Assembling actin filaments for protrusion

Klemens Rottner^{1,2,*} and Matthias Schaks^{1,2}

¹Zoological Institute, Braunschweig University of Technology, Spielmannstrasse 7, 38106 Braunschweig, Germany;

²Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany

*To whom correspondence should be addressed:

Phone: +49 531 391 3255

Fax: +49 531 391 3222

Email: k.rottner@tu-braunschweig.de

Cell migration entails a plethora of activities combining the productive exertion of protrusive and contractile forces to allow cells to push and squeeze themselves through cell clumps, interstitial tissues or tissue borders. All these activities require the generation and turnover of actin filaments that arrange into specific, subcellular structures. The most prominent structures mediating the protrusion at the leading edges of cells include lamellipodia and filopodia as well as plasma membrane blebs. Moreover, in cells migrating on planar substratum, mechanical support is being provided by an additional, more proximally located structure termed the lamella. Here, we systematically dissect the literature concerning the mechanisms driving actin filament nucleation and elongation in the best-studied protrusive structure, the lamellipodium. Recent work has shed light on open questions in lamellipodium protrusion, including the relative contributions of nucleation *versus* elongation to the assembly of both individual filaments and the lamellipodial network as a whole. However, much remains to be learned concerning the specificity and relevance of individual factors, their cooperation and their site-specific functions relative to the importance of global actin monomer and filament homeostasis.

Introduction

The discovery of actin expression in non-muscle cells in the middle of the last century ignited an ever-growing explosion of studies on how the cytoskeleton and in particular the acto-myosin system contributes to force development in migration and shape maintenance and change in non-muscle cells [1]. In spite of the relevance of microtubules for signalling and the regulation of cell morphology as well as their paramount contribution to neuronal architecture and growth, mesenchymal cell motility can occur without them [2]. In contrast, stability, maintenance and the vast majority of forward or rearward movements of the plasma membrane are not thinkable without the dynamic turnover of actin filaments. The rare but famous exceptions in eukaryotes include the MSP-mediated motility in nematode sperm [3] and the microtubule-based axopodia of *Heliozoa* protists [4], as well as in vertebrates the microtubule-mediated protrusions evoked by clostridial pathogens on infected epithelium [5].

The polar actin filaments in most cells of the animal kingdom are organizing into distinct subcellular domains, the most prominent of which are the actin cortex and plasma membrane protrusions such as lamellipodia, filopodia and blebs [6]. It is worth noting that all these terms originally coined decades ago based on morphological rather than functional or mechanistic features are increasingly drifting in meaning, as nicely summarized recently [7], but the structures discussed below exclusively refer to the canonical, actin-based versions of them. Rapid turnover of filaments *in vivo* is driven by a process generally referred to as treadmilling, the various steps of which depend on differential on/off kinetics of ATP- *versus* ADP-bound actin on the two filament ends, and are regulated by a plethora of actin-binding proteins [8,9]. The treadmilling cycle is initiated by addition of assembly-competent ATP-actin monomers onto the rapidly-growing barbed ends of filaments, followed by fast ATP hydrolysis and slow Pi-release off monomers within the filament, and dissociation of the latter from filament pointed

ends. As final step, ADP-actin monomers are then “re-charged” for a new round of assembly with ATP.

A quarter of a century ago, the discovery of Arp2/3 complex as a novel nucleator of actin filaments certainly initiated a new era of actin research that keeps surprising us up till today [10,11]. Its binding to a so called mother filament followed by association of additional actin monomer generates a daughter filament branch, the molecular details of which are just beginning to be unravelled [12]. The biochemistry of actin branching [13] has early been supported by electron micrographs showing branched, dendritic actin networks *in situ* [14], which is continuing to be extended to various, subcellular Arp2/3-containing locations [15-17]. Indeed, the Arp2/3 complex is now established to operate in processes as diverse as migration, autophagy [18], myoblast fusion [19] and DNA double-strand break repair [20,21]. Despite this diversity, the generation of branched actin filament networks by the Arp2/3 complex can mechanistically explain its specific involvement in each of them. Yet, recent discoveries include the notion that the heteroheptameric Arp2/3 complex has to be considered as family of diverse complexes due to the usage of two alternative isoforms in case of three out of the seven subunits in humans [22]. Moreover, Arp2/3 complex-mediated branching is also exploited by various bacteria and viruses interacting with the actin cytoskeleton [17,23-25], and was the first type of motility reconstituted by purified proteins *in vitro* [26].

Aside from Arp2/3 complex, similarly exciting have been studies discovering and characterizing additional classes of actin filament nucleators [27], of which the formins are presumably most relevant for actin-based protrusion, as discussed here (see also [6]). As opposed to Arp2/3 complex, most formins are famous for their capability to processively elongate actin filaments from their barbed ends, thus being capable of generating and sensing piconewton forces at the single filament level [28,29]. However, we know today that processive elongation of actin filaments is not restricted to formins, as Ena/VASP family members, for instance, the accumulation of which coincides with the extent of lamellipodial actin

polymerization [30], can also exert this activity, although molecular details differ. More specifically, and distinct from formins that nucleate and elongate single actin filaments as dimers and in a fashion dependent on the small actin monomer binding protein profilin, essential features designating Ena/VASP-specific actin filament assembly combine filament bundling and actin monomer delivery onto filament barbed ends by multimeric family member arms [31,32]. This combination appears so fundamental that it has even been mimicked by pathogenic host actin regulators [33].

In spite of the progress on individual biochemical activities of all these actin assembly factors, little is known about their relative relevance in protrusion and migration. Here, we will review most recent progress in our understanding of how these molecules work together during protrusion of the lamellipodium, the best-characterized model structure of Arp2/3 complex-driven actin network formation *in vivo*.

The lamellipodium and related actin structures

For decades, research on cell migration is intimately linked to studying the activity and movement of the most prominent structure formed by cells at their fronts, at least when growing on comparably solid substrata *in vivo*, the lamellipodium: Due to being formed by various cell types and in multiple conditions [34,35], it is not surprising that this structure manifests with high diversity and size dimensions. The lamellipodium was originally defined mostly based on structural parameters, i.e. as a network of actin filaments protruding ahead of the more stable lamella behind [6,35]. However, we can now clearly extend this definition of the lamellipodium to the dynamic, actin-containing structure missing from the cell periphery if eliminating the function of the small GTPase Rac (isogenes 1, 2 and 3 in mammals) and its downstream effectors [36-42]. Figure 1 summarizes the most frequently studied types of actin-based protrusions at the plasma membrane of animal cells, e.g. during developmental processes or

