The lamellipodium: where motility begins

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Lamellipodia, filopodia and membrane ruffles are essential for cell motility, the organization of membrane domains, phagocytosis and the development of substrate adhesions. Their formation relies on the regulated recruitment of molecular scaffolds to their tips (to harness and localize actin polymerization), coupled to the coordinated organization of actin filaments into lamella networks and bundled arrays. Their turnover requires further molecular complexes for the disassembly and recycling of lamellipodium components. Here, we give a spatial inventory of the many molecular players in this dynamic domain of the actin cytoskeleton in order to highlight the open questions and the challenges ahead.

A supplementary movie is available at: http://archive.bmn.com/supp/tcb/small.avi

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Thirty years ago, when concepts of non-muscle cell structure were rudimentary, Abercrombie identified the thin layer of cytoplasm (~0.2 μm thick) that protrudes at the front of spreading and migrating cells as the primary 'organelle' of motility. When such protrusions were parallel to the substrate, he referred to them as the 'leading lamella', the 'leading edge' or the 'lamellipodium' (Fig. 1); when they curled upwards, he referred to them as 'ruffles' [1]. Subsequent studies over the next two decades [2] revealed the presence of concentrated arrays of polar actin filaments in lamellipodia and demonstrated that protrusion was based on actin polymerization. Experiments in which fluorescent actin was injected into fibroblasts showed that lamellipodia were, in fact, the primary sites of actin incorporation [3], marking them as the major 'filament factory' of the cell [4]. Alongside their protrusive activity, lamellipodia serve other important roles. They are involved in the development of adhesions to the substrate and, as ruffles, serve in macropinocytosis and phagocytosis. They must therefore recruit all the components required for these functions. Also, adhesion itself entails reorganization of lamellipodium filaments, leading to the development of different classes of adhesion complexes.

As far as motility is concerned, interest currently focuses on how actin polymerization is localized and controlled. Because lamellipodia are not easily isolated for biochemical analysis, ideas on this front first developed from *in vitro* studies of actin polymerization and from the characterization of the proteins recruited by pathogens to enable their movement in cytoplasm [5,6]. From these studies, the Arp2/3 complex has emerged as an important player in the initiation of actin polymerization for actin-based pathogen motility [5,6], and other findings support a role for Arp2/3 in lamellipodium protrusion [7,8]. However, the Arp2/3 complex is just one player among many implicated in initiating, organizing and disassembling the lamellipodium network. More recent progress in characterizing other players has come in part from the use of green-fluorescent protein (GFP) to tag putative components, combined with live-cell microscopy to localize them *in vivo*. This approach, which has rapidly gained in importance, is particularly relevant to the question of lamellipodium organization because chemical fixation can easily lead to the loss of resident components and, under inappropriate conditions, to the gross distortion of lamellipodium structure; unfortunately, this is common in published pictures. Here, we attempt to produce a current molecular inventory of

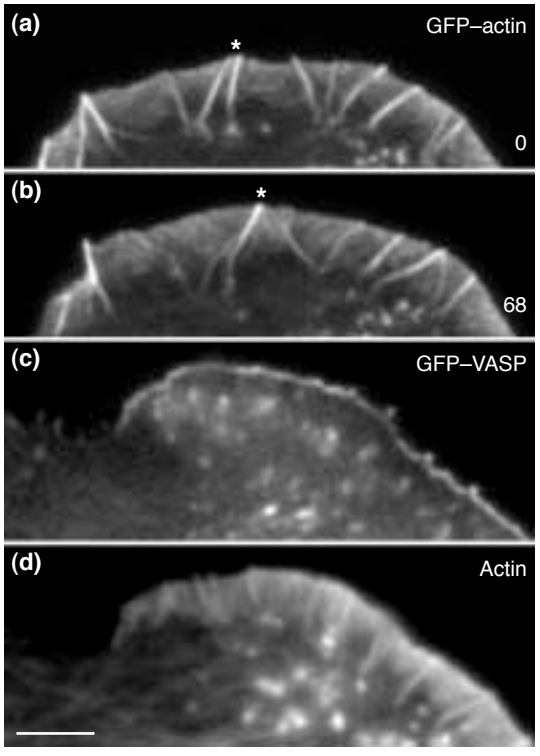

Fig. 1. *(a,b) The lamellipodium and associated microspikes seen in two video frames of a B16 mouse melanoma cell expressing green-fluorescent protein (GFP) fused to actin (see supplementary video at: http://archive.bmn.com/supp/tcb/small.avi). In addition to the forward translation of the lamellipodium, there is a lateral motion of microspikes, indicated by the asterisks: two microspikes (a) fuse into one (b). The numbers indicate time in seconds. (c,d) The localization of VASP at the tips of protruding lamellipodia. (c) The last video frame of a living cell expressing GFP–VASP before fixation with glutaraldehyde. (d) The fixed cell after labeling of actin with phalloidin. Bar, 5 μm.*

