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Review Article

Actin dynamics in cell migration

Matthias Schaks[1,2], Grégory Giannone[3,4] and ⬤ Klemens Rottner[1,2]

[1]Division of Molecular Cell Biology, Zoological Institute, Technische Universität Braunschweig, Spielmannstrasse 7, Braunschweig 38106, Germany; [2]Department of Cell Biology, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, Braunschweig 38124, Germany; [3]University of Bordeaux, Interdisciplinary Institute for Neuroscience, UMR 5297, Bordeaux F-33000, France; [4]CNRS, Interdisciplinary Institute for Neuroscience, UMR 5297, Bordeaux F-33000, France

Correspondence: Klemens Rottner (k.rottner@tu-braunschweig.de)

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Cell migration is an essential process, both in unicellular organisms such as amoeba and as individual or collective motility in highly developed multicellular organisms like mammals. It is controlled by a variety of activities combining protrusive and contractile forces, normally generated by actin filaments. Here, we summarize actin filament assembly and turnover processes, and how respective biochemical activities translate into different protrusion types engaged in migration. These actin-based plasma membrane protrusions include actin-related protein 2/3 complex-dependent structures such as lamellipodia and membrane ruffles, filopodia as well as plasma membrane blebs. We also address observed antagonisms between these protrusion types, and propose a model – also inspired by previous literature – in which a complex balance between specific Rho GTPase signaling pathways dictates the protrusion mechanism employed by cells. Furthermore, we revisit published work regarding the fascinating antagonism between Rac and Rho GTPases, and how this intricate signaling network can define cell behavior and modes of migration. Finally, we discuss how the assembly of actin filament networks can feed back onto their regulators, as exemplified for the lamellipodial factor WAVE regulatory complex, tightly controlling accumulation of this complex at specific subcellular locations as well as its turnover.

Introduction

Cell migration is a key function of life. With a few exceptions, this process relies on the dynamic assembly and disassembly of actin filaments. These filaments organize into distinct subcellular domains, of which plasma membrane protrusions like lamellipodia, filopodia, and blebs are highly complementary to cell-substratum adhesion structures such as focal complexes or focal contacts, the latter of which serve as anchorage sites for yet another prominent actin structure relevant for migration, the so-called stress fiber. As possibly oversimplified, but still useful, general scheme, one can say that the protrusive structures best formed at the migrating front edge initiate the process of migration by exploring future space, while the contractile structures such as stress fibers (not always as prominently organized as distinct bundles as in migrating fibroblast or epithelial cells) deliver the forces onto cell-substratum adhesions to drag the rest of the cell body and cell rear forward. In this respect, efficient migration requires a well-balanced coordination of both protrusive and retractile activities, with overactivity of the latter frequently being counterproductive for efficient migration.

In this review, we first summarize actin filament assembly processes and the structures that they induce, as well as their turnover. We also aim for understanding how cells use the distinct molecular mechanisms known today to drive actin polymerization to form either one of the three major protrusion types. The discussion on similarities and differences between different protrusions are complemented by the 'old' question of how Rac and Rho GTPases control polarization and protrusion [1]. Finally, we ask ourselves how activities and turnover of the Rac effector WASP-family verprolin-homologous protein (WAVE) regulatory complex (WRC) might be tuned at lamellipodia. This is crucial for migration, last not least due to the essential functions found for WRC in lamellipodial actin polymerization.
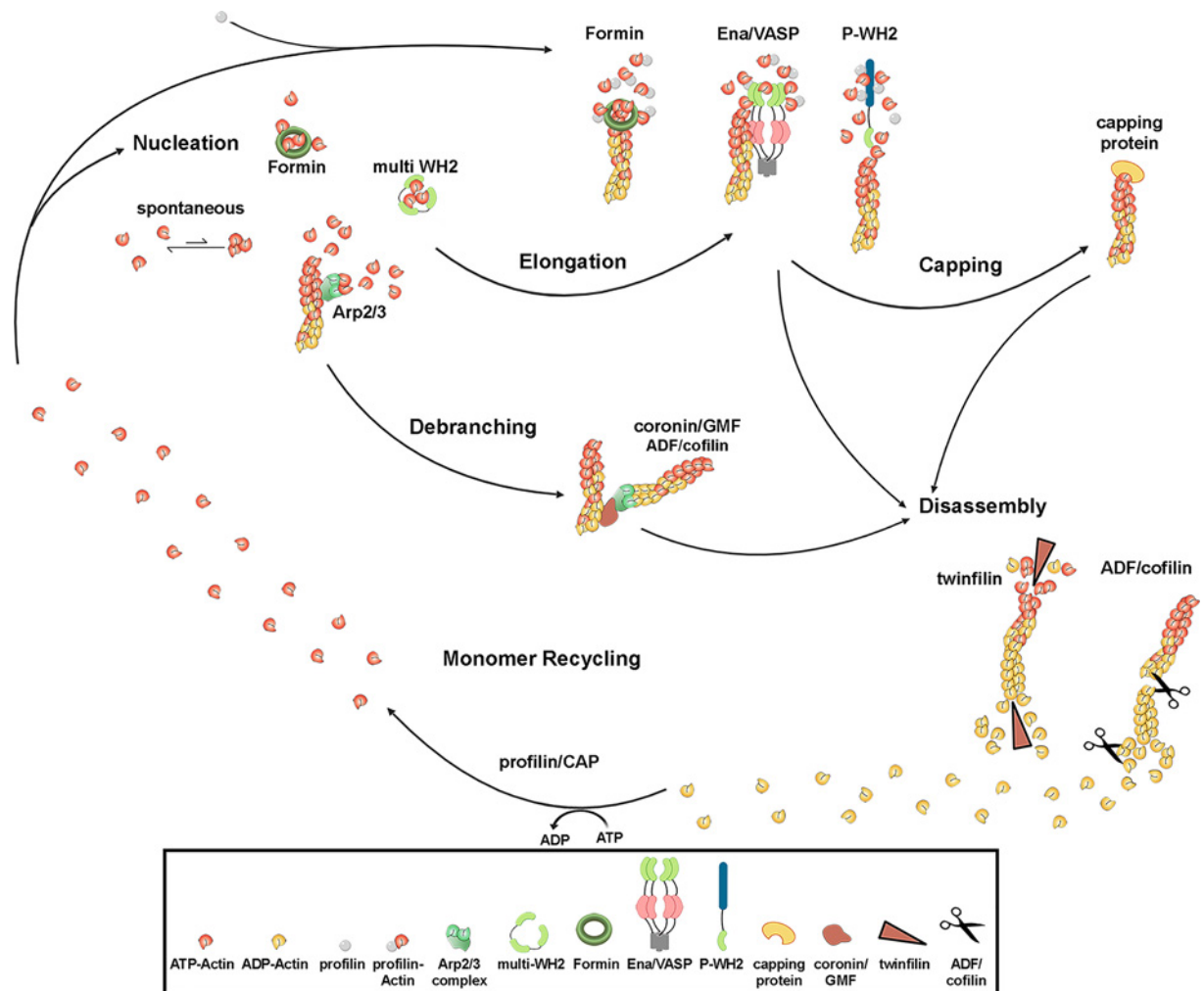
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Figure 1. Actin polymerization and turnover