essential activities of haematopoietic cells. We have refrained from including additional protrusion types, as induced for instance by bacterial or viral pathogens, as those have been covered in recent reviews [25,43]. The term ruffle today generally describes a lamellipodium-like structure potentially formed at two distinct subcellular locations in cells spread on two-dimensional surfaces. One type of structure corresponds to an up- or backwards-lifted lamellipodium at the cell periphery, and the second to a more complex structure known as circular dorsal ruffle (CDR), the closure of which co-incides with the formation of a macropinosome (Figure 1). The protrusion of lamellipodia and ruffles also coincides at least with the initiation of adherens junctions [44]. All those lamellipodia-like structures frequently display more bundled arrays that we call microspikes, which are to be separated as likely distinct in molecular regulation from filopodia that polymerize beyond the edges of lamellipodia or ruffles. Blebs are the only structures shown, the protrusion of which does not require active actin polymerization, but are formed instead by hydrostatic pressure at local actin cortex instabilities, and retracted by actin filaments polymerized inside the bleb subsequent to bleb expansion [45,46]. A consecutive inhibition and activation cycle of RhoA has recently been proposed to accompany the expansion and retraction phases during blebbing [47], but much remains to be learned concerning the details of their molecular regulation and the relation to other protrusion processes. Except for filopodia and blebs during their expansion, all these structures will employ Arp2/3 complex-mediated actin assembly to form, in spite of clear differences in relevance among distinct structures (see also below). Note that Arp2/3 complex activation in lamellipodia and ruffles is clearly dominated by WAVE regulatory complex (see also Box 1), whereas podosomes and invadopodia (invadosomes) are WASP/N-WASP-dependent [6]. In contrast, and although clearly involved, Arp2/3 complex displays a differential contribution to different types of phagocytosis [48]. Moreover, the literature harbours conflicting reports on the specificity of Arp2/3 complex activation during CDR formation [49,50]. Hence, the precise extent and mechanism of Arp2/3 complex activation in

CDRs or other actin-related structures such as the phagocytic cup [48] are yet to be established. At least as far known, specific formins and additional actin regulators are similarly broadly engaged in the formation of all these structures (see pathways in Figure 1 for best established examples). Below, we will dissect what's known on how all these factors collaborate, as exemplified by the lamellipodium, but likely extendable to other structures comprising mixtures of networks and filament bundles such as the lamellipodium.

Relative contributions of Arp2/3 complex and other actin assembly factors

The Arp2/3 complex comprises two actin-related proteins, Arp2 and Arp3, which are structurally highly similar to actin and co-assemble with five additional, smaller subunits called ArpC1-5. Arp2 and Arp3 together with one or two additional actin monomers form a template for daughter branch generation, in which the complex remains attached to the slowly growing, so called pointed end of the actin filament, leaving the rapidly growing, barbed end free for rapid polymerization. Based on classical electron microscopy studies, all filaments in the lamellipodial network are positioned with their barbed ends facing forwards [51]. Arp2/3 complex is intrinsically inactive, but can most effectively be activated by so called class I nucleation promoting factors (NPFs) [52]. The C-termini of the latter harbour the following domains, an actin monomer-binding WH2-domain (also called **W**; in some proteins present as two or three copies), and Arp2/3 complex-binding connector (**C**) and acidic (**A**) regions that together make up the so called **WCA**-domain. Class I NPFs comprise a whole family of factors today, of which the heteropentameric WAVE complex (also **WAVE** regulatory complex, WRC, see Box 1 for details on its regulation) is the one relevant for lamellipodia formation [6]. Due to WRC accumulation at the plasma membrane, Arp2/3 complex activation and hence its continuous incorporation into the network occurs at the very front, at the membrane – network interface, just like actin [53,54]. In various cell types, formation of lamellipodia and membrane

ruffles and at least haptotactic, integrin-dependent cell migration is intimately linked to the presence of functional Arp2/3 complex [41,48,55]. This means that both initiation of such actin networks, but also their continuous protrusion and turnover require Arp2/3 complex activity in an obligatory fashion [56]. Not even formins capable of nucleating actin filaments *in vitro* and stimulated to accumulate at the plasma membrane can restore lamellipodia formation in cells unable to properly activate Arp2/3 complex [57].

One open question concerns the potential source of mother filaments for Arp2/3 complex-dependent branching in lamellipodia. In theory, branching can occur in principle from any actin filament, irrespective of how it was nucleated. For instance, both mammalian FMNL2 and mDia1 formins have previously been described to be able to generate filaments used for Arp2/3 complex-dependent branching *in vitro* [58,59]. However, their role as mother filament generators in lamellipodia is questionable, albeit for distinct reasons: In case of mDia1, no accumulation could be found at the tips of lamellipodia, which would positively correlate with the dynamics of other actin assembly factors driving this process, such as WRC [38,40]. The lack of such an enrichment was described by various labs and using distinct experimental approaches, ranging from live cell imaging of active, EGFP-tagged mDia1 in B16-F1 melanoma [60] and superresolution microscopy of immunostained, endogenous mDia1 [59] to careful registration of fluctuating mDia1 signals with edge protrusion and retraction movements [61]. Interestingly, the authors of the latter study found mDia1 accumulation before the onset of protrusion, which was interpreted as indicating potential generation of mother filaments before Arp2/3 complex-mediated branching is initiated. This conclusion was emphasized even further based on RNA interference data [59] and the proposal that mDia1 operates in this process in a non-redundant fashion [62]. However, we are convinced that the view of an essential function of mDia1 as upstream filament generator for Arp2/3 complex-dependent lamellipodia formation is incompatible with apparent lamellipodia formation in mDia1-deficient fibroblasts, and the engagement of mDia1 and its *Dictyostelium* homologue ForA into

protein complexes operating at the cell rear rather than front [60]. Aside from these considerations and due to continuous Arp2/3 complex activation [see above; 56], mother filament generation could be required, in principle, during all stages of lamellipodium protrusion, and not just during their initiation, as argued in case of mDia1 [62]. If correct, this would require lamellipodium tip accumulation of the proposed mother filament generator to fully coincide with protrusion, as is the case for FMNL2 and FMNL3 [57]. However, RNA interference or genetic removal of both members of this formin subfamily significantly decreased actin filament mass in lamellipodia without affecting the extent of lamellipodial Arp2/3 complex incorporation. This strongly suggesting these formins to generate filament populations acting in a fashion separable from the Arp2/3 complex-dependent actin network [57]. Interestingly, branching at the periphery of cells has been observed from filaments or filament bundles at both retractile edges or experimentally-induced cytoplasmic wounds [63], raising the question as to whether specific filament primers are even needed *in vivo* [64]. Together, as opposed to the undebated, essential role of Arp2/3 complex in lamellipodial protrusion, there is no unambiguous experimental evidence for an essential function of any additional nucleator/elongator in this or any other Arp2/3 complex-mediated process at present [6,26]. Instead, the precise relative functions of lamellipodial formins and Ena/VASP family members have been less clear for long, in particular if considering the wealth of information gathered from various different systems and experimental approaches over decades (for Ena/VASP compare [31,65,66]). Hence, systematic side-by-side analyses of the relative impact of functional interference with all these factors and in the same cell type, both individually and in combination, will be required to shed more light on such questions.

Single filament *versus* collective behaviour of the network

In traditional models of cooperation between Arp2/3 complex and actin filament elongators in lamellipodia, excellently reviewed in a comprehensive fashion a few years back [67], the intuitive view – at least at first glance - was that a balance between branching and elongation of filaments determines the persistence *versus* speed of lamellipodial protrusion. This means that a dominance of branching over elongation should reduce protrusion speed of lamellipodia, but increase their persistence, and *vice versa* if elongation dominates. At the structural level, this would coincide with high network density in the high branching regime, and low network density and thus reduced protrusion stability in the low branching and thus high elongation regime [67], as previously observed upon CAAX-box-mediated Ena/VASP targeting to plasma membranes [65]. This model works if assuming that the activity of a given actin assembly factor on individual actin filaments *in vitro* can be directly translated into its activity on a collective of barbed ends at lamellipodia tips *in vivo*, as proposed previously [58,67].