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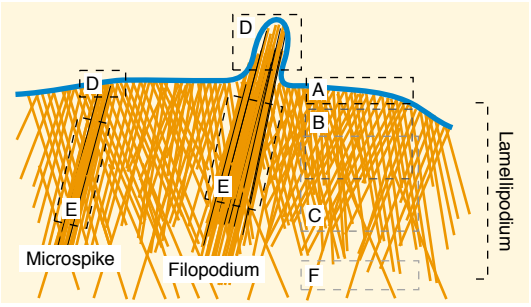

Fig. 2. *Schematic representation of subdomains in lamellipodia and filopodia: (A) tip of lamellipodium; (B) actin meshwork; (C) region of major disassembly; (D) tip of filopodium; (E) bundle; (F) undegraded filament that contributes to the cytoplasmic network. According to localization studies, zones B and C overlap considerably, but the activity of disassembly increases towards the base of the lamellipodium.*

lamellipodia, with the aim of highlighting their subdomains and composition (Figs 2–4) and to discuss the functional implications and open questions.

However, first, a note on nomenclature. Depending on cell type and condition, the lamellipodium can vary in breadth from ~1 μm to 5 μm and can exhibit highly variable numbers of radiating bundles 0.1–0.2 μm in diameter and many micrometers long. When contained within the breadth of the lamellipodium, the bundles have often been referred to as 'ribs' and, when they extend beyond the edge of the lamellipodium, as either 'microspikes' or 'filopodia'. Here, we use 'microspikes' (rather than ribs) [9] to describe bundles that do not project beyond the cell edge and 'filopodia' when they do. According to this nomenclature, microspikes are part of the lamellipodium and can be potential precursors of filopodia. The term 'cortical actin', often misused to describe lamellipodium networks, will be reserved for actin-associated complexes at the cell membrane, involving proteins such as spectrin, dystrophin and ezrin.

Lamellipodium tip engages protein complexes to drive actin polymerization

Pathogens that usurp the machinery of the cell to move in cytoplasm do so by recruiting to their surface the complexes involved in driving actin polymerization [6] (Table 1). A growing body of evidence indicates that the tips of lamellipodia and filopodia serve an analogous function of localizing and harnessing actin polymerization for cell motility. This was highlighted by studies of the dynamics of GFP-tagged vasodilator-stimulated phosphoprotein (VASP; a member of the Ena/VASP family of proteins) in melanoma cells. VASP, which binds to the surface protein ActA of *Listeria* [6], was found to accumulate at the tips of lamellipodia and filopodia (Fig. 1), corresponding to the sites where the fast-growing ends of the actin filaments abut the cell membrane. The amounts of VASP recruited to lamellipodium tips increased with the protrusion rate [10], pointing to a positive role of Ena/VASP proteins for actin assembly. Such a role for VASP was demonstrated for the actin-based motility of *Listeria* [11] and for phagocytosis [12]. The apparent incompatibility of these findings with the increased motility of cells lacking Ena/VASP proteins [13] might be explained by changes in their ruffling and adhesion dynamics, or by a combination of these, leading to more efficient net translocation. Nevertheless, VASP represents the first of a growing list of proteins marking lamellipodium tips as sites of assembly of protein complexes engaged in driving and regulating actin polymerization (Fig. 3, Table 1).

Actin-driven pathogen motility involves the activation of the Arp2/3 complex at the pathogen surface, but different pathogens recruit alternative combinations of molecular adaptors to achieve this [6]. Thus, ActA of *Listeria* can activate Arp2/3 directly, whereas *Shigella* and *Vaccinia* virus recruit the Wiskott–Aldrich-syndrome protein family member N-WASP to activate Arp2/3. For vertebrate cells, another family member (Scar/WAVE) has been implicated in activating Arp2/3 in lamellipodium formation [8,14]; this is supported by the localization of Scar/WAVE1 at lamellipodium tips [15,16]. Scar/WAVE proteins interact directly with the Abelson tyrosine kinase, c-Abl [17], which has previously been implicated in actin dynamics, suggesting that Scar/WAVE proteins might recruit this kinase to lamellipodia. In addition to Scar/WAVE-proteins, c-Abl also interacts with a protein family termed Abi proteins (Abl-interacting proteins), which localize exclusively to the tips of lamellipodia and filopodia [18]. Because fibroblasts deficient in Abl and its relative Arg (Abl-related gene) can still form lamellipodia [19], c-Abl is not essential but might serve in the modulation of lamellipodium protrusion. This would be in line with findings implicating this kinase in cell motility [19,20]. Another protein that accumulates at the surface of *Listeria* and at the tips of lamellipodia and filopodia is profilin [21], which is known to enhance the treadmilling of actin filaments *in vitro* by shuttling monomeric actin to the barbed ends of actin filaments [5]. Its localization here is thus consistent with bulk addition of actin monomer close to the membrane [22].

