Actin filament structures in migrating cells

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ABSTRACT

Cell migration is necessary for several developmental processes in multicellular organisms. Furthermore, many physiological processes such as wound healing and immunological events in adult animals are dependent on cell migration. Consequently, defects in cell migration are linked to various diseases including immunological disorders as well as cancer progression and metastasis formation. Cell migration is driven by specific protrusive and contractile actin filament structures, but the types and relative contributions of these actin filament arrays vary depending on the cell-type and the environment of the cell. In this chapter, we introduce the most central actin filament structures that contribute to mesenchymal and amoeboid cell migration modes, and discuss the mechanisms by which the assembly and turnover of these structures are controlled by various actin-binding proteins.

1. INTRODUCTION

Cell migration is a complex, dynamic process that has fundamental role in the development and physiology of multicellular animals. Cell migration is also critical for the survival of many unicellular organisms. For example, unicellular protozoa migrate to hunt their prey, whereas immune cells travel in animal tissues to seek pathogens. Furthermore, antigen-presenting dendritic cells, which explore the tissues, migrate into the lymph nodes to present peripherally acquired antigens to T cells upon exposure to infection or inflammatory stimuli. In addition to immunological processes, cell migration is important for survival of animals, because for example wound healing depends on migration of different cell-types to the injured area. Finally, apart from physiological processes in adult tissues, cell migration is crucial for the majority of developmental processes, including gastrulation and organ formation (Nourshargh et al. 2010; Petrie and Yamada 2012; Doyle et al. 2013).

Due to its fundamental roles in developmental and physiological processes, it is not surprising that defects in cell migration are linked to a variety of human disorders, including problems in the function of the immune system. For example, phagocytic cells with a mutation in an actin-binding protein WASP, which is important regulator of cell motility, have a poor chemotactic response toward inflammatory chemoattractants. Also WASP-deficient T cells display a marked migration deficiency (Moulding et al. 2013). Conversely, uncontrolled cell migration is linked to cancer invasion and metastasis. In the case of carcinomas, these processes initiate with a delamination of otherwise non-migratory epithelial cells from epithelial sheets, followed by migration of carcinoma cells across basal lamina and underlying cell layers into blood or lymph vessels. Both normal and cancer cells use similar machineries for migration, but cancer cells appear to lack the ’stop signals’ important for anchoring the cells. Also their ability to properly read the chemical and mechanical signals of the environment is typically lost (Friedl and Alexander 2011; Petrie and Yamada 2012).

For cells to move, they must extend plasma membrane at the leading edge, subsequently move the cell body, and retract the tail of the cell (Ridley 2011). The force for these events is provided by actin, which is a ubiquitous protein found in all eukaryotes. In cells, actin exists both in monomeric and filamentous forms. Actin filaments are polar structures that contain two biochemically and structurally distinct ends, named the *barbed end* and *pointed end*. Under steady state conditions, actin filament assembly takes mainly place at filament barbed ends, and strongly favors ATP-bound actin monomers. Actin filament itself functions as an ATPase, which leads to enrichment of ADP-Pi and ADP-actin subunits toward the filament pointed end, where the net disassembly of actin filaments occurs. These features result in simultaneous ATP-dependent elongation of actin filaments at their barbed ends and shortening at the pointed ends. This process, named *actin filament treadmilling*, provides force for a number of cellular processes involving membrane dynamics. In addition to treadmilling, actin filaments generate force through a fundamentally different mechanism involving myosin-family motor proteins. Here, the actin filaments serve as tracks for myosin molecules that, depending on the type of myosin, can move either towards the barbed or

pointed end of the actin filament, and transport different types of cargo or form bi-polar bundles that can induce the contraction of actin filament arrays (Pollard and Cooper 2009). To ensure the formation of desired actin filament arrays at correct location of the cell, the organization and dynamics of actin filaments in cells are controlled by a large array of actin-binding proteins. The activities of these proteins are linked to various intracellular and extracellular biochemical and mechanical signals through their regulation by phosphorylation, Rho-family small GTPases, and plasma membrane phospholipids (Heasman and Ridley 2008; Saarikangas et al. 2010).

In the following chapters we will introduce the functions and compositions of the three actin filament structures (lamellipodia, filopodia and stress fibers) that contribute to cell migration in two-dimensional environment. We will also introduce the types of actin structures that drive cell migration specifically in a complex three-dimensional tissue environment.

2. ACTIN-RICH STRUCTURES INVOLVED IN ADHESION-DEPENDENT CELL MIGRATION

In tissue environment, cells can use various different modes of cell migration. Common to most of these, excluding the collective migration of epithelial sheets, is that they require protrusion formation at the cell front that is coupled to movement of cell body and retraction of the tail. Cell migration can either depend on adhesion to the extracellular matrix (ECM) or be independent of adhesion. In a two-dimensional environment such as on a tissue culture plate, most cell-types display mainly adhesion-dependent migration mode. Three different actin filament structures contribute to this type of cell migration. *Lamellipodial*, branched actin filament arrays at the leading edge of the cell provide the force for generation of wide plasma membrane protrusions that drive the advancement of the leading edge. Thin *filopodial* actin filament bundles form finger-like plasma membrane protrusions that function as ‘sensory organs’ at the leading edge of cells. In addition to these actin treadmilling – dependent structures, also contractile myosin II -containing actin filament bundles, named *stress fibers*, contribute to adhesion-dependent cell migration. However, the function of stress fibers in cell migration varies depending on the cell-type, ECM density, and stiffness of the matrix (Ridley 2011; Tojkander et al. 2012).