However, functional interference with lamellipodial formins FMNL2 and FMNL3, both of which drive nucleation and profilin-mediated, processive elongation of actin filaments *in vitro*, generated results that were fully compatible with their subcellular dynamics, regulation and activity [57], but inconsistent with aforementioned model. First, shifting the balance towards Arp2/3 complex-mediated branching by FMNL2 and -3 removal decreased actin network density instead of increasing it, and softened rather than stiffened these lamellipodia, at least if considering their force development measured by atomic force microscopy [57]. Moreover, in spite of decreased protrusion, rates of network polymerization were largely unchanged, which can only be explained by increased rearward flow rates of the more slowly protruding lamellipodia lacking FMNL2 and FMNL3. Importantly, recent mathematical modelling of all these activities and experimental conditions revealed that a reduction of lamellipodial heights caused by lowered actin filament densities upon FMNL2/3 removal can cause both reduced protrusion and increased flow rate, explaining largely unchanged actin assembly rates in these conditions [68]. These data thus call for a revision of our over-simplified views on how

polymerization of individual filaments embedded into lamellipodia affect the behaviour of the collective of filaments within the network, but they are not questioning the biochemical activities of the actin assembly factors measured *in vitro* [31,57,58].

So what do FMNL formins do in lamellipodia? In our current view, FMNL2 and -3 displaying slightly differential activities concerning nucleation *versus* elongation *in vitro* [57], generate subsets of filaments in lamellipodia, optimizing their protrusion efficiency, extent of filament bundling and force development. This is also consistent with loss of function studies in more complex model systems, such as zebrafish and mice [69,70].

Notably, it is not fully understood how potential, differential polymerization of filaments within the lamellipodium network is homogenized, since the edge membrane usually protrudes forwards in a highly smooth and continuous fashion. However, the adaptability of lamellipodial actin networks has recently been highlighted by examining the relation of load and network structure [71]. Interestingly, branched actin networks were previously observed *in vitro* to adapt to elevated resistance by increasing filament densities without changing the stoichiometry of their constituents, including Arp2/3 complex [72]. This force feedback mechanism occurred without changes in average filament lengths. It was based instead on an increase of both the number of growing filaments and their packing density, as opposed to an increase in branching activity per unit actin filament [72]. Fully consistent with this, the work by Mueller et al. now explains how the simple geometry of Arp2/3-dependent actin networks can enable such adaptation mechanisms *in vivo* and thus tune lamellipodial protrusion in response to mechanical force [71]. In essence, an elevation of load will cause the protection of filaments from termination by heterodimeric capping protein [73] at increased ranges of angles relative to the edge membrane, culminating in an enhanced density of the Arp2/3-dependent network. Conversely, a load decrease will outcompete shallower over perpendicular filaments, thereby decreasing network density at increased protrusion speed [71]. Based on these results, it is tempting to speculate that potential differences in individual growth rates of filaments will be

suppressed or compensated for by such network-intrinsic, geometrical features. For instance, FMNL formins (and perhaps additional elongators) could both promote the nucleation of more orthogonal, lamellipodial filaments, as observed previously [57,74], and simultaneously protect the capping of shallower ones, increasing overall filament density by a factor of two [57]. However, these additional filaments cannot enhance the overall assembly rate of the lamellipodium, except perhaps if locally exceeding a critical number for protrusion of a filopodium (Figure 1). Rates of lamellipodial network polymerization might thus less directly be controlled by the inventory of actin polymerases residing at their tips than previously thought (see also below) [57,58,67,75]. Future work will have to experimentally validate such hypotheses.

Actin monomer to filament ratio and global *versus* local regulation

A recent surface structuration assay allowing the mimicry of lamellipodium protrusion with differential NPF patterning and concentration established the connection between Arp2/3-dependent network architecture or density and growth efficiency, and how these parameters may affect steering of the lamellipodium during cell migration [76]. The artificial, lamellipodia-like structures studied in this case are simplified, as their assembly is completely fed into rearward flow due to controlled nucleation of the network off substratum-coated NPFs, i.e. no *bona-fide* protrusion possible. However, in spite of the importance of high and homogeneous actin filament density for effective actin assembly, excess local NPF concentrations and thus branching activity reduced effective actin assembly simply by local actin monomer depletion [76]. It is conceivable that similar mechanisms are at play in cells to steer protrusions based on actin monomer availability and signalling-induced, local organization of actin networks driving migration, although the details remain to be established.

In general, regulation of actin assembly in cells at the level of actin monomers has been receiving increasing attention in the past few years [77]. The most-prominent actin monomer binding protein, profilin, is still a major focus, although exciting novel research indicates that one of its most popular functions, the re-charging of ADP-actin monomer with ATP, might equally well be taken over by cyclase-associated protein (CAP) [78]. For instance, profilin has been proposed as key regulator of Arp2/3-dependent *versus* –independent actin assembly pathways, because it can promote the latter while counteracting Arp2/3-dependent branching [79,80]. Inspired by these and other results, Kovar and colleagues formulated an actin network competition model truly centred on profilin as key determinant of a limiting pool of polymerizable actin monomer [81]. The molecular mechanism was proposed to reflect a simple competition for actin monomer between profilin and the W-domain(s) N-terminal to CA within class I NPFs [10]. Indeed, there is several indications for internetwork competition in the literature, as for instance in case of lamellipodia *versus* filopodia [56,82,83] or in case of epithelial cell polarization regulated by myosin II-dependent contractile bundles *versus* branched actin-networks [84]. In addition, we have recently found the protrusion and actin assembly rate of lamellipodia to be reduced by excess, ectopic polymerization of filaments in the cytoplasm [75], demonstrating that we can generate experimental scenarios *in vivo* that display monomer depletion as recently observed *in vitro* [76].

However, the simple inhibition of Arp2/3 complex by profilin has been challenged by a recent *in vitro* study that suggested that the proline-rich region N-terminal to WCA commonly present in class I NPFs [10,52,85] can alleviate this competition by passing on actin for efficient, WCA-mediated branching via proline-rich sequences (P) [86]. This exciting new study also showed that the PWCA-modules of class I NPFs can actually tether barbed ends and promote their elongation through both W-mediated actin and P-mediated profilin-actin recruitment, thereby acting as what was coined a “distributive network polymerase” [86]. To what extent profilin-dependent and –independent delivery of actin monomers to barbed ends at the lamellipodium

tip may be directly mediated by the class I NPF WRC (Box 1) *versus* additional actin polymerases residing at these sites [67] remains to be established (also see Figure 2).

In addition, profilin is not just an actin monomer binding protein, but has also been found to track the barbed end [87], potentially competing with various barbed end binders in the lamellipodium, including polymerases and capping protein. Based in part on these and additional results, Carlier and Shekhar have concluded actin turnover and differential actin array sizes in experimentally manipulated cells to derive from differential signalling rather than a finite pool of profilin-actin [88]. In their “global treadmilling model”, inhibition of Arp2/3 complex *versus* formin-mediated actin assembly has strong effects on the respective other network, without major changes of profilin-actin concentration. Careful future experiments in cells are required to distinguish between all these models.

Finally, actin monomer has recently been concluded to concentrate at lamellipodia tips [89], like in a sink towards accelerated actin polymerization, but leading edge targeting from cytosolic pools was proposed to be mediated by the monomer sequestering factor thymosin β 4 rather than profilin [90]. Clearly, future efforts will have to precisely determine the cellular concentrations of all species of polymerizable actin and how they interact with the multitude of factors recycling and employing them for effective and continuous, lamellipodial assembly (Figure 2).