Signaling at the tip through Rho GTPases

The assembly of actin-based membrane projections is regulated by small GTPases of the Rho family [23,24]. Two members of this family, Rac1 and Cdc42, signal the formation of lamellipodia and filopodia, respectively [25]. The activation of Rac and Cdc42 can be mediated by stimulation of both growth factor [23] and integrin [26] receptors and requires GDP–GTP exchange factors (GEFs), many of which have been described [27,28]. Rho GTPases are synthesized as cytosolic proteins but can be targeted to membranes by a series of posttranslational modifications [29]. General membrane localization cannot explain the

focal induction of lamellipodia or filopodia at the cell periphery, and so it is tempting to speculate that Rac and Cdc42 might be locally activated to induce these protrusions. Indeed, a fluorescence resonance energy transfer (FRET) approach to visualizing GTP-bound Rac1 in live cells recently revealed an accumulation of the activated state of this GTPase in membrane ruffles upon growth factor stimulation [30].

Upon ligand binding, growth-factor receptors can activate phosphoinositide 3-kinases, a product of

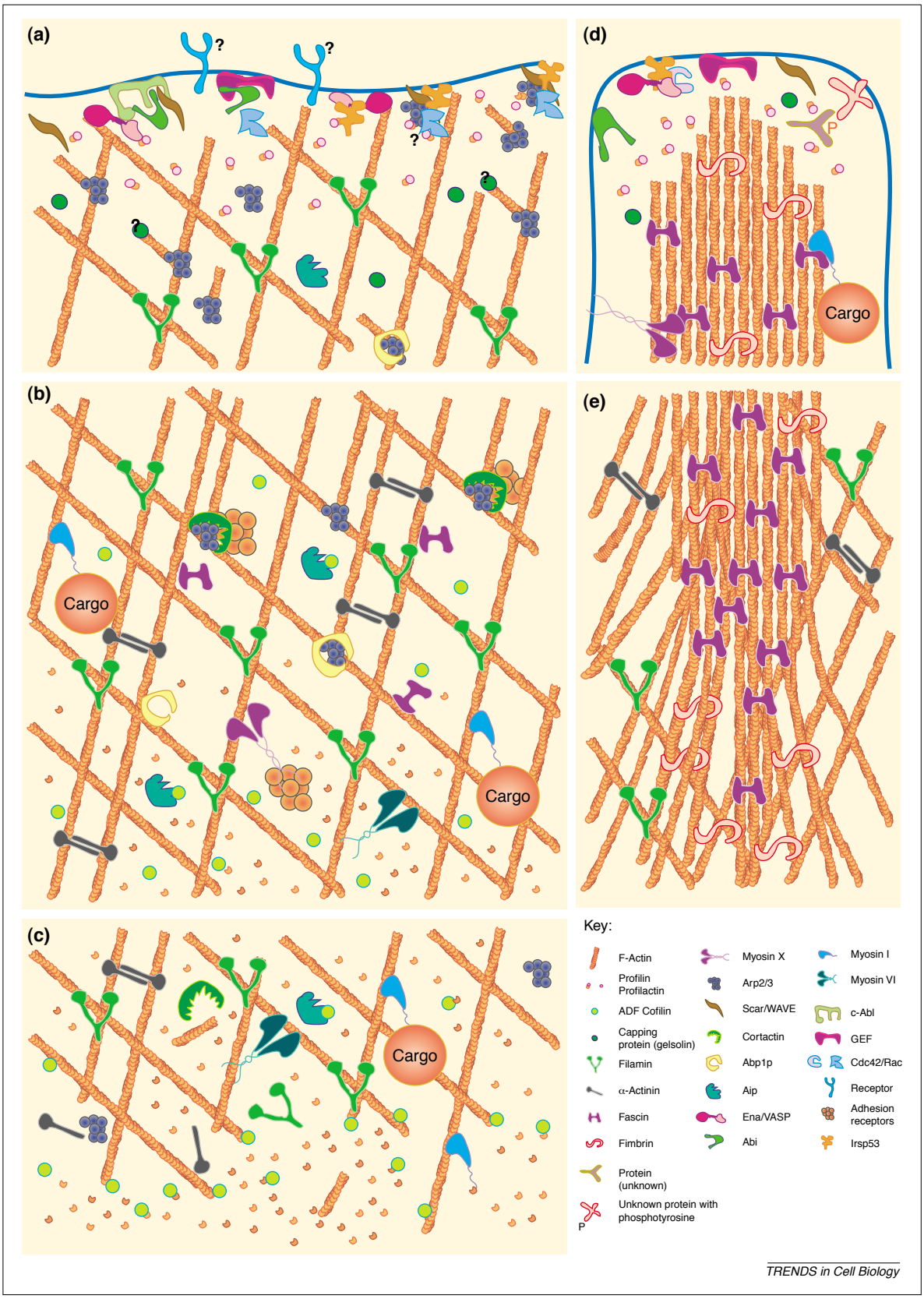

Fig. 3. *The locations of molecules and complexes in the zones corresponding to those depicted in Fig. 2: (a) tip of lamellipodium, (b) actin meshwork, (c) region of major disassembly, (d) tip of filopodium, and (e) bundle.*

which – phosphatidylinositol (3,4,5)-trisphosphate – in turn activates GEFs such as Vav [31] and Sos [28]. Interestingly, lipid products of phosphoinositide 3-kinases [such as phosphatidylinositol (3,4,5)-trisphosphate] accumulate in a polarized way at the protruding membranes of chemotactic leukocytes and are thought to contribute to the spatial activation of Rho GTPases [32]. It is therefore an exciting question whether GEFs are present at the sites of actin assembly or whether the Rho GTPases are recruited to these sites after being activated by GEFs elsewhere in the cell. An indication that the first of these is true comes from the recent demonstration that Vav-1, a GEF for both Rac and Cdc42, is recruited to the tips of filopodia [33].

