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Assembling actin filaments for protrusion

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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.

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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-bound 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 biochemisty 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: Because of 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, that is 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, for example 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-lifted or backwards-lifted lamellipodium at the cell periphery, and the second to a more complex structure known as **c**ircular **d**orsal **r**uffle (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