Diverse factors, such as Arp2/3 complex, various formins or proteins containing multiple actin monomer binding WH2 domains can catalyze nucleation of filaments from ATP-loaded actin monomers. Subsequently, elongation of nascent filaments is achieved by either formins, Ena/VASP proteins or proteins harboring a polyproline-WH2 (P-WH2) module. Elongation can be terminated by capping proteins, for instance heterodimeric capping protein. Filament branches, created by Arp2/3 complex, can be disconnected by proteins of the coronin/GMF as well as cofilin families, whereas twinfilin catalyzes the disassembly of filament ends. Moreover, ADF/cofilin family members are best known for promoting filament disassembly by severing and depolymerization. Recycling of ADP-actin to polymerization-competent ATP-actin is executed by profilin or cyclase-associated protein.

The making and breaking of actin filaments

Polymerization of actin filaments at physiological concentrations is occurring rapidly *in vitro*. Thereby, filaments become polar, forming a fast polymerizing barbed (+) end and a slower polymerizing pointed (-) end. Cells, however, have been evolving various means to tightly control polymerization and depolymerization of actin filaments to their needs (Figure 1). It is commonly agreed that the vast majority of actin monomers is bound by monomer binding or sequestering factors such as members of the profilin family or thymosin β4, thereby strongly reducing the concentration of polymerization-compatible actin monomers in cells [2]. For nucleation of actin filaments, the most critical step is the formation of a trimer or tetramer. All actin nucleators have in common that they mimic this trimer/tetramer state. The most prominent factor to date nucleating actin filaments is the actin-related protein (Arp) 2/3 complex. This heteroheptameric protein complex comprises two actin related proteins, Arp2 and Arp3 that are highly similar to actin, as well as smaller subunits called ArpC1-ArpC5. Arp2 and Arp3 together with two actin monomers most efficiently form a nucleus for daughter filament generation, meaning that it must bind to a pre-existing filament, giving

rise to large arrays of branched actin meshworks [3]. Arp2/3 complex is intrinsically inactive but can be activated by at least eight factors presently known in mammals, which frequently are regulated within protein complexes and are collectively called nucleation promoting factors (NPF) of class I. These have in common that they harbor a so-called WCA-domain. This module contains an actin monomer binding WH2- (or W-) region and Arp2/3 complex-binding connector (C) and acidic (A) domains [3,4]. Two NPF molecules most efficiently activate one Arp2/3 complex [5–7]. Other prominent examples for actin nucleators are proteins of the formin family. In contrast with Arp2/3 complex, they act by stabilizing an actin dimer using their conserved formin homology 2 (FH2) domain and subsequently recruit actin or profilin-actin complexes [8,9]. Yet another group of actin nucleators constitutes proteins with multiple WH2 domains or other actin-binding modules capable of bringing three or four actin monomers together, the close proximity of which can overcome the kinetically non-favorable nucleation [4].

Processive filament elongation can also be achieved by many formin family members, which remain associated with and can thus ride on the growing, barbed ends of actin filaments. This activity appears to be dependent on the presence of profilin-actin complexes (profilin-actin) that are bound by the proline-rich FH1-domain of most formins [8]. However, proteins of the Ena/VASP family can processively elongate filaments as well, although as opposed to formins acting as dimers, Ena/VASP proteins operate in tetrameric complexes, thereby combining features of filament bundling and elongation, independently of profilin [10,11]. Aside from the role as Arp2/3 complex activators, NPFs have recently also been proposed to function in filament elongation, by accepting profilin-actin complexes and transferring actin monomers in a distributive and non-processive fashion onto barbed ends [12]. The activity counteracting filament elongation is most prominently represented by heterodimeric capping protein, capping filament barbed ends, and thus terminating their elongation [13].