Recently, potential pathways for the transduction of signals from active Rac and Cdc42 to actin polymerization into lamellipodia and filopodia have been uncovered. Of the many effector proteins that interact specifically with GTP–Cdc42, only the haematopoietic Wiskott–Aldrich-syndrome protein (WASP) and its ubiquitous family member N-WASP provide a direct link to actin assembly through activation of the nucleating activity of the Arp2/3 complex. *In vitro*, phosphatidylinositol (4,5)-bisphosphate and GTP–Cdc42 can activate N-WASP in a cooperative manner, and it has therefore been proposed that N-WASP could, upon recruitment to and activation at the membrane, effect the protrusion of filopodia and/or lamellipodia [5]. However, recent studies of cells derived from N-WASP-knockout models demonstrated that N-WASP is not essential for Cdc42-based filopodium formation [34,35] and therefore call for a revision of current models of N-WASP function in actin assembly [5,20].

As opposed to the direct interaction of N-WASP and Cdc42, Scar/WAVE (which transduces Rac-mediated lamellipodium formation via the Arp2/3 complex) cannot bind to Rac directly [14]. A search for the link between Rac and Scar/WAVE led to the identification of the insulin receptor substrate Irsp53 (also known as IRS-58) [36]. This adaptor protein links activated Rac and Scar/WAVE to induce lamellipodia [36] but is recruited to the tips of both lamellipodia and filopodia (H. Nakagawa and J.V. Small, unpublished). In line with the additional localization of Irsp53 at filopodium tips, overproduction of this protein has been found to induce the formation of Cdc42-based filopodia [37]. More recently, a direct interaction of Irsp53 with Cdc42 was attributed to a partial Cdc42/Rac interactive binding (CRIB) motif, and filopodium formation was proposed to involve binding of Irsp53 to the Ena/VASP-family protein Mena [38]. Together, these results led to the proposal of a novel, Arp2/3-independent, pathway for filopodium induction, which would be consistent with the findings from N-WASP-defective fibroblasts [34].

Another signaling pathway implicated in lamellipodium formation involves the p21-activated kinase (PAK) protein family. These serine/threonine kinases were identified as direct downstream effectors of Rac and Cdc42. PAKs are engaged in multiple signaling pathways, some of which might be coupled directly to lamellipodium protrusion. For instance, PAK interaction with Cdc42/Rac increases the levels of phosphorylated myosin light chain (MLC) thought to be required for the anchorage of lamellipodia. In addition, PAKs were shown more recently to activate Lim kinases to phosphorylate and thereby block the severing/depolymerizing activity of cofilin, which is proposed to effect lamellipodium turnover [39,40].