2.1. Structure and function of lamellipodium

Lamellipodia are thin (100-160 nm), sheet-like plasma membrane protrusions at the leading edge of migrating cells. These dynamic, actin-rich structures extend for several micrometers behind the leading edge of cell. They tend to protrude and retract constantly creating membrane ruffles (Abercrombie et al. 1970a; Abercrombie et al. 1970b). Extracted lamellipodia and lamella of fish keratocytes lacking microtubules, nuclei and most other organelles are still able to undergo directional motility (Euteneuer and Schliwa 1984), indicating that these structures harbor all necessary functions required for cellular movement.

In migrating cells, lamellipodia are thought to serve as anchors for cells to move through the tissue. They can extend over long distances in front of the cell body, attach to extracellular matrix, and pull the cell body through the tissue (Giannone et al. 2007). Although cells are able to migrate also without lamellipodia (Gupton et al. 2005), these structures are crucial for persistent directional migration, indicating that lamellipodia are responsible for sensing environmental cues for migration (Wu et al. 2012; Suraneni et al. 2012).

Actin filaments form a dense, branched network in lamellipodia with more than 100 filaments in one micrometer. Filament density is highest at the distal region of the leading edge and gradually decreases towards the rear of the lamellipodium (Svitkina et al. 1997; Abraham et al. 1999). All actin filament barbed ends in lamellipodia are facing towards plasma membrane, forming brush-like structures. Practically only very few filament pointed ends are observed in the lamellipodium as they elongate from Y-shaped junctions formed by the Arp2/3 complex, which nucleates the formation of new actin filaments from the sides of pre-existing mother filaments (Mullins et al. 1998; Svitkina and Borisy 1999).

2.2. Regulation of actin dynamics in lamellipodium

The dendritic nucleation model proposes that an array of new daughter filaments is nucleated at the leading edge of cell close to the plasma membrane (Mullins et al. 1998; Pollard and Borisy 2003). On the other hand, actin monomers dissociate from filaments mainly at the proximal region of the lamellipodium through filament severing and depolymerization (Iwasa and Mullins 2007; Lai et al. 2008). The complex meshwork of branched actin filaments is under tight regulation of a large number of actin-regulatory proteins. The activities of these proteins are precisely controlled by a variety of signaling proteins, including the small GTPase Rac1, which is the master regulator of lamellipodia formation in many animal cell-types. By activating those proteins that induce the nucleation and polymerization of branched actin filament network, and inhibiting those proteins that drive filament disassembly close to the plasma membrane, Rac1 ensures the formation of proper lamellipodial branched actin filament network at the leading edge (Ridley 2015) (Fig. 1).

2.2.1. Nucleation of new filaments

The Arp2/3 complex, one of the key components in lamellipodial actin regulation, is composed of seven subunits, including two actin-related proteins, Arp2 and Arp3 (Machesky et al. 1994). It is present throughout the lamellipodium where it nucleates new actin filaments, stays associated at pointed ends of the newly formed filaments and is released only after their dissociation (Lai et al. 2008). The initiation of new branched actin filaments occurs close to the plasma membrane, where the Arp2/3 complex binds to pre-existing mother filaments and subsequently nucleates a new daughter filament. Daughter filaments elongate from the sides of mother filaments, forming characteristic 70° +/- 7° angles between the mother and daughter filaments (Mullins et al. 1998; Svitkina and Borisy 1999; Blanchoin et al. 2000). Formation of these branched filaments is critical for the generation of dendritic lamellipodial network. This has been also addressed by Arp2/3 depletion and loss-of-function

experiments, which resulted in the disappearance of lamellipodia and to loss of directional cell migration (Rogers et al. 2003; Wu et al. 2012; Suraneni et al. 2012).

The Arp2/3 complex alone is not sufficient to initiate filament formation, because it needs both pre-existing mother filaments and specific activators to be functional. Activation of the Arp2/3 complex takes place at the plasma membrane and is catalyzed by nucleation promoting factors (NPFs) such as WASP (Wiskott-Aldrich syndrome proteins) family proteins and SCAR/WAVE complexes. The activities of these NPFs are in turn controlled by Rho-family small GTPases and membrane phospho-inositides to ensure accurate spatial and temporal regulation of the Arp2/3-mediated assembly of lamellipodial actin filament meshworks (Bear et al. 1998; Machesky et al. 1999; Campellone and Welch 2010).