Ageing of actin monomers incorporated into filaments, which is mediated by hydrolysis of bound ATP, favors the association with disassembly factors thereby driving disassembly toward filament pointed ends [14]. Disassembly has been described to occur through various means: Arp2/3 complex-containing networks can most prominently be debranched by proteins of the coronin and GMF families [15], but also by ADF/cofilin [16], although the latter protein family is best known for severing [17] or depolymerizing individual filaments [18]. Whereas ADF/cofilin is traditionally agreed to shift the balance toward disassembly at filament pointed ends [19], challenged though by a recent, divergent view [20], twinfilin family proteins are commonly thought to promote the disassembly at both filament ends [21,22]. Finally, ADP-bound actin monomers can be recharged by either profilin proteins [23–25] or by cyclase-associated protein (CAP) [26], thereby completing the cycle and providing fresh, polymerization-competent actin monomers (Figure 1).

How differential signaling and actin assembly factor activities translate into distinct protrusion types

In principle, protrusion of the plasma membrane can be achieved by at least three different modes or mechanisms involving actin filaments (Figure 2). One mechanism is the generation of a branched Arp2/3 complex-containing network, such as the lamellipodium. Formation of this structure is frequently associated with mesenchymal migration. The lamellipodium constitutes a flat structure that can protrude on solid surfaces both *in vitro* and *in vivo*, but also lift up into the third dimension dependent on signaling conditions and adhesion capacity to the underlying substratum [14]. Such 3D lamellipodia are also termed ruffles, but are considered indistinguishable in mechanisms of actin turnover to lamellipodia protruding on flat surfaces. The lamellipodium is formed at the cell periphery by continuous branching of the Arp2/3 complex, which creates forces that overcome membrane tension and enable forward movement of the plasma membrane. In this structure, forces arising from actin polymerization driving protrusion are uncoupled from myosin II-based contractility [27]. Aside from ruffles at the cell periphery or those emerging as so-called circular ruffles at the dorsal cell surface, comparable structures involving Arp2/3 complex-dependent actin remodeling include the podosomes of hematopoietic cells and invadopodia of tumor cells [28,29]. In the latter two structures, however, which are occasionally grouped together as 'invadosomes', specific differences to lamellipodia and ruffles do exist. First, the molecular inventory of invadosomes is comparable, but not identical to lamellipodia [28,29], and second, the protrusive activity of invadosomes is tightly coupled not only to adhesion to the extracellular matrix, but also to degradation of the latter.

The second mechanism of plasma membrane protrusion involves the growth of small linear bundles of actin filaments, called filopodia, which also create forces toward the membrane through filament polymerization, but independent of Arp2/3 complex [30–33]. The space between individual filopodial bundles is commonly filled by the contractile activity mediated by myosin II [30]. As elaborated on in several, excellent reviews in more detail recently [34–36], this group of protrusions includes various types of bundled structures displaying significant differences in
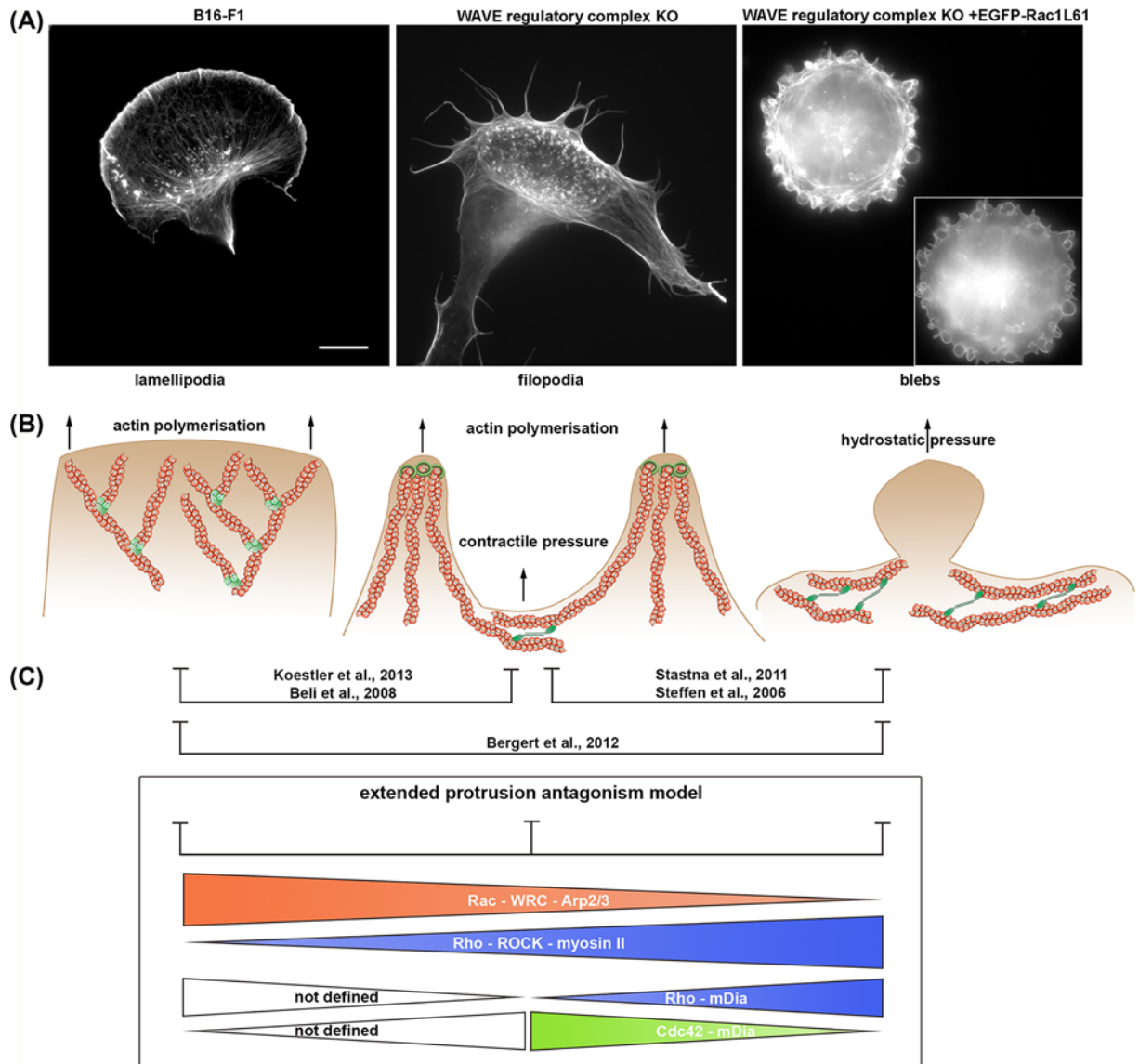
Figure 2. Types of actin-based protrusions