Forming and stabilizing the actin network

Actin polymerization at the lamellipodium tip must be tightly coupled to the establishment of molecular linkages that constrain the generated actin filaments within a membrane sheet, through filament–filament and filament–membrane interactions. Emphasis has recently been placed on the possible role of the Arp2/3 complex in initiating and structuring actin networks. *In vitro* experiments have shown that Arp2/3 can promote the branching of actin filaments, but conflicting models have been proposed for how this occurs [41,42]. Nevertheless, evidence for the *in vivo* relevance of filament branching by Arp2/3 has been extracted from appealing images of lamellipodium meshworks prepared for electron microscopy by an improved critical-point drying method [43]. Accordingly, a dendritic branching model of actin-based protrusion has been widely accepted to explain cell motility [43,44]. Although it is attractive, this model still requires rigorous testing, especially by the use of alternative methods of electron microscopy to re-evaluate the existence and frequency of filament branching in lamellipodia.

Two of the proteins shown to bind to and activate the Arp2/3 complex *in vitro*, cortactin and Abp1, also co-distribute with Arp2/3 across the lamellipodium. Because cortactin can activate Arp2/3 when bound to F-actin and inhibits debranching of *in vitro* Arp2/3–actin complexes, it has been suggested to serve as a stabilizer of the putative actin filament branches in the lamellipodium [45]. Cortactin is also found along the length of actin comet tails of pathogens, where it might play a similar role in network stabilization [46]. As a potential receptor linker, cortactin might couple actin flow to receptors on the surface of the lamellipodium [47]. Abp1 has similar properties to cortactin, and complementary data from yeast and mammalian cells suggest that it might link actin polymerization with endocytosis [48,49]. The localization of the related protein drebrin in the lamellipodium and in close proximity to the plasma membrane [50] is consistent with such a role.

Other candidates for actin network stabilization are the classical actin crosslinking proteins filamin and α-actinin. A structural role for filamin in