2.2.2. Regulation of filament elongation

There are several enhancers and suppressors controlling actin filament elongation following the Arp2/3-mediated nucleation. Heterodimeric capping protein localizes in the lamellipodia near plasma membrane where it, by binding to filament barbed ends, prevents the assembly and disassembly of actin monomers to/from filaments (Isenberg et al. 1980; Mejillano et al. 2004; Lai et al. 2008). Capping protein is vital for the Arp2/3-dependent cell motility and formation of proper lamellipodial protrusions. Depletion of capping protein in mammalian cells leads to the loss of lamellipodia and explosive formation of filopodia (Mejillano et al. 2004). Capping protein may thus limit the amount of free actin filament barbed ends, thus funneling actin monomers to a smaller number of assembly-competent barbed ends at the lamellipodium (Loisel et al. 1999).

The counterparts for capping protein constitute from a family of formins and Ena/VASP proteins (Reinhard et al. 1992; Sagot et al. 2002; Pruyne et al. 2002). These proteins protect filament barbed ends from being capped, increase the rate of filament elongation, and reduce the amount of branches (Skoble et al. 2001; Kovar et al. 2003; Romero et al. 2004; Breitsprecher et al. 2011a). Fibroblasts lacking lamellipodial Ena/VASP proteins display abnormally short and branched actin filaments, whereas Ena/VASP over-expression leads to longer and less branched filaments (Bear et al. 2002). Unlike Ena/VASP proteins, formins are also able to nucleate linear actin filaments. Formins remain associated at the barbed ends after the nucleation, walking along filaments as they elongate (Romero et al., 2004). Depletion of certain formins inhibits lamellipodial protrusions, and consequently reduce cell migration (Yang et al. 2007; Block et al. 2012).

2.2.3. Actin filament disassembly and monomer recycling

Sustained actin polymerization and formation of new protrusions at the leading edge cannot continue for a long time without regeneration of the pool of polymerization-competent actin monomers. Therefore, it is necessary that cells can rapidly disassemble actin filaments and recycle monomers for next elongation cycle. The members of ADF/cofilin protein family are the most critical regulators of actin filament disassembly in cells. Majority of ADF/cofilins

bind both monomeric and filamentous actin with a preference for ADP-containing actin subunits (Carlier et al. 1997). Importantly, ADF/cofilins promote rapid actin filament disassembly by severing actin filaments and thus increasing the number of filament pointed ends from where actin monomers can dissociate (Andrianantoandro and Pollard 2006). Depletion of ADF/cofilins indeed leads to problems in proper lamellipodium formation due to decreased filament disassembly and consequent depletion of the pool of assembly-competent actin monomers (Hotulainen et al. 2005; Kiuchi et al. 2007). Recently, it has been shown that ADF/cofilins do not work alone in cells, but that at least three proteins; cyclase-associated protein (CAP), Aip1, and coronin enhance the activity of ADF/cofilins to promote rapid disassembly of actin filaments (Chaudhry et al. 2013; Jansen et al. 2015; Gressin et al. 2015). Furthermore, even following severing by ADF/cofilins, spontaneous depolymerization of actin filaments at their pointed ends is slow compared to what is needed for efficient filament turnover in lamellipodia (Pollard 1986; Watanabe and Mitchison 2002). Twinfilin, another member of ADF-H domain protein family, is able to enhance the depolymerization rate of actin filaments when working together with CAP. Therefore, cofilin, CAP and twinfilin appear to work in concert to rapidly disassemble actin filaments in cells (Johnston et al. 2015).

In addition to proteins promoting filament severing and depolymerization, specific proteins catalyzing the dissociation of Arp2/3-stabilized filament branches evolved in eukaryotes. Most potent debranching factor is glia-maturation factor (GMF), which is a small globular protein that, similarly to ADF/cofilins, is composed of a single ADF-H domain (Gandhi et al. 2010). However, unlike ADF/cofilins, GMF does not bind or sever actin filaments, but instead binds to and catalyzes the dissociation of Arp2/3-nucleated filament branches. In migrating cells, depletion of GMF leads to decreased lamellipodial dynamics and consequent defects in directional cell migration (Poukkula et al. 2014; Haynes et al. 2015).

Newly polymerized actin filaments consist of ATP-bound actin monomers, whereas monomers dissociating from the pointed ends predominantly contain ADP in the nucleotide-binding cleft. Thus, ADP in actin monomers has to be exchanged to ATP prior to the new round of filament assembly. CAPs and profilin are considered as the main cellular factors that promote the nucleotide exchange on actin monomers. From these, especially CAP is well-suited for promoting nucleotide-exchange in cells because it binds both ADP- and ATP-actin monomers with high affinity and efficiently catalyzes nucleotide exchange on actin monomers (Mattila et al. 2004; Quintero-Monzon et al. 2009). Depletion of CAP leads to problems in lamellipodia formation and dynamics in migrating cells (Bertling et al. 2004). CAP also interacts with profilin and can potentially deliver ATP-actin monomers to this protein (Bertling et al. 2007). Profilin can promote the assembly of ATP-actin monomers to free filament barbed ends, and localize ATP-actin monomers to Ena/VASP and formin family proteins. However, profilin inhibits the spontaneous nucleation of actin filaments, and is thus well-suited to function as a gatekeeper of actin filament nucleation and assembly of actin monomers to desired actin filament arrays (Pantaloni and Carlier 1993; Rotty et al. 2015; Suarez et al. 2015).

