial actin filament structures. Based on correlative light and electron microscopy analysis, the authors also suggested that filopodia in cells that express a constitutively active Dia2 arise through the gradual convergence of long Dia2-nucleated lamellipodial filaments into bundles[13]. Together, these data suggest that several different mechanisms regulate the elongation and organization of actin filaments during filopodial formation, and that the relative importance of these mechanisms varies between different organisms and between distinct cell types.

A working model for the initiation and elongation of a filopodium is presented in FIG. 3. According to this model, filopodia formation is initiated by the convergence of uncapped or formin-nucleated actin filament barbed ends at the plasma membrane by the activity of myosin-X (FIG. 3a). The elongation of these protected actin filament barbed ends towards the plasma membrane provides the force for membrane deformation during filopodial elongation. In addition, I-BAR domain proteins, such as IRSp53, might directly deform the plasma membrane to enhance the formation of a tubular filopodial plasma-membrane protrusion (FIG. 3b). The elongating actin filaments are subsequently crosslinked by fascin and perhaps also by the ENA/VASP proteins to generate a stiff filopodial actin filament bundle (FIG. 3c). In addition to its role in barbed-end convergence, myosin-X seems to be involved in the localization of filopodial components to the tip and might therefore regulate the elongation rate, stability and adhesive properties of the filopodium. The relative importance of each of these components in filopodia formation would then depend on multiple factors such as the availability of the dendritic actin filament network as well as other proteins, which also contribute to filopodial formation but whose functions during this process have not yet been extensively characterized.

Conclusions and future directions

Extensive studies by several laboratories have identified a key set of proteins that have an important role in filopodia formation in various cell types. These include signalling proteins, actin-crosslinking proteins, factors that regulate actin filament nucleation and elongation, motor proteins and proteins that are capable of tubulating the plasma membrane. However, the relative importance of each of these proteins in filopodia formation seems to vary between different organisms and their cell types. This might provide an explanation for the differences in the morphology and dynamics of filopodia-like membrane protrusions in different cells.

It is likely that other, currently unidentified, factors have a central role in filopodia formation, and so there is a need for the systematic identification and analysis of

filopodial components. Furthermore, several important questions concerning the functions of already identified filopodial components exist. These include, for example, the characterization of the exact role of ENA/VASP proteins during filopodia initiation and elongation as well as understanding the function of myosin-X and its cargo-binding domain in filopodial tips. In addition, elucidating the mechanisms by which adhesion structures are formed in the filopodia of different cell types will provide important challenges for future research. Finally, the comparison of filopodia components and their relative importance in filopodia formation between different cell types will probably enlighten our understanding of the contradictory data concerning the roles of the ARP2/3-nucleated lamellipodial actin network and ENA/VASP proteins in filopodial formation.

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DATABASES
Interpro: http://www.ebi.ac.uk/interpro
BAR | IMD
UniProtKB: http://beta.uniprot.org
CDC42 | EPS8 | FAK | IRSp53 | MTSS1 | N-WASP | paxillin | Rac1 | RhoA | talin | WASP

FURTHER INFORMATION
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