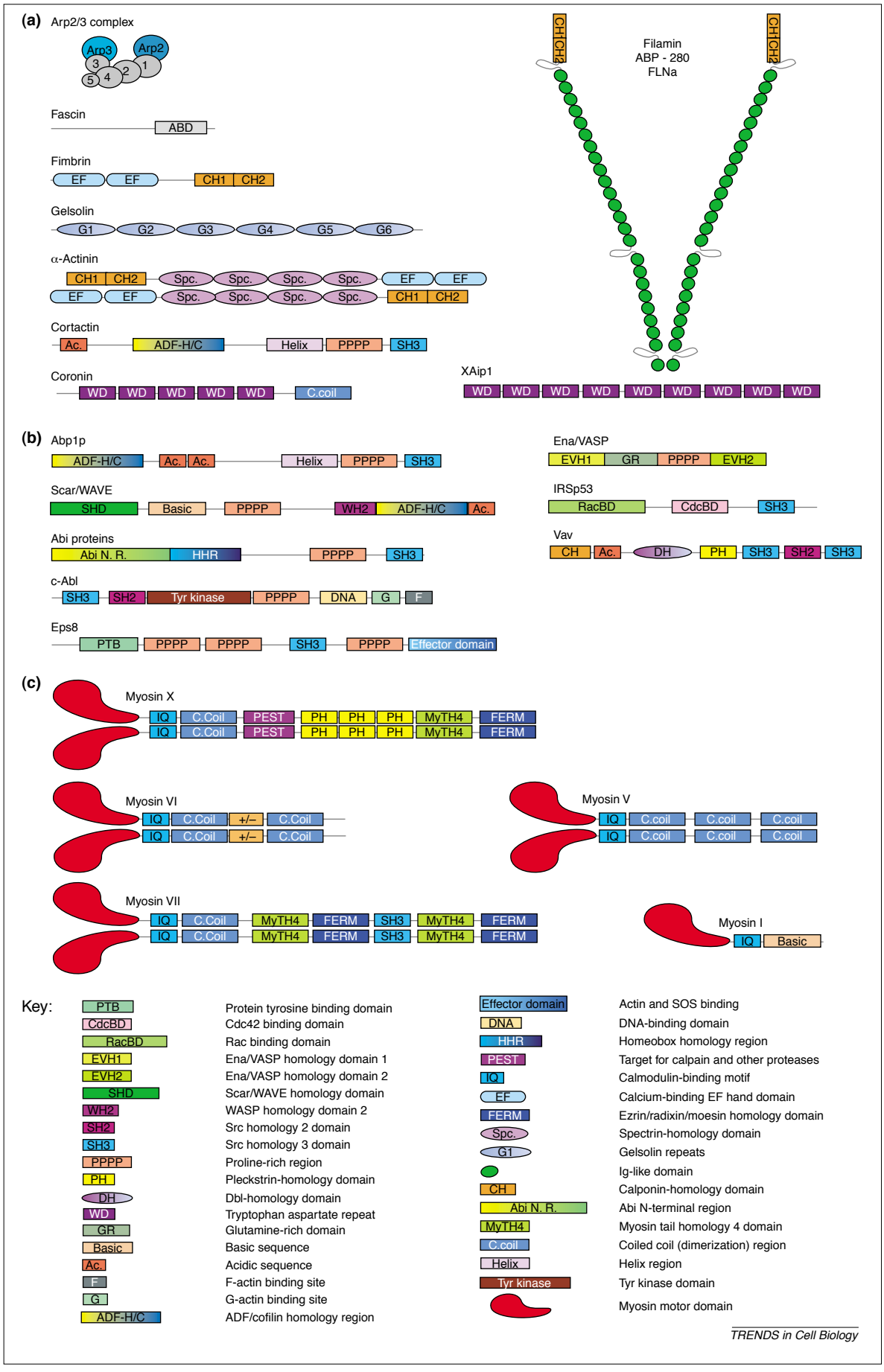

Fig. 4. *Domain organization of proteins in lamellipodia and filopodia. (a) Actin-binding and -remodeling proteins. (b) Modular structure of signaling proteins. (c) Modular structure of myosin motors.*

Table 1. Comparative localization of proteins involved in actin-based motility

Protein	Lam.	Fil.	Pathogen	Putative functions	Selected binding partners	Refs
Scar/WAVE	*Tip*	*Tip*	*n.d.*[a]	*Activates Arp2/3.*	*Arp2/3 complex, G-actin*	*[15,16]*
Profilin IIa	*Tip*	*Tip*	*Pole*	*Also in focal adhesions. Shuttles actin monomers onto filaments.*	*WASp/Scar, Ena/VASP, G-actin, Arp2/3 complex, dynamin*	*[21]*
Ena/VASP	*Tip*	*Tip*	*Pole*	*Also on stress fibers and in focal adhesions Modulate actin polymerization.*	*Zyxin, vinculin, profilin, F-actin, c-Abl*	*[10,13, 16]*
Irsp53	*Tip*	*Tip*	*n.d.*	*Links Rho GTPases to effector proteins.*	*Rac1, Cdc42, Scar/WAVE, Mena*	*[36]* –[b]
Abi	*Tip*	*Tip*	*n.d.*	*Adaptor protein engaged in multiple complexes.*	*Abl, Arg, Eps8, Sos, Nap*	*[18]*
c-Abl	*Tip*	*n.d.*	*n.d.*	*Tyrosine kinase that modulates actin protrusions.*	*Abi, Ena/VASP, Scar/WAVE*	–[c]
Vav-1	–	*Tip*	*n.d.*	*GEF for Rho GTPases.*	*Rac1, Cdc42, Rho, Nck, Ack, Fyn*	*[33]*
Arp 2/3 complex	*Tip and Mesh.*	–	*Tail*	*Nucleates and branches actin filaments.*	*WASp/Scar cortactin, Abp1, profilin, actin*	*[8,43]*
Coronin	*Mesh.*	*n.d.*	*Tail*	*Promotes actin polymerization.*	*F-actin*	*[62,60]*
Cofilin	*Mesh.*	*n.d.*	*Tail*	*Severs and depolymerizes actin filaments.*	*F-actin, G-actin, Aip, Lim kinase*	*[62]*
Cortactin	*Mesh.*	*n.d.*	*Tail*	*c-Src substrate. Stabilizes Arp2/3-induced actin filament network.*	*F-actin, Arp2/3, dynamin, c-Src, shank-2*	*[46,47]*
Capping proteins (CapZ)	*Mesh.*	*n.d.*	*Tail*	*Block growth from actin filament barbed ends.*	*F-actin*	*[62]*
Fascin	*Mesh.*	*Microspike*	*n.d.*	*Regulated by phosphorylation. Bundles actin filaments.*	*F-actin*	*[65,66]*
Fimbrin (Plastin)	*Mesh.*	*Microspike*	*Tail*	*Also found in microvilli. Ca^{2+} sensitive. Bundles actin filaments.*	*F-actin, Ca^{2+}*	*[69]*
Talin	*Front zone*	*Tip region*	*Tail*	*Links receptors to the actin cytoskeleton.*	*Layilin (in lamellipodium)*	*[98,99]*
Filamin	*Mesh.*	*Microspike*	*Tail*	*Stabilizes actin filament meshworks.*	*Small GTPases, integrins, RalA, Trio*	*[98]*
α-Actinin	*Mesh.*	*Microspike*	*Tail*	*Bundles actin filaments, links receptors to actin cytoskeleton.*	*Integrins, F-actin, Ca^{2+}, PIP2*	*[56]*