(**A**) Representative examples of different types of plasma membrane protrusion, lamellipodia, filopodia, and blebs. Panels show B16-F1 cells wildtype (left) or upon specific experimental treatments (middle and right), as indicated on top of images, followed by fixation and staining for the actin cytoskeleton with phalloidin. Middle and right panels represent WAVE regulatory complex KO (Sra-1/PIR121 KO clone #3, as in [80]) and example of the same cell type transiently expressing EGFP-tagged, constitutively active Rac1, respectively (EGFP-Rac1L61; inset). Scale bar, 10 μm. (**B**) Mechanisms of formation of actin-dependent plasma membrane protrusions as shown in A. Protrusion of the plasma membrane is frequently achieved by forming an Arp2/3 complex-dependent lamellipodial actin network. Disruption of lamellipodia formation by for instance eliminating WRC expression (WRC KO) forces cells to migrate using a bunch of filopodial actin bundles, with the space between them being filled by contractile activity of myosin II (middle panel). Conversely, overexpression of active Rac in WRC KO cells interferes with filopodia formation and stimulates bleb formation (see also [80]). Plasma membrane blebbing relies on high myosin II activity in the contractile actin cortex creating intracellular, hydrostatic pressure. Local cortex – plasma membrane detachment or cortex rupture can lead to the protrusion of an actin-free plasma membrane bleb. (**C**) Models explaining antagonisms between protrusion types: we propose a model wherein Rac-WRC-Arp2/3 complex versus Rho-ROCK-myosin II signaling reciprocally control the formation of lamellipodia versus blebs, respectively [73]. On top of this, we suggest that spatial activation by either Rho or Cdc42 GTPases tunes output signals for mDia formins controlling filopodia or bleb formation at decreased Rac signaling conditions.
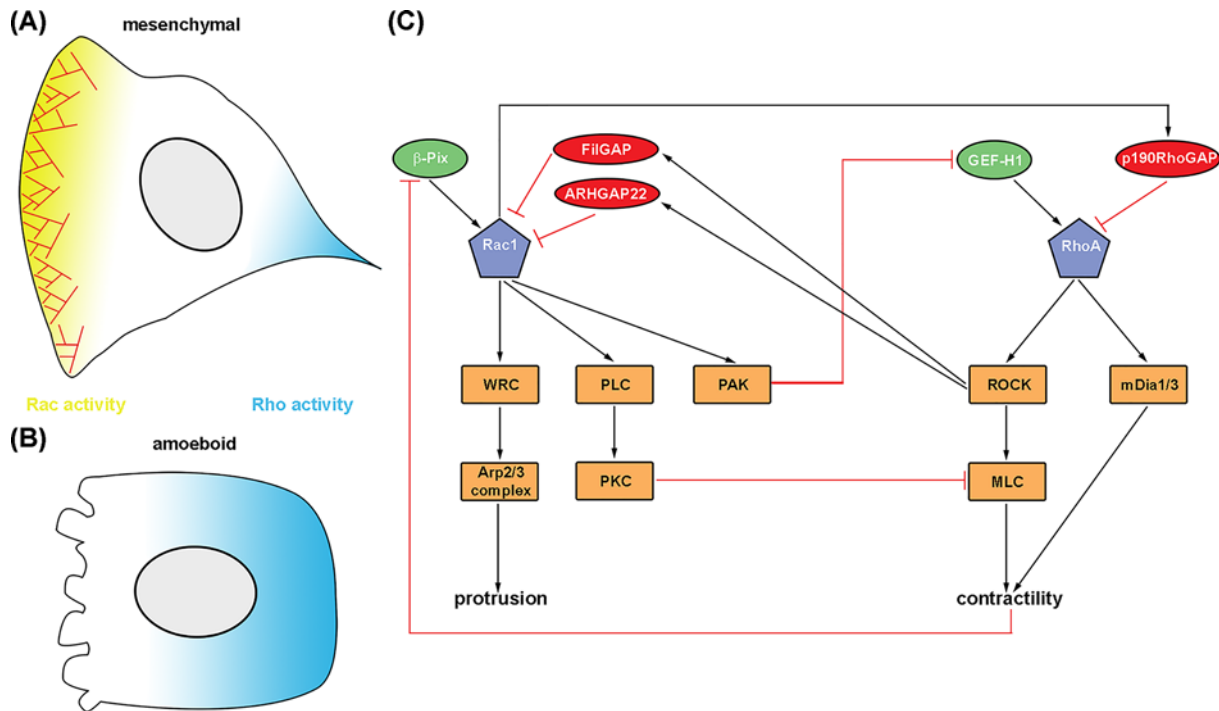
Figure 3. Establishment of polarity by Rac/Rho
(**A**) The small GTPases Rac and Rho (or at least their best studied representatives in mammals, Rac1 and RhoA) ultimately control different migration modes, i.e. mesenchymal migration by Rac and amoeboid migration by Rho. During mesenchymal migration, Rac activity dominates at the leading edge (yellow), while Rho signaling is enhanced toward the cell rear (blue), leading to the formation of a lamellipodium at the leading edge. (**B**) Amoeboid movement is characterized by high levels of active Rho and decreased Rac signaling, causing elevated contractility giving rise to plasma membrane blebbing. (**C**) Rac and Rho signaling displays a mutual antagonism at multiple levels. For details see text.