2.3. Structure and functions of filopodia

Filopodia are thin, dynamic plasma membrane protrusions that are filled with actin filament bundles. They are considered to function as sensory organs at the leading edge of motile cells by sensing e.g. growth factors, chemokines, and extracellular matrix (Mattila and Lappalainen 2008). Filopodia are also often oriented towards the gradient of chemoattractants in migrating cells (Bentley and Toroian-Raymond 1986). In addition to motility, filopodia are involved in cell-to-cell and cell-to-matrix adhesion and maturation of neurons (Vasioukhin et al. 2000; Gallo and Letourneau 2004; Galbraith et al. 2007). Furthermore, filopodia have essential role in activation of T lymphocytes in antigen-presenting cells, and they function as phagocytic tentacles in macrophages (Al-Alwan et al. 2001; Kress et al. 2007).

The diameter of filopodia is typically approximately 0.1-0.3 µm and their length can be over 10 µm as measured from the cell cortex. However, during sea-urchin embryo gastrulation, filopodia can extend even up to 80 µm from the cell cortex (Miller et al. 1995). In contrast, filopodial-like protrusions called microspikes are hardly visible (Taylor and Robbins 1963). Thus, a huge variation exists between the morphological and dynamic parameters of different types of filopodial protrusions, and it is likely that membrane protrusions generally classified as 'filopodia' consist of several functionally different groups of thin plasma membrane protrusions with distinct assembly pathways and molecular components (Mattila and Lappalainen 2008). Here, we focus on filopodia that are present at the leading edge of migrating cells.

In the leading edge filopodia, individual actin filaments extend from cell cortex to the tip of filopodium, and they form unipolar bundles with rapidly-growing barbed ends facing towards the tip of the filopodium. The core of a filopodium typically consists of 15-30 individual filaments, which are tightly packed and arranged in parallel to each other (Small and Celis 1978; Medalia et al. 2007). At least in mammalian cells, filopodial actin filaments can be generated either from the pre-existing branched lamellipodial actin network (Svitkina et al. 2003), or through *de novo* nucleation of new actin filaments at specific foci at the plasma membrane (Small and Celis 1978; Medalia et al. 2007). The formation, morphology and dynamics of filopodia are controlled by an array of actin-binding proteins. The activities of the actin-binding proteins are in turn controlled by various signaling proteins from which the small GTPase Cdc42 functions as the master regulator of filopodia formation in many animal cells (Ridley 2015) (Fig. 2).

2.4. Actin dynamics in filopodia

Filopodia are not stable protrusive structures, but often undergo constant extension and retraction, especially in migrating cells and during neuronal growth cone path-finding (Bentley and Toroian-Raymond 1986). Balance between extension and retraction is controlled by the rate of actin filament assembly at the tips of filaments (Mallavarapu and

Mitchison 1999; Bornschlögl 2013) as well as by filament disassembly through ADF/cofilin –mediated severing at the base of filopodia (Breitsprecher et al. 2011b).

2.4.1. Nucleation and elongation of filaments

Based on electron microscopy and genetic studies (Svitkina et al. 2003; Schirenbeck et al. 2005; Medalia et al. 2007), two alternative models for filament nucleation in filopodia have been suggested. *The convergent elongation model* proposes that actin filaments in filopodia arise from lamellipodial actin network, whereas *the tip nucleation model* suggests that short individual filaments in a 'terminal cone' of filopodia act as a nucleation site for continuous filament bundles (Mattila and Lappalainen 2008; Yang and Svitkina 2011).

Depending on model, either the Arp2/3 complex or formins are the best candidates for nucleating actin filaments for filopodia. From the tip nucleation point of view, formin mDia2 is the primary candidate to nucleate, elongate and protect filament assembly in filopodia (Peng et al. 2003; Schirenbeck et al. 2005; Yang et al. 2007). Also other formins such as DAAM1 have been linked to filopodia formation (Jaiswal et al. 2013). However, defects in Arp2/3 expression, function or localization lead to significant problems in filopodial formation in cultured neurons, HeLa cells, *Caenorhabtidis elegans* and *Drosophila melanogaster* (Machesky and Insall 1998; Korobova and Svitkina 2008; Norris et al. 2009), supporting the convergent elongation model. Since cells contain different types of filopodia with distinct molecular compositions and dynamics, it is likely that both convergent elongation and tip nucleation model are relevant, and that their relative contributions for filament nucleation vary depending on the cell-type and type of filopodium.

How linear, parallel actin filaments of filopodia could then be nucleated by the Arp2/3 complex? This is possible if filament barbed ends are uncapped and protected from capping by formins or Ena/VASP proteins (Svitkina et al. 2003; Yang et al. 2007). Interestingly, heterodimeric capping protein and Ena/VASP regulate lamellipodial and filopodial modes of leading edge actin network in concert. Capping protein silencing leads to loss of lamellipodia and increased formation of filopodia, but when capping protein is depleted simultaneously with Ena/VASP, leading edge ruffles are formed (Mejillano et al. 2004). Capping protein is present also in filopodia in low levels but its role in filopodial actin network regulation remains to be elucidated (Sinnar et al. 2014).