Conclusions and future perspectives

In our current thinking, the actin assembly machineries operating in lamellipodia can be considered as role model for various, Arp2/3 complex-dependent structures formed in cells or during pathogenic processes. This holds true in particular for those structures operating at plasma membranes, such as pathogen-induced actin structures, phagocytic cups or CDRs driving micropinocytosis. Moreover, all this concerns principal mechanisms of tuning the

relative contributions of Arp2/3-dependent branching *versus* other mechanisms of nucleation and elongation, and regarding the potential regulation by actin monomer (see above). We consider the lamellipodium as branched actin network supported by filaments or filament bundles of Arp2/3-independent origin [57,74] that can be switched on and off by Rho GTPase-triggered signalling (Figure 1), and that is continuously turning over by assembly at the front and disassembly throughout the structure (Figure 2), as independently found in various model systems [54,67,89,91]. Although WRC/Arp2/3 complex-dependent branching is agreed to be indispensable for initiation and maintenance of this structure [6], the functions of additional players in the family of actin assembly factors are just beginning to emerge [57,67,68], including the relevance of profilin, profilin-bound actin and the precise nature in distinct tissues and cell types of the “re-charging” activity of ADP-actin monomers with ATP [78]. Moreover, we have refrained from emphasizing the relevance of coronin and members of the **actin depolymerizing factor homology (ADF-H)** family, including GMF, which have been excellently covered in recent reviews [92,93]. Notably, the relative relevance of all these factors in actin network remodelling and/or disassembly remains to be established. In addition, it will be challenging in the future to integrate currently conflicting observations into our model, such as rapid actin turnover (partially by actin oligomers) throughout the lamellipodium [94] or RhoA (and not Rac) as protrusion initiator [95].

Acknowledgements

We would like to thank all members of our lab for continuous and fruitful discussions, and the Deutsche Forschungsgemeinschaft for funding (GRK2223 to K.R.).

The authors declare no conflict of interest.

BOX 1

WAVE regulatory complex – an update on its regulation

Exciting recent research has uncovered some of the intricacies of regulation of heteropentameric WRC by its major activator in animals, the small GTPase Rac, and its implication for Arp2/3 complex-mediated lamellipodia formation in cells. It is commonly believed that WRC comprises 5 subunits, which operate as functional unit to drive Scar/WAVE-mediated Arp2/3 complex activation in different processes at the plasma membrane. In each tissue and organism, individual subunits (except for HSPC300) may be encoded by several isogenes that are assembled based on isogene expression. In mammals, the Rac interaction surface is encoded by Specifically **Rac**-associated protein 1 (Sra-1) or its isogene PIR121. In landmark research by the Rosen-group, it had originally been shown that interaction of Rac with Sra-1 causes relief of an inhibitory interaction of the WCA-domain of WAVE with WRC surfaces (in particular on Sra-1) [96]. More recent work then describing the structure of WRC-Rac1 complex as determined by cryo-electron microscopy, suggested a second binding site for Rac of even higher affinity, with the previously characterized site now called A site (for adjacent to the WCA-binding site), and the new site D site (for WCA-domain distant site) [97]. WRC was consequently proposed to be activated by simultaneous engagement of two Rac1 molecules, potentially endowing cells with the ability to sense the density of Rac1 signals for precise control of WRC-mediated actin assembly. *In vivo* work analysing cells exclusively harbouring WRCs lacking one of these binding sites or both of them revealed the situation to be more complex, with the formerly described, A site being established as the major activation site, and the second, high-affinity binding D site contributing to WRC-mediated actin assembly efficiency [38]. Interestingly, although the presence of either one of these sites is required for

WRC activation, they are crucial but not obligatory for recruitment of activated WRC, suggesting additional mechanisms of WRC accumulation in lamellipodia to be at play. In contrast, binding of WRC to previously defined WIRS (WRC interacting receptor sequence) peptides [98] is dispensable for this process [38]. Note, however, that aside from Rac, WRC positioning at the plasma membrane has been proposed to be mediated by multiple signals, including phosphatidylinositol (3,4,5)-trisphosphate (PIP3) binding to the basic domain of WAVE [99] or WRC binding to the lamellipodial actin assembly factor Lamellipodin (Lpd) [100]. The precise relevance of all these interactions is incompletely understood, as mutation of the PIP3-binding basic domain of Scar/WAVE in *Dictyostelium* generates a hyperactive Scar/WAVE variant rather than a compromised one [101], and as opposed to WRC, Lpd is not essential for embryonic development [100].

Figure Legends

Figure 1: Actin-dependent arrays of protrusion at the plasma membrane and essential signalling pathways that induce them. The majority of cell edge protrusions comprises branched actin filament networks (shown in red) formed by Arp2/3 complex, as in lamellipodia (1) or ruffles if lifting up- and backwards (2). Ruffling can also occur at the dorsal surface of cells growing on 2D-surfaces, which are then called circular dorsal ruffles coinciding with micropinocytosis (3). Additional branched actin structures are the phagocytic cups of haematopoietic cells (4), as well as the adherens junctions mediating cell-cell-contact in epithelial or endothelial cells (5). All these structures employ WAVE regulatory complex (WRC) as critical Arp2/3 complex activator, except for invadosomes (podosomes and invadopodia), which protrude at ventral cell surfaces of osteoclasts and macrophages (for details see text; 6). Filopodia are bundled structures (shown in green), and can either be found distally to lamellipodia or independently of the latter (7). The bundled, green structure embedded into the lamellipodium illustrates a microspike. If the actin cortex is ruptured (blue), the plasma membrane protrudes a bleb driven by hydrostatic pressure (8), which is subsequently retracted in an actin-dependent fashion (not shown). The process of dorsal ruffling and accompanied macropinocytosis is shown with two consecutive time points (t_0 and t_1), and dorsal and peripheral ruffles as well as invadosomes are shown as sagittal sections (dashed rectangles).

Figure 2: Lamellipodial actin network assembly and disassembly. Assembly of the lamellipodial actin network at the plasma membrane (top) is driven by the continuous branching activity of the Arp2/3 complex and elongation of branched and non-branched actin filaments by various polymerases. WAVE regulatory complex (WRC) continuously activates Arp2/3 complex at the lamellipodium tip, which is essential per definition for actin network assembly and maintenance. In addition, the C-terminus of WAVE and thus WRC as functional unit was

recently suggested to potentially also function in filament tethering (right, in the absence of sufficient actin or profilin-actin) and filament elongation (middle). This “distributive elongation activity” might occur both in a profilin-dependent and –independent fashion. FMNL formins also contribute to lamellipodial network density by nucleating filaments independently of Arp2/3 complex, and elongating them in a processive, profilin-dependent fashion. In analogy to formins, Ena/VASP proteins will also tether and elongate filaments in a processive manner, but as opposed to formins, processivity can occur with and without profilin. Capping protein stochastically terminates filament elongation and can travel rearwards with the network dependent on life time of capped filaments. This may be limited by disassembly and debranching of the network gradually increasing towards the rear of the lamellipodium, and effected by disassembly factors such as ADF/cofilin and debranching factors like coronin or GMF (for details see text).