structural organization and dynamics, but ranging from sequentially protrusive and retractile or occasionally kinking filopodia to the much more ordered and apparently static microvilli of epithelia or the stereocilia in cochlear hair cells.

The third mechanism of plasma membrane protrusion is the one that does not require active actin polymerization [37,38]. The plasma membrane of eukaryotic cells is generally thought to be attached to a thin cortex of actin filaments. This cortex harbors contractile properties, based on myosin II activity, enabling cells at least within the animal kingdom to create hydrostatic pressure pushing against the plasma membrane [39]. A nucleus for a bleb can be formed, in principal, by two different ways. Either a local cortex-plasma membrane detachment or a rupture of the actin-cortex itself [37,38]. Anyhow, it appears that high myosin II activity is critical for the formation of blebs, indicating that intracellular, hydrostatic pressure is the driving force [37]. The type of migration mediated by these structures is called amoeboid motility.

Establishment of cell polarity by Rac and Rho GTPases

While exhibiting these three modes of protrusion, either exclusively or mixed at variable ratios, cells rely on their ability to polarize in order to allow the cell body to move forward. The small GTPases Rac1 and RhoA are crucial for initiating and maintaining cell polarity and directed movement, but appear to play opposing roles during these processes. Their spatiotemporal activities have been extensively studied using fluorescence resonance energy transfer microscopy, with both being active at leading edge and cell rear, in principle, although with differential patterns and magnitudes [40–42]. Although not absent in protrusion, which still constitutes a matter of vivid debate [42–45], it appears that RhoA signaling is at least enhanced at retraction sites [40,41]. Indeed, there is mutual inhibition at multiple levels between Rac1 and RhoA signaling, which can lead to a bistable system [46]. Figure 3 summarizes the antagonism between Rac1 and RhoA. At the leading edge, Rac likely dominates RhoA signaling and *vice versa* at the rear to achieve polarity and maintain directed movement. While Rac GTPases signal through WRC to achieve Arp2/3
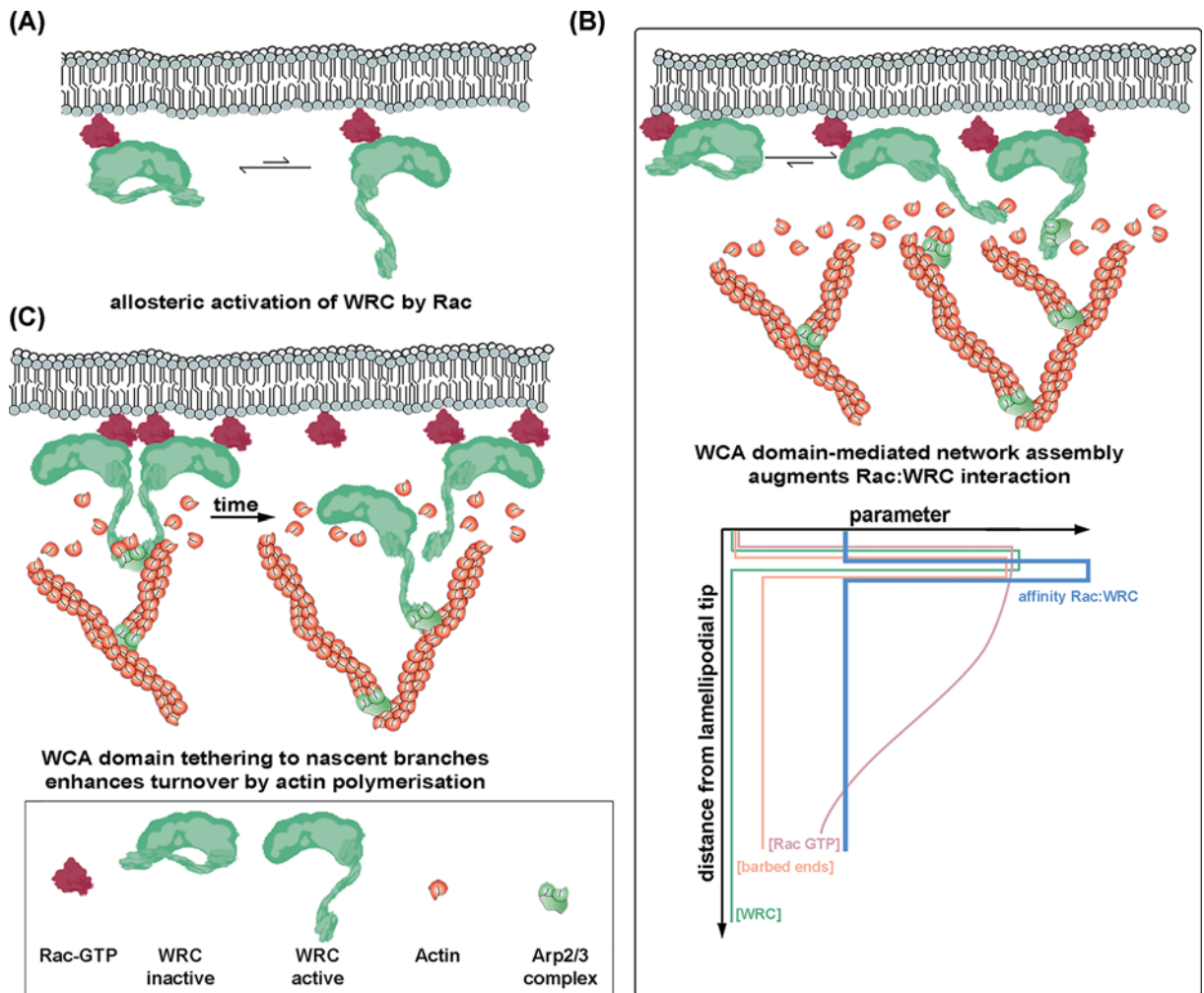
Figure 4. WRC accumulation and turnover at lamellipodia