2.4.2. Filament cross-linking proteins

In filopodia, actin filaments form tight, unipolar bundles. Bundling is necessary to overcome the resistance of the plasma membrane because individual filaments are too flexible to push the membrane forward. It has been mathematically estimated that a bundle of over 10 filaments has enough rigidity to overcome the membrane resistance and that an optimal number of filaments in a bundle is around 30 (Mogilner and Rubinstein 2005). Fascin is an actin bundling protein essential for generation of actin filament bundles in filopodia. It localizes along the entire length of filopodium, and its depletion reduces the number of

filopodia in cells. Interaction of fascin with actin filaments is dynamic, which allows efficient coordination of filament elongation and bundling during filopodia protrusion (DeRosier and Edds 1980; Vignjevic et al. 2006). In addition to fascin, also fimbrin, α-actinin and filamin are able to support actin bundling-dependent motility (Brieher et al. 2004). Interestingly, also Ena/VASP protein of *Dictyostelium discoudeum* seems to be involved in filament bundling in filopodia (Schirenbeck et al. 2006).

2.4.3. Actin - plasma membrane interactions and membrane curvature

Members of the BAR (Bin-Amphiphysis-Rvs) protein family are important regulators of membrane curvature, and can additionally link the actin cytoskeleton to the plasma membrane. N-BAR and most F-BAR domain proteins generate positive membrane curvature and induce plasma membrane invaginations, whereas the I-BAR domain proteins generate negative membrane curvature and can thus induce the formation of plasma membrane protrusions (Peter et al. 2004; Suetsugu et al. 2006; Mattila et al. 2007; Shimada et al. 2007). Interestingly, the diameter of membrane tubules induced by I-BAR domains is similar to the diameter of filopodia in cells, and over-expression of I-BAR domain proteins or their isolated I-BAR domains can induce filopodia-like protrusions at the plasma membrane (Saarikangas et al. 2009).

From the I-BAR domain proteins, the insulin receptor substrate protein of 53 kDa (IRSp53) has been intimately linked to filopodia formation. In addition to sensing or generating negative membrane curvature, IRSp53 functions as a scaffolding protein for several actin regulating proteins, such as Rho GTPases, Arp2/3 complex activator WAVE2, Ena/VASP, and actin filament bundling/capping protein Eps8. Thus, IRSp53 can either sense or generate membrane curvature at the tip of the filopodium through its membrane-binding I-BAR domain, and additionally promote actin filament assembly at this region by interacting with actin-binding proteins through its other domains (Krugmann et al. 2001; Disanza et al. 2006; Lim et al. 2008; Prévost et al. 2015).

2.5. Organization and functions of stress fibers

The stress fibers were observed over 90 years ago under plain bright-field microscope and were initially thought to be fibers that reversibly build-up in response to cytoplasmic tension or stress (Lewis and Lewis 1924). However, it is now well established that stress fibers do not form due to internal stress, but rather reflect and respond to the physical rigidity of the extracellular matrix (Riveline et al. 2001; Costa et al. 2002; Discher et al. 2005; Tojkander et al. 2015). In cell-types that experience strong external shearing forces, such as the endothelial cells of vasculature, stress fibers are distinctively prominent (Franke et al. 1984). Moreover stationary cells display thicker and more stable actomyosin bundles compared to highly motile cells to better resist and respond to the external mechanical forces or to remodel the surrounding tissue (Hinz et al. 2001; Pellegrin and Mellor 2007). The role of stress fibers in cell migration varies depending on the cell-type and the extracellular environment of the cell. In two-dimensional environment, stress fibers are believed to be involved in regulating cell

polarity and promoting retraction of the tail of migrating cell, whereas in cells migrating in a three-dimensional extracellular matrix (ECM), stress fiber–like actomyosin bundles may be more directly involved in adhesion to the matrix and advancement of the leading edge (Tojkander et al. 2012).

The core of contractile stress fibers is built up from short actin filaments that are organized in a bipolar manner. This resembles the sarcomeric units in muscles and enables the movement of bipolar myosin II filaments along the actin bundles to create contractility. However, whereas muscle myofibrils are entirely composed of bipolar arrays of actin filaments, stress fibers display often a mixed polarity and are composed of both unipolar and bipolar actin filament arrays (Sanger et al. 1983; Cramer et al. 1997; Svitkina et al. 1997).

Stress fibers are often coupled to the extracellular matrix via focal adhesions. These are large, multi-protein structures that enable cells to communicate with the extracellular matrix as they migrate (Geiger et al. 2009). Diverse family of integrins and other focal adhesion components anchor the extracellular matrix to the cytoplasmic stress fibers (Geiger and Yamada 2011). The assembly and dynamics of stress fibers are controlled both at focal adhesions as well as along the stress fiber network by a large array of actin- and myosin-binding proteins. The activities of these proteins are in turn controlled by mechanical forces and various signaling proteins, from which the RhoA small GTPase is often considered as the master regulator of stress fiber assembly (Ridley and Hall 1992) (Fig. 3).