References

1. Pollard TD: **Tribute to Fumio Oosawa the pioneer in actin biophysics.** *Cytoskeleton (Hoboken)* 2017, **74**:446-449.
2. Euteneuer U, Schliwa M: **Persistent, directional motility of cells and cytoplasmic fragments in the absence of microtubules.** *Nature* 1984, **310**:58-61.
3. Italiano JE, Jr., Roberts TM, Stewart M, Fontana CA: **Reconstitution in vitro of the motile apparatus from the amoeboid sperm of *Ascaris* shows that filament assembly and bundling move membranes.** *Cell* 1996, **84**:105-114.
4. Tilney LG, Porter KR: **Studies on the microtubules in heliozoa. II. The effect of low temperature on these structures in the formation and maintenance of the axopodia.** *J Cell Biol* 1967, **34**:327-343.
5. Schwan C, Stecher B, Tzivelekidis T, van Ham M, Rohde M, Hardt WD, Wehland J, Aktories K: **Clostridium difficile toxin CDT induces formation of microtubule-based protrusions and increases adherence of bacteria.** *PLoS Pathog* 2009, **5**:e1000626.
6. Rottner K, Faix J, Bogdan S, Linder S, Kerkhoff E: **Actin assembly mechanisms at a glance.** *J Cell Sci* 2017, **130**:3427-3435.
7. Fritz-Laylin LK, Lord SJ, Kakley M, Mullins RD: **Concise Language Promotes Clear Thinking about Cell Shape and Locomotion.** *Bioessays* 2018, **40**:e1700225.
8. Pollard TD, Borisy GG: **Cellular motility driven by assembly and disassembly of actin filaments.** *Cell* 2003, **112**:453-465.
9. Shekhar S, Carlier MF: **Enhanced Depolymerization of Actin Filaments by ADF/Cofilin and Monomer Funneling by Capping Protein Cooperate to Accelerate Barbed-End Growth.** *Curr Biol* 2017, **27**:1990-1998 e1995.
10. Campellone KG, Welch MD: **A nucleator arms race: cellular control of actin assembly.** *Nature reviews. Molecular cell biology* 2010, **11**:237-251.
11. Machesky LM, Atkinson SJ, Ampe C, Vandekerckhove J, Pollard TD: **Purification of a cortical complex containing two unconventional actins from *Acanthamoeba* by affinity chromatography on profilin-agarose.** *J Cell Biol* 1994, **127**:107-115.
12. Smith BA, Padrick SB, Doolittle LK, Daugherty-Clarke K, Correa IR, Jr., Xu MQ, Goode BL, Rosen MK, Gelles J: **Three-color single molecule imaging shows WASP detachment from Arp2/3 complex triggers actin filament branch formation.** *Elife* 2013, **2**:e01008.
13. Mullins RD, Heuser JA, Pollard TD: **The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of filaments.** *Proc Natl Acad Sci U S A* 1998, **95**:6181-6186.
14. Svitkina TM, Borisy GG: **Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia.** *The Journal of cell biology* 1999, **145**:1009-1026.
15. Collins A, Warrington A, Taylor KA, Svitkina T: **Structural organization of the actin cytoskeleton at sites of clathrin-mediated endocytosis.** *Curr Biol* 2011, **21**:1167-1175.
16. Efimova N, Svitkina TM: **Branched actin networks push against each other at adherens junctions to maintain cell-cell adhesion.** *J Cell Biol* 2018, **217**:1827-1845.
17. Mueller J, Pfanzelter J, Winkler C, Narita A, Le Clairche C, Nemethova M, Carlier MF, Maeda Y, Welch MD, Ohkawa T, et al.: **Electron tomography and simulation of baculovirus actin comet tails support a tethered filament model of pathogen propulsion.** *PLoS Biol* 2014, **12**:e1001765.
18. Coutts AS, La Thangue NB: **Regulation of actin nucleation and autophagosome formation.** *Cell Mol Life Sci* 2016, **73**:3249-3263.

19. Hamp J, Lower A, Dottermusch-Heidel C, Beck L, Moussian B, Flotenmeyer M, Onel SF: **Drosophila Kette coordinates myoblast junction dissolution and the ratio of Scar-to-WASp during myoblast fusion.** *J Cell Sci* 2016, **129**:3426-3436.
20. Caridi CP, D'Agostino C, Ryu T, Zapotoczny G, Delabaere L, Li X, Khodaverdian VY, Amaral N, Lin E, Rau AR, et al.: **Nuclear F-actin and myosins drive relocation of heterochromatic breaks.** *Nature* 2018.
21. Schrank BR, Aparicio T, Li Y, Chang W, Chait BT, Gundersen GG, Gottesman ME, Gautier J: **Nuclear ARP2/3 drives DNA break clustering for homology-directed repair.** *Nature* 2018.
22. Abella JV, Galloni C, Pernier J, Barry DJ, Kjaer S, Carlier MF, Way M: **Isoform diversity in the Arp2/3 complex determines actin filament dynamics.** *Nat Cell Biol* 2016, **18**:76-86.
23. Egile C, Loisel TP, Laurent V, Li R, Pantaloni D, Sansonetti PJ, Carlier MF: **Activation of the CDC42 effector N-WASP by the Shigella flexneri IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility.** *J Cell Biol* 1999, **146**:1319-1332.
24. Welch MD, Rosenblatt J, Skoble J, Portnoy DA, Mitchison TJ: **Interaction of human Arp2/3 complex and the Listeria monocytogenes ActA protein in actin filament nucleation.** *Science* 1998, **281**:105-108.
25. Welch MD, Way M: **Arp2/3-mediated actin-based motility: a tail of pathogen abuse.** *Cell host & microbe* 2013, **14**:242-255.
26. Loisel TP, Boujemaa R, Pantaloni D, Carlier MF: **Reconstitution of actin-based motility of Listeria and Shigella using pure proteins.** *Nature* 1999, **401**:613-616.
27. Chesarone MA, Goode BL: **Actin nucleation and elongation factors: mechanisms and interplay.** *Current opinion in cell biology* 2009, **21**:28-37.
28. Kovar DR, Pollard TD: **Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces.** *Proc Natl Acad Sci U S A* 2004, **101**:14725-14730.
29. Yu M, Yuan X, Lu C, Le S, Kawamura R, Efremov AK, Zhao Z, Kozlov MM, Sheetz M, Bershadsky A, et al.: **mDia1 senses both force and torque during F-actin filament polymerization.** *Nat Commun* 2017, **8**:1650.
30. Rottner K, Behrendt B, Small JV, Wehland J: **VASP dynamics during lamellipodia protrusion.** *Nat Cell Biol* 1999, **1**:321-322.
31. Bruhmann S, Ushakov DS, Winterhoff M, Dickinson RB, Curth U, Faix J: **Distinct VASP tetramers synergize in the processive elongation of individual actin filaments from clustered arrays.** *Proc Natl Acad Sci U S A* 2017, **114**:E5815-E5824.
32. Winkelman JD, Bilancia CG, Peifer M, Kovar DR: **Ena/VASP Enabled is a highly processive actin polymerase tailored to self-assemble parallel-bundled F-actin networks with Fascin.** *Proc Natl Acad Sci U S A* 2014, **111**:4121-4126.
33. Benanti EL, Nguyen CM, Welch MD: **Virulent Burkholderia species mimic host actin polymerases to drive actin-based motility.** *Cell* 2015, **161**:348-360.
34. Caswell PT, Zech T: **Actin-Based Cell Protrusion in a 3D Matrix.** *Trends Cell Biol* 2018.
35. Small JV, Stradal T, Vignat E, Rottner K: **The lamellipodium: where motility begins.** *Trends Cell Biol* 2002, **12**:112-120.
36. Kunda P, Craig G, Dominguez V, Baum B: **Abi, Sra1, and Kette control the stability and localization of SCAR/WAVE to regulate the formation of actin-based protrusions.** *Curr Biol* 2003, **13**:1867-1875.
37. Leithner A, Eichner A, Muller J, Reversat A, Brown M, Schwarz J, Merrin J, de Gorter DJ, Schur F, Bayerl J, et al.: **Diversified actin protrusions promote environmental exploration but are dispensable for locomotion of leukocytes.** *Nat Cell Biol* 2016, **18**:1253-1259.