(**A**) Allosteric activation of WRC requires active Rac GTPase prior to lamellipodia formation. (**B**) During lamellipodium protrusion, WRC's WCA domain continuously binds actin and Arp2/3 complex monomers, likely inhibiting its inactivation, thereby in turn fostering binding to Rac, and enhancing its accumulation at the tips of protruding lamellipodia. The panel at the bottom displays gradients of Rac-GTP levels (rosy) versus concentrations of actin filament barbed ends (orange) or WRC (green) from distal to proximal lamellipodial regions, as indicated, proposed to cause an affinity increase between Rac and WRC at the lamellipodium tip (blue). Illustration inspired by models of actin or actin binding protein distributions at the leading edge published recently [12]. Note that sharp edges of parameter changes from distal to proximal lamellipodial regions are not intended to indicate true abrupt changes, but aid illustration purposes. (**C**) Replacement and thus turnover of WRCs upon successful branch formation may be achieved (or at least aided) by sustained binding of its WCA domain to nascent branches presumed to travel rearward during lamellipodium protrusion [108,109]. This may provide a mechanism for pulling WRC off the membrane and into the network, leading to its retrograde motion and inactivation (the latter not shown). Note that for the sake of simplicity, this model of WRC regulation by Rac, actin and Arp2/3 complex binding (for details see text) does not include WRC interactions with additional lamellipodial regulators, such as lamellipodin (Lpd, see [93]) or Ena/VASP family members, which were likewise reported to contribute to WRC activation and stabilization [110].

complex-dependent lamellipodial protrusion, Rho signaling activates actomyosin-based contractility via ROCK and mDia formins at the cell rear [47–50]. However, there are multiple levels of cross-talk. For instance, polarization of myosin II activity to the rear appears to rely on WRC, as knockdown of the hematopoietic subunit Hem-1 results in activation of myosin II at the protruding front [51]. Likewise, acute inhibition of Arp2/3 complex causes rearrangement of lamellipodial actin filaments into antiparallel arrays sufficient to trigger myosin recruitment both in cells [52,53] and *in vitro* [54].

On top of this, there is extensive negative cross-talk between Rac and Rho. This mutual antagonism is driven, at least in part, by activation or inactivation of GAPs and GEFs, respectively. For instance, Rac has been shown to directly activate p190RhoGAP [55] or to inhibit the RhoA-GEF GEF-H1 via its effector PAK [46,56]. On the contrary, the RhoA effector ROCK can activate the Rac-GAP FilGAP through phosphorylation [57] or indirectly activate the Rac-GAP ArhGAP22 [58]. Furthermore, the focal adhesion-localized Rac-GEF β-Pix is sensitive to tension and thereby negatively regulated by RhoA [59].

Despite the multitude of molecular mechanisms mediating antagonistic activities by directly impacting on Rho-GTPase regulation, phospholipase C gamma (PLC-γ) activity has also been shown to be essential for mesenchymal chemotaxis toward PDGF by selective inhibition of the respective downstream effector machinery of RhoA, more specifically myosin II at the leading edge [60]. Downstream of PLC-γ, this pathway involves PKCα activation through diacylglycerol production and subsequent inhibitory phosphorylation of myosin II. Although PLC isozymes are commonly activated downstream of G protein-coupled receptors, it is again Rac amongst the Rho-family GT-Pases that has also been shown to stimulate PLC-β and -γ activity in cells. Here, Rac operates by binding to the PH and split-PH domain of PLC-$β_2$ and PLC-$γ_2$ isozymes, respectively [61,62], while other Rho GTPases such as RhoA and Cdc42 fail to do so. The crystal structures of Rac GTPases in complex with PLC-β2 or PLC-γ2 revealed the structural basis for this selectivity [63,64]. In addition, the Rac-PLC-γ2 interaction has been shown to amplify B cell receptor-induced Ca^{2+} signaling by means of rescue in PLC-γ2-deficient cells with a Rac binding-deficient PLC-γ2 mutant [65]. Notwithstanding this, future studies are required to determine the precise role of Rac-PLC interactions for motile processes such as lamellipodia formation and chemotaxis.