[a] *Abbreviations: Lam., lamellipodium; Fil., filopodium; Mesh., lamellipodial actin meshwork; n.d.: not determined; (–), not localized; PIP2, phosphatidylinositol (4,5)-bisphosphate.*
[b] *H. Nakagawa and J.V. Small, unpublished.*
[c] *P. Hahne and J.V. Small, unpublished.*

lamellipodia is supported by findings with cells of a human line deficient in the filamin isoform FLNa, which spread poorly and bleb actively at their edges but revert to normal morphology on transfection with *FLNa* cDNA [51]. Recent studies reconfirm the localization of filamin in the actin filament network of lamellipodia and raise the question of the relative contributions of filamin and Arp2/3 in network formation and stabilization [52]. In addition to binding to F-actin, filamin can associate with transmembrane proteins through its C-terminal region [53,54]. Thus, filamin could serve as a linker between the membrane and the cytoskeleton to recruit signaling proteins to the vicinity of sites of actin polymerization and remodeling. The reported association of filamin with the Rho GEF Trio [55] and small GTPases [53] supports an involvement of filamin as a docking site for signaling molecules, although the significance is unclear. α-Actinin crosslinks actin filaments into bundles and networks *in vitro* and localizes throughout the lamellipodium [56]. Different isoforms are partially segregated between different actin compartments, with actinin 1 and actinin 4 in ruffles [57]. α-Actinin-null cells of *Dictyostelium* show no motility defects except in a null background of the filamin homolog ABP120 [58], suggesting structural complementation between these crosslinkers in the lamellipodium. In synthetic comet tails of actin [11], a lack of α-actinin results in a less compacted tail, supporting a crosslinking function for this protein.

Coronin, an actin-binding and crosslinking protein, is similarly homogeneously distributed in lamellipodia, and its deletion in *Dictyostelium* leads to decreased motility and impaired cytokinesis [59]. *Xenopus* coronin remains bound to fibroblast cytoskeletons after Triton extraction, and coronin overproduction amplifies lamellipodium formation [60]. The nature of the interplay between coronin and other actin-binding proteins is unknown, but the β-propeller-forming WD domains in coronin could mediate interactions with such partners [61]. Coronin is present throughout the *Listeria* actin tail [62], consistent with a membrane-independent structural function.

Microspikes and filopodia

According to antibody labeling [43], Arp2/3 is excluded from filopodia and microspikes. This situation might reflect the elongation of pre-existing filaments in filopodia during protrusion [63] with no new filament generation, as in lamellipodia. Microspikes and filopodia are probably generated by bundling of lamellipodium filaments; fascin and fimbrin (plastin), which both bundle actin filaments *in vitro*, have been implicated in this process [64]. Fascin is a ubiquitous protein involved in stabilizing actin bundles in prominent cellular processes, including stereocilia and hair bristles [64], where additional crosslinkers cooperate in bundling. Bundling of actin by fascin is inhibited by serine phosphorylation *in vitro* [65] and also *in vivo* [66],

but the details remain to be clarified [67]. An additional regulatory pathway of bundle formation is suggested by the inhibition of the actin binding of fascin by drebrin [68]. Bundling by fimbrin might be regulated by calcium or phosphorylation, but we must admit that almost nothing is known about the way filopodia are assembled and disassembled *in vivo*.

However, because fascin and fimbrin are found not only in microspike bundles but also in the intervening lamellipodium network [66,69], we assume that they exist in these two locations in dormant and active states. A further interesting aspect is the often-observed rapid lateral mobility of microspikes and filopodia (see supplementary video at: http://archive.bmn.com/supp/tcb/small.avi). How does this occur? A simple explanation is provided by the geometry of the lamellipodium network, which suggests that there is a lateral flow of filaments during protrusion [70]. Taking this idea one step further, the rate of lateral movement of bundles in neuronal growth cones increases with their angle to the lamellipodium front [71]. From correlated measurements of retrograde flow rate, the lateral movement could readily be explained by the extension of bundles by polymerization at the tip, whereby the polymerization rate increased with angle [71].

An important feature distinguishing microspikes and filopodia from lamellipodia is the difference in the complement of proteins at their tips. For example, microspike and filopodium tips harbor an unknown protein that is heavily phosphorylated [72], lack Scar/WAVE-1 [15] and selectively recruit Vav [33]. Further characterization of the proteins specifically resident at filopodium tips should contribute to a clarification of the targets downstream of Cdc42 that specify filament bundling and filopodium protrusion.