38. Schaks M, Singh SP, Kage F, Thomason P, Klünemann T, Steffen A, Blankenfeldt W, Stradal TE, Insall RH, Rottner K: **Distinct interaction sites of Rac GTPase with WAVE regulatory complex have non-redundant functions *in vivo*.** Available at: <http://dx.doi.org/10.2139/ssrn.3188452> 2018.
39. Steffen A, Ladwein M, Dimchev GA, Hein A, Schwenkmezger L, Arens S, Ladwein KI, Margit Holleboom J, Schur F, Victor Small J, et al.: **Rac function is crucial for cell migration but is not required for spreading and focal adhesion formation.** *Journal of cell science* 2013, **126**:4572-4588.
40. Steffen A, Rottner K, Ehinger J, Innocenti M, Scita G, Wehland J, Stradal TE: **Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation.** *EMBO J* 2004, **23**:749-759.
41. Suraneni P, Rubinstein B, Unruh JR, Durnin M, Hanein D, Li R: **The Arp2/3 complex is required for lamellipodia extension and directional fibroblast cell migration.** *J Cell Biol* 2012, **197**:239-251.
42. Wu C, Asokan SB, Berginski ME, Haynes EM, Sharpless NE, Griffith JD, Gomez SM, Bear JE: **Arp2/3 is critical for lamellipodia and response to extracellular matrix cues but is dispensable for chemotaxis.** *Cell* 2012, **148**:973-987.
43. Stradal TEB, Schelhaas M: **Actin dynamics in host-pathogen interaction.** *FEBS Lett* 2018.
44. Yamada S, Nelson WJ: **Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion.** *J Cell Biol* 2007, **178**:517-527.
45. Cunningham CC: **Actin polymerization and intracellular solvent flow in cell surface blebbing.** *J Cell Biol* 1995, **129**:1589-1599.
46. Paluch EK, Raz E: **The role and regulation of blebs in cell migration.** *Curr Opin Cell Biol* 2013, **25**:582-590.
47. Ikenouchi J, Aoki K: **Membrane bleb: A seesaw game of two small GTPases.** *Small GTPases* 2017, **8**:85-89.
48. Rotty JD, Brighton HE, Craig SL, Asokan SB, Cheng N, Ting JP, Bear JE: **Arp2/3 Complex Is Required for Macrophage Integrin Functions but Is Dispensable for FcR Phagocytosis and In Vivo Motility.** *Dev Cell* 2017, **42**:498-513 e496.
49. Legg JA, Bompard G, Dawson J, Morris HL, Andrew N, Cooper L, Johnston SA, Tramountanis G, Machesky LM: **N-WASP involvement in dorsal ruffle formation in mouse embryonic fibroblasts.** *Mol Biol Cell* 2007, **18**:678-687.
50. Suetsugu S, Yamazaki D, Kurisu S, Takenawa T: **Differential roles of WAVE1 and WAVE2 in dorsal and peripheral ruffle formation for fibroblast cell migration.** *Developmental cell* 2003, **5**:595-609.
51. Small JV, Isenberg G, Celis JE: **Polarity of actin at the leading edge of cultured cells.** *Nature* 1978, **272**:638-639.
52. Welch MD, Mullins RD: **Cellular control of actin nucleation.** *Annu Rev Cell Dev Biol* 2002, **18**:247-288.
53. Iwasa JH, Mullins RD: **Spatial and temporal relationships between actin-filament nucleation, capping, and disassembly.** *Curr Biol* 2007, **17**:395-406.
54. Lai FP, Szczodrak M, Block J, Faix J, Breitsprecher D, Mannherz HG, Stradal TE, Dunn GA, Small JV, Rottner K: **Arp2/3 complex interactions and actin network turnover in lamellipodia.** *EMBO J* 2008, **27**:982-992.
55. King SJ, Asokan SB, Haynes EM, Zimmerman SP, Rotty JD, Alb JG, Jr., Tagliatela A, Blake DR, Lebedeva IP, Marston D, et al.: **Lamellipodia are crucial for haptotactic sensing and response.** *J Cell Sci* 2016, **129**:2329-2342.
56. Koestler SA, Steffen A, Nemethova M, Winterhoff M, Luo N, Holleboom JM, Krupp J, Jacob S, Vinzenz M, Schur F, et al.: **Arp2/3 complex is essential for actin network**