In spite of the regulation of actin network polymerization and actomyosin contraction in a polar fashion, Rac and Rho also participate in the regulation of phosphoinositide asymmetry. There is a well-described feed forward loop between Rac and class I PI3K that favors the production of phosphatidylinositol-3,4,5-trisphosphate at the protruding cell front [66]. On the contrary, Rho signaling can activate the PI3K antagonist PTEN via ROCK [67,68].

Relation and interconversion between lamellipodia, filopodia, and bleb protrusions

There are several examples in the literature in which interference with lamellipodia formation promotes plasma membrane blebbing. Inhibition of lamellipodia through blocking WRC or Arp2/3 complex function promotes formation of plasma membrane blebs in various cell types in distinct organisms [69–74]. Interestingly, it was found that the induction of blebs upon knockdown of WRC depended on the formin mDia2 [74], which had previously been proposed to operate in cortical actin assembly [75]. On the contrary, inhibition of ROCK or myosin II that interferes with blebbing can enhance lamellipodia formation at least transiently [27,73,76]. However, there are also examples where disruption of Rac, WRC, or Arp2/3 complex, and hence removal of lamellipodia causes excessive filopodia formation instead of blebbing [30,77–79]. Interestingly, in the recent past, we have found means to switch the protrusive behavior of B16-F1 melanoma cells between all these three types of protrusion. While B16-F1 cells typically form lamellipodia when plated on laminin, knockout of Rac GTPases or WRC blocks lamellipodia and induces filopodia formation [77]. Intriguingly, however, when WRC-KO cells overexpress constitutively active Rac, the formation of filopodia appears to be impaired and cells start forming blebs ([80] and Figure 2A). A similar pattern has been found in spreading fibroblastoid cells, in which the Arp2/3 complex was disrupted [30]. Filopodia induced by this treatment appeared compromised upon additional inhibition with the antiformin compound SMIFH2, again leading to cell blebbing [30]. One explanation for this observation could be that Rac is interfering with filopodia/bundling formation. Indeed, microinjection of active Rac leads to immediate suppression of filopodia [78]. Interestingly, SMIFH2 treatments were also described to switch cell protrusion from lamellipodia to blebs [81]. Furthermore, specific isoforms of the formins mDia2 and FMNL1 are known to be capable of switching between filopodia and bleb induction [82,83]. In a 'wildtype' context, mDia2 is a well-established inducer of filopodia [84–86]. Whereas filopodia induction by WRC knockdown required mDia2, WAVE2 overexpression in these conditions promoted blebbing, again dependent on mDia2 [74]. This indicates that mDia2 activity might drive filopodia formation or membrane blebbing, dependent on context. Along these lines, the Rho GTPase Cdc42 was also observed to mediate a switch from bleb to filopodia formation, as its microinjection as active variant into blebbing, WRC-depleted cells triggered filopodia at the expense of blebbing ([31], and K.R., unpublished data). As already emphasized previously [87], mDia formins can be activated by both Cdc42 and RhoA, which have been linked to filopodia formation and actin cortex assembly, respectively. It is tempting to speculate that the spatial activation of respective mDia formins by either RhoA or Cdc42 and their subsequent engagement into locally established signaling complexes could determine whether they engage in filopodia formation or actin cortex assembly. Notably, as opposed to mDia2, active mDia1 accumulates at the rear and appears much less

active than mDia2 concerning filopodia induction in otherwise comparable conditions [47,85,86]. Whether or not this can be solely explained by potential, differential regulation through RhoA versus Cdc42 signaling will have to be worked out in detailed, future studies. Irrespective of the outcome and inspired by previous models based on the apparent antagonism between Arp2/3-dependent, lamellipodia-based protrusion and contractility-dependent blebbing [73], we propose that specific cell context, meaning given protein repertoire and signaling status will define whether low Arp2/3 complex activity at the plasma membrane finally translates into blebbing or alternatively filopodia formation (Figure 2).

Regulation of WRC localization, activity, and turnover

Amongst the different mechanisms of protrusion at migrating cell fronts, the lamellipodium constitutes a structure particularly relevant for effective migration, at least in case of mesenchymal cells migrating on flat, adhesive structures, both *in vitro* and *in vivo* [28,88]. In recent years, we and others have thus systematically dissected the core actin assembly machinery in lamellipodia (i.e. Rac-WRC-Arp2/3 complex) because continuous protrusion of these structures and associated force development is driven by actin polymerization regulated by this machinery.

During initiation of lamellipodia protrusion, Rac function appears to be essential for activation of WRC. WRC possesses two binding sites for Rac [89,90] and although the so-called A site appears to be more crucial for allosteric activation in cells, both sites are required for optimal lamellipodial protrusion [77]. Furthermore, the Arp2/3 complex is thought to be most effectively activated by simultaneous engagement of two WCA domains [5–7], meaning four GTP-bound Rac molecules may be required for efficient activation of one Arp2/3 complex unit during the initiation phase of lamellipodia formation. Interestingly, Rac GTPase was recently shown to form nanoclusters in protruding regions of polarized cells [91,92]. It is tempting to speculate therefore that nanoclustering promotes protrusion by elevating local Rac concentrations above thresholds required for optimal Arp2/3 activation, while simultaneously enhancing the likelihood of spatially restricted signaling, ensuring establishment and maintenance of polarity.