Lamellipodium disassembly

In a steadily migrating lamellipodium, the actin meshwork remains essentially constant in breadth (Fig. 1 and supplementary video at: http://archive.bmn.com/supp/tcb/small.avi), indicating a balance between assembly at the front and disassembly at the rear. Protrusion and retraction rates can be regulated at the level of actin assembly, apparently through the recruitment or dissociation of regulatory scaffolds [10,15,18]. Disassembly is thought to be achieved by proteins of the ADF/cofilin family [73] and possibly severing proteins like gelsolin, probably in cooperation with factors that break filament crosslinks [5]. These ideas stem more or less entirely from *in vitro* data but receive circumstantial support from localization studies. Depolymerization of actin by cofilin is inhibited through phosphorylation by LIM kinase [73] and enhanced by the actin-interacting protein Aip1 [74]. Cofilin localizes throughout the lamellipodium in *Dictyostelium* [75] and in fibroblasts [43]. This suggests that depolymerization is not restricted to the rear of the lamellipodium [43], consistent with a graded distribution of filament lengths from front to rear [76].

So that unproductive actin polymerization away from the cell edge is avoided, it has been suggested that any fast-growing free filaments ends that might be exposed in the lamellipodium network (by severing, for example) are excluded from the polymerization pool through 'capping' by the actin filament capping protein [5]. This suggestion is consistent with the presence of capping protein in lamellipodia and membrane ruffles [77]. For the *in vitro* propulsion of *Listeria* or *Shigella* [11], capping protein has been successfully used to limit the polymerization of actin to the pathogen surface. Also, in this assay, capping protein can be functionally replaced by gelsolin, implying that gelsolin family members might play a complementary capping role in lamellipodia [78]. However, is such a role required? It must be admitted that the problems of free plus-ends of actin filaments away from the cell edge is most simply solved if they do not exist at all. Indeed, their existence in lamellipodia has yet to be convincingly demonstrated.

Shunting to the front with myosin motors

Several non-filament-forming members of the myosin family have been localized in lamellipodia and filopodia, following the first observation of myosin I in *Dictyostelium* [79]. Myosin is not required for *Listeria* motility *in vitro* [11], suggesting a specific need for myosin-linked processes in the membrane leaflet environment of the lamellipodium. In addition to myosin I, myosins V and VI [80], VII [81], and X [82] localize to lamellipodia and membrane ruffles. Myosin I proteins in both budding yeast and *Dictyostelium* bind through their Src-homology 3 (SH3) domains, directly or indirectly, with Arp2/3 and other components of the actin polymerization machinery [83–86], lending support to the idea that these myosins might act by carrying cargo to the plus-ends of actin filaments, thus acting as cofactors in protrusion. Both myosin V and myosin VI localize to the lamellipodia of human carcinoma cells after stimulation with epidermal growth factor [80]. Myosin V has been generally implicated in vesicle transport [87], but a role in protrusion has also been suggested by an antibody-linked approach to disable it through chromophore-assisted laser inactivation (CALI) [88]. Significantly, myosin VI moves in a direction on actin that is opposite to that of all other known myosins [89], raising interesting questions about its function. Because there is rapid retrograde flow in lamellipodia linked to actin treadmilling, presumably potentiated by plus-end-directed myosin [90], there seems to be little need for a retrograde myosin motor for transport in lamellipodia. An alternative role for myosin VI could be organizing actin filaments [91],

perhaps as a cofactor in 'zipping' filaments together from the tip in the generation of filopodia.

Myosin X localizes to lamellipodia and to the tips of filopodia in epithelial MDCK cells [82], and myosin VII, its close relative in *Dictyostelium*, is found in lamellipodia, filopodia and phagocytic cups [81]. Deletion of the gene encoding myosin VII in *Dictyostelium* leads to inhibition of filopodium formation and decreased substrate adhesion. Taken together with the presence of FERM domains in the myosin tail (which can bind to transmembrane proteins), myosin VII has been attributed a role in the assembly and disassembly of adhesion proteins at the plasma membrane [81]. We suggest that some adhesion proteins are incorporated into adhesion sites by first targeting lamellipodia and filopodium tips, through myosin. Subsequently, complex formation and linkage to retrograde flow could transport these components to the base of lamellipodia and filopodia, to initiate adhesion. This route is suggested by the dual localization of proteins such as VASP [10], talin [92] and integrin α6β1 [93] at or towards [92] the front of the lamellipodium, as well as in adhesion complexes.

Concluding remarks

Resolving the mechanism of protrusion of the lamellipodium leaflet is central to reaching an understanding of actin-based cell motility. Already, studies of isolated proteins, *in vitro* and *in vivo* models, and pathogen systems [6,94], as well as theoretical treatments [95], have brought us a long way towards this aim. Nevertheless, because lamellipodia and filopodia do not exhibit the structural regularity found in more stable bundled arrays of actin [64], future advances in unveiling structure–function relationships must include the development of improved methods to visualize actin networks by electron microscopy and to localize the proteins associated with these networks [43,52,96]. Also, new ways of eliminating proteins from cells [97] or of modifying their interactions with binding partners will be pivotal, together with live-cell microscopy, in characterizing the roles of known and new proteins in lamellipodium function.

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