- treadmilling as well as for targeting of capping protein and cofilin. *Molecular biology of the cell* 2013, **24**:2861-2875.
57. Kage F, Winterhoff M, Dimchev V, Mueller J, Thalheim T, Freise A, Bruhmann S, Kollasser J, Block J, Dimchev G, et al.: **FMNL formins boost lamellipodial force generation.** *Nat Commun* 2017, **8**:14832.
 58. Block J, Breitsprecher D, Kuhn S, Winterhoff M, Kage F, Geffers R, Duwe P, Rohn JL, Baum B, Brakebusch C, et al.: **FMNL2 drives actin-based protrusion and migration downstream of Cdc42.** *Current biology : CB* 2012, **22**:1005-1012.
 59. Isogai T, van der Kammen R, Leyton-Puig D, Kedziora KM, Jalink K, Innocenti M: **Initiation of lamellipodia and ruffles involves cooperation between mDia1 and the Arp2/3 complex.** *J Cell Sci* 2015, **128**:3796-3810.
 60. Ramalingam N, Franke C, Jaschinski E, Winterhoff M, Lu Y, Bruhmann S, Junemann A, Meier H, Noegel AA, Weber I, et al.: **A resilient formin-derived cortical actin meshwork in the rear drives actomyosin-based motility in 2D confinement.** *Nat Commun* 2015, **6**:8496.
 61. Lee K, Elliott HL, Oak Y, Zee CT, Groisman A, Tytell JD, Danuser G: **Functional hierarchy of redundant actin assembly factors revealed by fine-grained registration of intrinsic image fluctuations.** *Cell Syst* 2015, **1**:37-50.
 62. Isogai T, Danuser G: **Discovery of functional interactions among actin regulators by analysis of image fluctuations in an unperturbed motile cell system.** *Philos Trans R Soc Lond B Biol Sci* 2018, **373**.
 63. Vinzenz M, Nemethova M, Schur F, Mueller J, Narita A, Urban E, Winkler C, Schmeiser C, Koestler SA, Rottner K, et al.: **Actin branching in the initiation and maintenance of lamellipodia.** *J Cell Sci* 2012.
 64. Achard V, Martiel JL, Michelot A, Guerin C, Reymann AC, Blanchoin L, Boujemaa-Paterski R: **A "primer"-based mechanism underlies branched actin filament network formation and motility.** *Curr Biol* 2010, **20**:423-428.
 65. Bear JE, Svitkina TM, Krause M, Schafer DA, Loureiro JJ, Strasser GA, Maly IV, Chaga OY, Cooper JA, Borisy GG, et al.: **Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility.** *Cell* 2002, **109**:509-521.
 66. Trichet L, Sykes C, Plastino J: **Relaxing the actin cytoskeleton for adhesion and movement with Ena/VASP.** *J Cell Biol* 2008, **181**:19-25.
 67. Krause M, Gautreau A: **Steering cell migration: lamellipodium dynamics and the regulation of directional persistence.** *Nat Rev Mol Cell Biol* 2014, **15**:577-590.
 68. Dolati S, Kage F, Mueller J, Musken M, Kirchner M, Dittmar G, Sixt M, Rottner K, Falcke M: **On the relation between filament density, force generation and protrusion rate in mesenchymal cell motility.** *Mol Biol Cell* 2018:mbcE18020082.
 69. Wakayama Y, Fukuhara S, Ando K, Matsuda M, Mochizuki N: **Cdc42 mediates Bmp-induced sprouting angiogenesis through Fmnl3-driven assembly of endothelial filopodia in zebrafish.** *Developmental cell* 2015, **32**:109-122.
 70. Woodham EF, Paul NR, Tyrrell B, Spence HJ, Swaminathan K, Scribner MR, Giampazolias E, Hedley A, Clark W, Kage F, et al.: **Coordination by Cdc42 of Actin, Contractility, and Adhesion for Melanoblast Movement in Mouse Skin.** *Curr Biol* 2017, **27**:624-637.
 71. Mueller J, Szep G, Nemethova M, de Vries I, Lieber AD, Winkler C, Kruse K, Small JV, Schmeiser C, Keren K, et al.: **Load Adaptation of Lamellipodial Actin Networks.** *Cell* 2017, **171**:188-200 e116.
 72. Bieling P, Li TD, Weichsel J, McGorty R, Jreij P, Huang B, Fletcher DA, Mullins RD: **Force Feedback Controls Motor Activity and Mechanical Properties of Self-Assembling Branched Actin Networks.** *Cell* 2016, **164**:115-127.

73. Edwards M, Zwolak A, Schafer DA, Sept D, Dominguez R, Cooper JA: **Capping protein regulators fine-tune actin assembly dynamics.** *Nat Rev Mol Cell Biol* 2014, **15**:677-689.
74. Rottner K, Kage F: **Actin Networks: Adapting to Load through Geometry.** *Curr Biol* 2017, **27**:R1274-R1277.
75. Dimchev G, Steffen A, Kage F, Dimchev V, Pernier J, Carlier MF, Rottner K: **Efficiency of lamellipodia protrusion is determined by the extent of cytosolic actin assembly.** *Mol Biol Cell* 2017, **28**:1311-1325.
76. Boujemaa-Paterski R, Suarez C, Klar T, Zhu J, Guerin C, Mogilner A, Thery M, Blanchoin L: **Network heterogeneity regulates steering in actin-based motility.** *Nat Commun* 2017, **8**:655.
77. Skrubber K, Read TA, Vitriol EA: **Reconsidering an active role for G-actin in cytoskeletal regulation.** *J Cell Sci* 2018, **131**.
78. Kotila T, Kogan K, Enkavi G, Guo S, Vattulainen I, Goode BL, Lappalainen P: **Structural basis of actin monomer re-charging by cyclase-associated protein.** *Nat Commun* 2018, **9**:1892.
79. Rotty JD, Wu C, Haynes EM, Suarez C, Winkelman JD, Johnson HE, Haugh JM, Kovar DR, Bear JE: **Profilin-1 serves as a gatekeeper for actin assembly by Arp2/3-dependent and -independent pathways.** *Dev Cell* 2015, **32**:54-67.
80. Suarez C, Carroll RT, Burke TA, Christensen JR, Bestul AJ, Sees JA, James ML, Sirotkin V, Kovar DR: **Profilin regulates F-actin network homeostasis by favoring formin over Arp2/3 complex.** *Dev Cell* 2015, **32**:43-53.
81. Suarez C, Kovar DR: **Inter-network competition for monomers governs actin cytoskeleton organization.** *Nat Rev Mol Cell Biol* 2016, **17**:799-810.
82. Beli P, Mascheroni D, Xu D, Innocenti M: **WAVE and Arp2/3 jointly inhibit filopodium formation by entering into a complex with mDia2.** *Nature cell biology* 2008, **10**:849-857.
83. Mejillano MR, Kojima S, Applewhite DA, Gertler FB, Svitkina TM, Borisy GG: **Lamellipodial versus filopodial mode of the actin nanomachinery: pivotal role of the filament barbed end.** *Cell* 2004, **118**:363-373.
84. Lomakin AJ, Lee KC, Han SJ, Bui DA, Davidson M, Mogilner A, Danuser G: **Competition for actin between two distinct F-actin networks defines a bistable switch for cell polarization.** *Nat Cell Biol* 2015, **17**:1435-1445.
85. Rottner K, Hanisch J, Campellone KG: **WASH, WHAMM and JMY: regulation of Arp2/3 complex and beyond.** *Trends in cell biology* 2010, **20**:650-661.
86. Bieling P, Hansen SD, Akin O, Li TD, Hayden CC, Fletcher DA, Mullins RD: **WH2 and proline-rich domains of WASP-family proteins collaborate to accelerate actin filament elongation.** *EMBO J* 2018, **37**:102-121.
87. Pernier J, Shekhar S, Jegou A, Guichard B, Carlier MF: **Profilin Interaction with Actin Filament Barbed End Controls Dynamic Instability, Capping, Branching, and Motility.** *Dev Cell* 2016, **36**:201-214.
88. Carlier MF, Shekhar S: **Global treadmilling coordinates actin turnover and controls the size of actin networks.** *Nat Rev Mol Cell Biol* 2017, **18**:389-401.
89. Lee CW, Vitriol EA, Shim S, Wise AL, Velayutham RP, Zheng JQ: **Dynamic localization of G-actin during membrane protrusion in neuronal motility.** *Curr Biol* 2013, **23**:1046-1056.
90. Vitriol EA, McMillen LM, Kapustina M, Gomez SM, Vavylonis D, Zheng JQ: **Two functionally distinct sources of actin monomers supply the leading edge of lamellipodia.** *Cell Rep* 2015, **11**:433-445.