2.5.1. Different stress fiber subtypes

Stress fibers can be generally divided into three categories: dorsal stress fibers, transverse arcs, and ventral stress fibers (Heath 1983; Small et al. 1998). *Dorsal stress fibers* are non-contractile actin bundles that associate with a focal adhesion at their distal end, and extend towards the cell center through actin polymerization at focal adhesions. Dorsal stress fibers display uniform polarity near the focal adhesion anchorage site, but appear to contain mixed polarity actin filaments towards the proximal part of the fiber (Cramer et al. 1997). Although dorsal stress fibers do not contain myosin II and are unable to contract, they can connect the other stress fiber types to focal adhesions. Dorsal stress fibers appear also important for cell migration at least in a two-dimensional tissue culture environment (Hotulainen and Lappalainen 2006; Tojkander et al. 2011; Kovac et al. 2013).

Transverse arcs are contractile, myosin II -containing actin bundles that are generated from the lamellipodial actin filament network. Transverse arcs are not directly associated with focal adhesions, but are linked to these cell - extracellular matrix contact sites through dorsal stress fibers. As the transverse arcs contract, they flow towards the cell center with a characteristic speed of ~0,3 μm/min (Hotulainen and Lappalainen 2006; Tee et al. 2015). Although transverse arcs have not been directly linked to cell migration, they are important for the formation of the flat lamellum at the leading edge of motile cells due to their association with the plasma membrane at the dorsal side of the cell (Burnette et al. 2014; Jiu et al. 2015).

Dorsal stress fibers and transverse arcs serve as precursors of *ventral stress fibers*, which are usually oriented perpendicular to the direction of migration and are connected to focal adhesions from their both ends (Small et al. 1998; Tojkander et al. 2012). Ventral stress fibers contain a sarcomeric array of bipolar myosin II and actin filament bundles, and are thus able to contract and apply tensile force to focal adhesions located at the ends of the bundle. Therefore, ventral stress fibers are important for cell adhesion, morphogenesis and mechanosensing (Tojkander et al. 2015). Ventral stress fibers are also responsible for retraction of the tail in many migrating cell-types (Kolega 2003).

2.6. Mechanisms of stress fiber assembly

Compared to the lamellipodial and filopodial actin filament arrays, assembly of contractile stress fibers is a complex process and involves many actin nucleation mechanisms and several different actin filament populations. The assembly of stress fibers is regulated mainly by Rho-family GTPases, whose activation promotes stress fiber formation/stabilization through a number of actin-binding proteins. RhoA also enhances stress fiber contractility through activation of myosin II by myosin light chain (MLC) and ROCK kinases (Heasman and Ridley 2008).

In stress fiber assembly, lamellipodial actin filaments serve as building blocks for generation of transverse arcs (Hotulainen and Lappalainen 2006). During this process, Arp2/3 complex -nucleated lamellipodial actin filaments assemble with formin-nucleated, myosin II -decorated actin filaments at the proximal end of the lamellipodium to generate transverse arcs (Hotulainen and Lappalainen 2006; Tojkander et al. 2011). Lamellipodia undergo cycles of protrusions and retractions in migrating cells. The appearance of transverse arc precursors coincides with the retraction phase, when actin filaments condense from the more criss-crossed meshwork into bundled filament precursors (Burnette et al. 2011). Additionally, material from filopodial filaments can be recycled for generating stress fibers (Nemethova et al. 2008; Anderson et al. 2008). Dorsal stress fibers, on the other hand, are nucleated and elongated by different formins (including at least Dia1 and INF2) and Ena/VASP family proteins at focal adhesions (Hotulainen and Lappalainen 2006; Gateva et al. 2014; Skau et al. 2015; Tojkander et al. 2015). As the dorsal stress fibers elongate, they associate with transverse arcs through a mechanism that is currently unknown. Thus, dorsal fibers and transverse arcs create an entwined network in the lamellum, where individual arcs can be connected to several different dorsal stress fibers (Tojkander et al. 2011). Ventral stress fibers can be subsequently generated from the network of dorsal stress fibers and transverse arcs through a complex mechanosensitive process if the cell is on a rigid matrix of if external forces are applied to the cell (Tojkander et al. 2015). Finally, it is important to note that stress fibers are dynamic structures that undergo continuous assembly and disassembly as well as organization of the network (Tojkander et al. 2012).

2.6.1. Actin filament cross-linking proteins in stress fibers

To date over 20 different protein classes have been reported to possess actin cross-linking activity. Most actin cross-linking proteins exist either as dimers or contain two actin binding domains. α-actinin and fascin are the most well-characterized, abundant cross-linking proteins of the contractile fibers. Homodimeric α-actinin links adjacent actin fibers through a bivalent binding mechanism with its two actin binding sites (Puius et al. 1998; Edlund et al. 2001; Türmer et al. 2015). Monomeric fascin links actin filaments in much more compact manner, forming tightly packed parallel actin bundles that have been recently shown to be important in the termini of mature stress fibers, close to focal adhesions (Otto et al. 1979; Elkhatib et al. 2014). Co-operative crosslinking by α-actinin and fascin was shown to produce more elastic bundles than either of the two proteins could generate individually (Tseng et al. 2001). α-actinin crosslinks align periodically in transverse arcs and ventral fibers, interposing with the two other central components of the contractile fibers, myosin II and tropomyosin, which also display a periodical localization pattern along stress fibers. Thus, the two types of contractile stress fibers display similar periodic α-actinin – myosin II –pattern that is characteristic to muscle myofibrils (Tojkander et al. 2012).