Once an Arp2/3 complex-mediated lamellipodial actin network has formed, it still relies on continuous Rac/WRC signaling. While Rac appears active up to several micrometers from the edges of lamellipodia into more proximal parts of the plasma membrane [40,42], WRC shows specific accumulation at lamellipodia tips [70,77]. This suggests that active Rac alone is not sufficient for WRC localization at the lamellipodium tip. In fact, previously published work suggested interactions with both proteins and lipids to aid WRC accumulation at this site, e.g., with IRSp53, lamellipodin (Lpd) and the leading edge phospholipid phosphatidylinositol-3,4,5-trisphosphate [93–95]. Along these lines, recent single molecule tracking experiments of Rac at the lamellipodium tip confirmed surprisingly short residency times as compared to WRC, but they also revealed Rac immobilization to positively correlate with its activity state and effector binding, including WRC [96].

Although short-lived and local, continuous Rac interactions may thus still contribute to the maintenance of WRC mediating protrusion. Together, multivalency of WRC interactions, repeatedly either with Rac or additional WRC binding proteins and lipids appears crucial for its effective targeting and function as Arp2/3 complex activator in continuous lamellipodium protrusion.

In spite of aforementioned considerations, an alternative interpretation of existing data would be that Rac also contributes to localising WRC at the lamellipodium tip, last not least because constitutively active WRC lacking functional Rac binding sites shows strong impairment in lamellipodia induction and accumulation at their tips [77,80]. This conclusion is not necessarily incompatible with comparably transient residency times of Rac at lamellipodia tips [96]. Indeed, there is growing evidence that WRC accumulation and/or maintenance at protruding tips requires loose or transient interactions with lamellipodial building blocks, i.e. Arp2/3 complex and actin monomers that may positively feed back onto WRC-Rac interactions and can be considered crucial for the following reasons: I.) Actin monomers were described to accumulate at the tips of lamellipodia [97,98]. II.) Constitutively active WRC, i.e. WRC harboring a WCA domain released from transinhibition normally mediated by Sra-1/PIR121-binding shows strongly enhanced residency time at lamellipodial tips, which depended on both Rac binding sites [77]. III.) Constitutively active WRC has increased affinity for Rac [89,90]. IV. Immobilization of Rac at lamellipodial tips is mediated, at least in part, by WRC-binding [96]. Similar observations were previously made with the WAVE-related NPF N-WASP, reported to constitute a critical Arp2/3 complex activator in distinct subcellular structures. Its two actin binding WH2 domains had previously been implicated in filament barbed end capture [99], and WH2 domain-mutation decreased the dwell time of N-WASP in vaccinia virus-induced actin tails, which usurps this protein as essential Arp2/3 complex activator in viral actin tail formation functioning in viral spread at the plasma membrane [100–102]. Dwell time reduction of N-WASP close to the virus coincided with reduced tail formation frequency, indicating N-WASP stabilization to positively correlate at least with tail initiation [102].

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In the alternative view proposed here, transient interactions of WRC's WCA domain with filament barbed ends as well as Arp2/3 complex and actin monomers at the lamellipodium tip may thus delay or counteract its inactivation, thereby enhancing WRC affinity toward Rac (Figure 4B). Similar positive feedback mechanisms have been suggested for lamellipodin, VASP and FMNL2, all of which accumulate at lamellipodia tips and for which actin filament binding at least contributes to lamellipodial targeting ([103,104], and Frieda Kage, personal communication).

Finally, turnover of WRC at the lamellipodium, at least in part, may be regulated by a mechanism also involving its WCA domain. For daughter filament generation, NPF-bound Arp2/3 complex has to stably attach to a mother filament. Using elegant triple-colour TIRF assays, it was recently shown that N-WASP detachment from Arp2/3 complex at nascent branches takes several seconds *in vitro* [105]. Transferring this into a context of a growing network where the NPF stays attached to the protruding membrane such as in the lamellipodium would mean that WRC might be exposed to tension, created by the polymerising network, pulling it away from the lamellipodium tip. Consistent with this would be that a fraction of WRC was previously observed to undergo retrograde flow along with the lamellipodial network [106]. Furthermore, blocking actin polymerization was described to interfere with turnover of both WRC at the lamellipodium tip and N-WASP at Vaccinia virus surfaces, and deleting the Arp2/3 complex binding CA-region of N-WASP dramatically enhanced the residency time of the latter on the virus [102,107]. Together, all these data suggest that active, Arp2/3-dependent actin assembly may actually significantly contribute to NPF removal from sites of Arp2/3 complex activation, and thus directly contribute to NPF turnover at respective sites, but how this occurs precisely, remains to be established.

Summary

- Actin filament assembly and disassembly is regulated by a variety of proteins in need of tight coordination.

- Actin-dependent plasma membrane protrusions differentially involve polymerization of actin networks, bundles or contractile structures to exert forces onto and thus shape the plasma membrane.

- Rac and Rho GTPases antagonize each other at multiple levels, and control cell polarization.

- Accumulation and turnover of WAVE regulatory complex is controlled by complex positive and negative feedback loops, tuning the efficiency of lamellipodial actin network formation and turnover.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
Arp, actin-related protein; FRET, fluorescence resonance energy transfer; NPF, nucleation promoting factor; WASP, Wiskott–Aldrich syndrome protein; WAVE, WASP-family verprolin-homologous protein; WRC, WAVE regulatory complex.

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