91. Renkawitz J, Schumann K, Weber M, Lammermann T, Pflücke H, Piel M, Polleux J, Spatz JP, Sixt M: **Adaptive force transmission in amoeboid cell migration.** *Nat Cell Biol* 2009, **11**:1438-1443.
92. Chan KT, Creed SJ, Bear JE: **Unraveling the enigma: progress towards understanding the coronin family of actin regulators.** *Trends Cell Biol* 2011, **21**:481-488.
93. Goode BL, Sweeney MO, Eskin JA: **GMF as an Actin Network Remodeling Factor.** *Trends Cell Biol* 2018.
94. Raz-Ben Aroush D, Ofer N, Abu-Shah E, Allard J, Krichevsky O, Mogilner A, Keren K: **Actin Turnover in Lamellipodial Fragments.** *Curr Biol* 2017, **27**:2963-2973 e2914.
95. Machacek M, Hodgson L, Welch C, Elliott H, Pertz O, Nalbant P, Abell A, Johnson GL, Hahn KM, Danuser G: **Coordination of Rho GTPase activities during cell protrusion.** *Nature* 2009, **461**:99-103.
96. Chen Z, Borek D, Padrick SB, Gomez TS, Metlagel Z, Ismail AM, Umetani J, Billadeau DD, Otwinowski Z, Rosen MK: **Structure and control of the actin regulatory WAVE complex.** *Nature* 2010, **468**:533-538.
97. Chen B, Chou HT, Brautigam CA, Xing W, Yang S, Henry L, Doolittle LK, Walz T, Rosen MK: **Rac1 GTPase activates the WAVE regulatory complex through two distinct binding sites.** *Elife* 2017, **6**.
98. Chen B, Brinkmann K, Chen Z, Pak CW, Liao Y, Shi S, Henry L, Grishin NV, Bogdan S, Rosen MK: **The WAVE regulatory complex links diverse receptors to the actin cytoskeleton.** *Cell* 2014, **156**:195-207.
99. Oikawa T, Yamaguchi H, Itoh T, Kato M, Ijuin T, Yamazaki D, Suetsugu S, Takenawa T: **PtdIns(3,4,5)P3 binding is necessary for WAVE2-induced formation of lamellipodia.** *Nat Cell Biol* 2004, **6**:420-426.
100. Law AL, Vehlow A, Kotini M, Dodgson L, Soong D, Theveneau E, Bodo C, Taylor E, Navarro C, Perera U, et al.: **Lamellipodin and the Scar/WAVE complex cooperate to promote cell migration in vivo.** *J Cell Biol* 2013, **203**:673-689.
101. Ura S, Pollitt AY, Veltman DM, Morrice NA, Machesky LM, Insall RH: **Pseudopod growth and evolution during cell movement is controlled through SCAR/WAVE dephosphorylation.** *Curr Biol* 2012, **22**:553-561.

Papers of particular interest, published in the past 2 years, have been highlighted as:

- of special interest
- of outstanding interest

- 31. Bruhmann S, Ushakov DS, Winterhoff M, Dickinson RB, Curth U, Faix J: **Distinct VASP tetramers synergize in the processive elongation of individual actin filaments from clustered arrays.** *Proc Natl Acad Sci U S A* 2017, **114**:E5815-E5824.

Using clustered arrays of the Ena/VASP family member VASP and total internal reflection fluorescence (TIRF) imaging, the authors show how distinct VASP tetramers can collaborate to elongate individual actin filaments. In such clusters potentially reflecting the *in vivo* situation at sites of VASP accumulation, elongation rates are independent of the number of free actin monomer binding sites, whereas in solution they are.

- 48. Rotty JD, Brighton HE, Craig SL, Asokan SB, Cheng N, Ting JP, Bear JE: **Arp2/3 Complex Is Required for Macrophage Integrin Functions but Is Dispensable for FcR Phagocytosis and In Vivo Motility.** *Dev Cell* 2017, **42**:498-513 e496.

Genetic deletion of the Arp2/3 complex subunit ArpC2 demonstrates integrin-dependent processes such as haptotaxis and complement-mediated phagocytosis (but not chemotaxis or FcR phagocytosis) to be severely compromised in macrophages lacking functional Arp2/3 complex.

- 57. Kage F, Winterhoff M, Dimchev V, Mueller J, Thalheim T, Freise A, Bruhmann S, Kollasser J, Block J, Dimchev G, et al.: **FMNL formins boost lamellipodial force generation.** *Nat Commun* 2017, **8**:14832.

Study precisely defining the role of Cdc42-effectors and lamellipodial formins FMNL2 and FMNL3 in protrusion and actin filament generation in lamellipodia. Both formins are responsible for generating and/or maintaining an Arp2/3 complex-independent population of actin filaments relevant for lamellipodial stability and force development.

- 71. Mueller J, Szep G, Nemethova M, de Vries I, Lieber AD, Winkler C, Kruse K, Small JV, Schmeiser C, Keren K, et al.: **Load Adaptation of Lamellipodial Actin Networks.** *Cell* 2017, **171**:188-200 e116.

Landmark paper showing for the first time how the geometry of lamellipodial actin networks mediates adaptation of these networks to differential loads: The molecular mechanism confirmed by mathematical modelling involves differential barbed end capping of filaments subtending the leading edge at distinct angles. High loads protect filaments with a broadened range of angles leading to high network densities, whereas low loads favour filaments towards perpendicular angles (at the expense of flat angles) leading to sparse networks.

- 75. Dimchev G, Steffen A, Kage F, Dimchev V, Pernier J, Carlier MF, Rottner K: **Efficiency of lamellipodia protrusion is determined by the extent of cytosolic actin assembly.** *Mol Biol Cell* 2017, **28**:1311-1325.

Study showing that simple increase of actin polymerases (VASP or active formins) in the lamellipodium increases actin filament density but not protrusion. Moreover, different actin assembly factors differentially reduce protrusion dependent on the extent of induction of cytosolic actin assembly.

- 76. Boujemaa-Paterski R, Suarez C, Klar T, Zhu J, Guerin C, Mogilner A, Thery M, Blanchoin L: **Network heterogeneity regulates steering in actin-based motility.** *Nat Commun* 2017, **8**:655.

Study on reconstituted lamellipodium-like actin network demonstrating that local consumption of actin monomers *in vitro* can become rate limiting for local actin assembly rates of filaments and thus steer the direction of actin network growth. However, aside from monomer depletion, the direction of steering is additionally controlled by network architecture.

- 78. Kotila T, Kogan K, Enkavi G, Guo S, Vattulainen I, Goode BL, Lappalainen P: **Structural basis of actin monomer re-charging by cyclase-associated protein.** *Nat Commun* 2018, **9**:1892.

Exciting new story convincingly demonstrating by X-ray crystallography and biochemical analyses cells how the C-terminal CARP-domain of cyclase-associated protein (CAP) associates with the nucleotide sensing region of actin and induces re-charging of actin with ATP. In an attractive working model, dimeric CAP is handing over recharged, ATP-actin to profilin instead of the latter being the main driver of ADP-ATP nucleotide exchange. The relevance of CAP-catalyzed nucleotide exchange on actin monomers is also confirmed in yeast cells.

- 86. Bieling P, Hansen SD, Akin O, Li TD, Hayden CC, Fletcher DA, Mullins RD: **WH2 and proline-rich domains of WASP-family proteins collaborate to accelerate actin filament elongation.** *EMBO J* 2018, **37**:102-121.

Carefully executed *in vitro* study illustrating how the C-termini of WASP family proteins (such as WAVE in the lamellipodium) could accelerate the rate of elongation of several filaments simultaneously (coined distributive polymerase activity). This new activity can occur through collaboration of the proline-rich and WH2 regions, and thus employ both profilin-actin and actin monomers.

- 97. Chen B, Chou HT, Brautigam CA, Xing W, Yang S, Henry L, Doolittle LK, Walz T, Rosen MK: **Rac1 GTPase activates the WAVE regulatory complex through two distinct binding sites.** *Elife* 2017, **6**.

Landmark paper describing the discovery of a second Rac binding site on the WRC subunit Sra-1, based on a new cryo-electron microscopy structure of WRC-Rac1 as well as biochemical and biophysical analyses.