2.6.2. Myosins and tropomyosins

Tropomyosins and myosin II bundles are important components of stress fiber function and contractility. Class II myosins are the major contractile proteins of the cardiac- and skeletal muscle tissues, but have specific isoforms also in the non-muscle cells that associate with stress fibers (Vicente-Manzanares et al. 2009). Mammalian non-muscle cells are able to utilize up to three different non-muscle myosin II (NMII) isoforms (myosin IIA, IIB and IIC) each displaying distinct characteristics in terms of catalytical activity, localization and role in various cellular functions (Katsuragawa et al. 1989; Kawamoto and Adelstein 1991; Golomb et al. 2004). Recent work demonstrated these isoforms are also able to co-assemble with each other into bipolar bundles in different stress fiber subtypes (Beach et al. 2014; Shutova et al. 2014). In a polarized cell, NMIIA and NMIIB are both central components of stress fibers, but are however enriched in the peripheral lamella and posterior contractile units, respectively (Maupin et al. 1994; Sandquist and Means 2008). The differential distribution reflects differences in the three isoforms regarding their rate of hydrolyzing ATP. NMIIB motor domain is able to hold the tension longer via its high affinity for ADP. Long upkeep of tension is well suited for more stable contractile fibers in the cortical and posterior part of the cell (Wang et al. 2000). In the more rapidly progressing cell front NMIIA, with markedly higher ATP hydrolysis rate and lower affinity for ADP, is able to exert tension more rapidly e.g. in response to extracellular signals (Wang et al. 2003).

Tropomyosins are a family of actin-binding proteins that function as coiled-coil dimers forming continuous polymers located in the grooves of filamentous actin. Canonical mechanism of function for tropomyosins is to regulate skeletal muscle contraction by steric blocking of the myosin binding to actin filaments prior to Ca^{2+} influx (Parry and Squire

1973). Tropomyosins also efficiently stabilize actin filaments and may functionally specify different actin filament populations (Hitchcock-DeGregori et al. 1988; Broschat et al. 1989; Gunning et al. 2015). Over 40 different tropomyosins can be generated by alternative splicing from four mammalian *tropomyosin* genes (Gunning et al. 2008). Interestingly, recent studies revealed that in migrating osteosarcoma cells, at least five functionally non-redundant tropomyosin isoforms localize to stress fibers and are important for their assembly and/or stability. Furthermore, some tropomyosin isoforms appear to be essential for myosin II -recruitment to stress fibers (Tojkander et al. 2011). Thus, tropomyosins are central components of stress fibers, where they seem to specify functionally distinct actin filament populations and recruit myosin II molecules to stress fiber precursors.

3. CELL MIGRATION IN A THREE-DIMENSIONAL ENVIRONMENT

In tissues of multicellular animals, cells migrate predominantly in a complex three-dimensional environment. Here the cells can crawl on or through extracellular matrix as well as migrate on top of each other. In some cases, such as during transcellular migration of leukocytes from blood vessels to the underlying tissues, cells can even move through other cells (Ridley 2011; Muller 2015). In tissue environment, migration of cells can also be guided by variable biochemical or mechanical signals. Chemical signals include growth factor gradients that can either attract or repel migrating cells during developmental processes and angiogenesis as well as chemokine-induced migration of lymphocytes. On the other hand, fibroblasts can read the mechanical properties of the environment and move towards the regions of highest substrate stiffness in a process called durotaxis (Majumdar et al. 2014; Haeger et al. 2015).

Compared to migration on a two-dimensional tissue culture plate, cells in a three-dimensional tissue environment display more variation in their migration modes. The most widely studied migration types are *mesenchymal* and *amoeboid* migration modes. In mesenchymal, adhesion-dependent migration mode, the cells moving along ECM are typically elongated and employ similar lamellipodial and filopodial structures for movement as used in a two-dimensional environment. In contrast, *amoeboid* migration does not require formation of adhesions or lamellipodial-like protrusive structures, but is instead employing myosin-dependent contractile actin cortex to form membrane blebs towards the direction of migration. Importantly, same cell can switch between different types of migration modes, depending on mechanical and chemical properties of the environment (Petrie and Yamada 2012; Paluch and Raz 2013). Cell migration in a three-dimensional tissue environment also employs other specific actin-dependent processes, such as invadopodia and podosomes, which are involved in matrix degradation to enable cells to move through the dense extracellular matrix (Linder and Wiesner 2015).

3.1. Podosomes and invadopodia

Cell movement in two-dimensional matrices is limited to planar level, whereas in a tissue environment all three dimensions are available. In three-dimensional environment, cells

however, need to often degrade the extracellular matrix in order to crawl. Special structures termed podosomes and invadopodia can be applied to degrade extracellular matrix during mesenchymal migration in a three-dimensional environment (Hoshino et al. 2013). Both structures initially appear as punctae on the plasma membrane, and utilize polymerization of actin filaments to breach substratum perpendicularly. Podosome architecture consists of an actin core where the Arp2/3-initiated branched actin network is surrounded by myosin II -containing unbranched actin filaments (Kaverina et al. 2003; Osiak et al. 2005; Bhuwania et al. 2012). Third structural unit in podosomes is clusters of focal adhesion -associated proteins such as paxillin or vinculin surrounding the actin core (Cox et al. 2012; van den Dries et al. 2013). Also integrins co-localize with maturing podosomal structures to establish linkage between the cell and extracellular matrix (Pfaff and Jurdic 2001).

In contrast to column -like podosomes, invadopodia are extended membrane protrusions invading into the extracellular matrix (Linder et al. 2011). Podosomal structures display rapid actin dynamics and significantly shorter life span with a typical turnover time of one minute, whereas invadopodia are able to persist over an hour (Destaing et al. 2003; Li et al. 2010). The tip of invadopodium inhabits parallely aligned actin filament bundles that precede branched actin meshwork on the bottom of the invadopodial structures. Arp2/3 and several filament-polymerizing formins are important for establishing nascent invadopodia that form mature structures after recruitment of matrix metalloproteases (Lizárraga et al. 2009; Wiesner et al. 2010). Interestingly invadopodia do not appear to extensively recruit integrins and it is still unknown whether they have adhesive contacts with the extracellular matrix (Mueller and Chen 1991; Deryugina et al. 2001).

3.2. Blebbing based cell migration

Many animal cells contain a contractile cell cortex under their plasma membrane. Although not considered to be a subtype of the stress fibers, it similarly constitutes from myosin II decorated actin filaments with mixed or random polarity, accompanied with other actin binding proteins (Bray and White 1988; Charras et al. 2006). Similarly to stress fibers, the formation of actin cortex requites both formins (Dia1) and the Arp2/3 complex (Bovellan et al. 2014). The cortical actin filament network is linked to the plasma membrane at least through ezrin-radixin-moesin (ERM) family proteins (Charras et al. 2006).

Contractile actin cortex has an important role in amoeboid cell migration, where propulsive blebs drive the cell migration, and connection points with the extracellular matrix are not utilized (Wolf et al. 2003; Pinner and Sahai 2008). Especially cancer cells are often highly contractile, making them prone to blebbing (Bergert et al. 2012). Furthermore, at least melanoma and breast cancer cells migrate by blebbing mouse tissues, suggesting that this is an important migration mode of many cancer cell-types *in vivo* (Tozluoğlu et al. 2013).

A bleb forms when actin cortex detaches from the plasma membrane or when the cortex itself is disrupted due to build-up of pressure and tension inside the cell caused by actomyosin contraction. Due to contraction of the remaining actomyosin cortex, cytosol flows into the

newly-formed bleb lacking the cortex. Actin filaments then begin to reform under the membrane in the bleb. This is followed by a retraction phase that is driven by myosin II-promoted contraction of the newly formed actomyosin cortex underlying the plasma membrane of the bleb (Cunningham 1995; Paluch et al. 2005; Charras et al. 2006; Charras and Paluch 2008). (Fig. 4). Cells confined in a non-adhesive environment often migrate in the direction of a very large 'leader bleb', whose formation requires the presence of actin filament bundling protein Eps8 (Logue et al. 2015).

Three distinct models for the role of blebbing in cell migration have been presented. The first model proposes that blebbing plays a critical role in forward migration by transporting the cytoplasm and thereby moving the center point of mass of the cell forward. Actin is linked to the cell-cell contacts formed by E-cadherins, which anchor the migrating cell to the extracellular matrix (Kardash et al. 2010). "The chimneying" model, named after rock climbing technique, proposes a migration mechanism in the total absence of adhesion with the environment. Either actin polymerization against the sides of a cell or backwards flow of cell cortex could generate force that is strong enough allowing cell to move forward. In either case, movement is supported by specific or nonspecific friction between cell and the substrate (Lämmermann et al. 2008; Hawkins et al. 2009; Poincloux et al. 2011). Finally, "The swimming model" suggests that cells could use blebbing in a swimming-fashion, where the cell body translocates after cell shape changes during blebbing (Lim et al. 2013).

4. FUTURE PERSPECTIVES

Cell migration requires several different actin-based structures and is finely tuned by large amount of regulatory proteins. Although actin dynamics and its role in cell migration have been intensively studied over several decades, many unanswered questions concerning the mechanisms underlying cell migration still remain. For example, although the principles of actin filament assembly and disassembly in lamellipodia are beginning to be relatively well established, we still do not have a complete picture about how filopodia and contractile actomyosin bundles, such as stress fibers, are assembled in cells. Furthermore, cell migration is not only controlled by chemical cues, but mechanical environment of the cell is also important in regulating the assembly and turnover of actin filament structures involved in cell migration. However, the principles by which different actin filament arrays respond to mechanical cues remain largely elusive. Finally, the organization, dynamics and regulation of actin filament structures in cells migrating in a three-dimensional tissue environment are poorly understood. However, rapid evolution of light and electron microscopy methods, together with the revolution of CRISPR/Cas9-based genome editing approaches, have now made it more feasible to examine actin dynamics also in normal as well as in cancer cells migrating in their 'native' three-dimensional tissue environment.

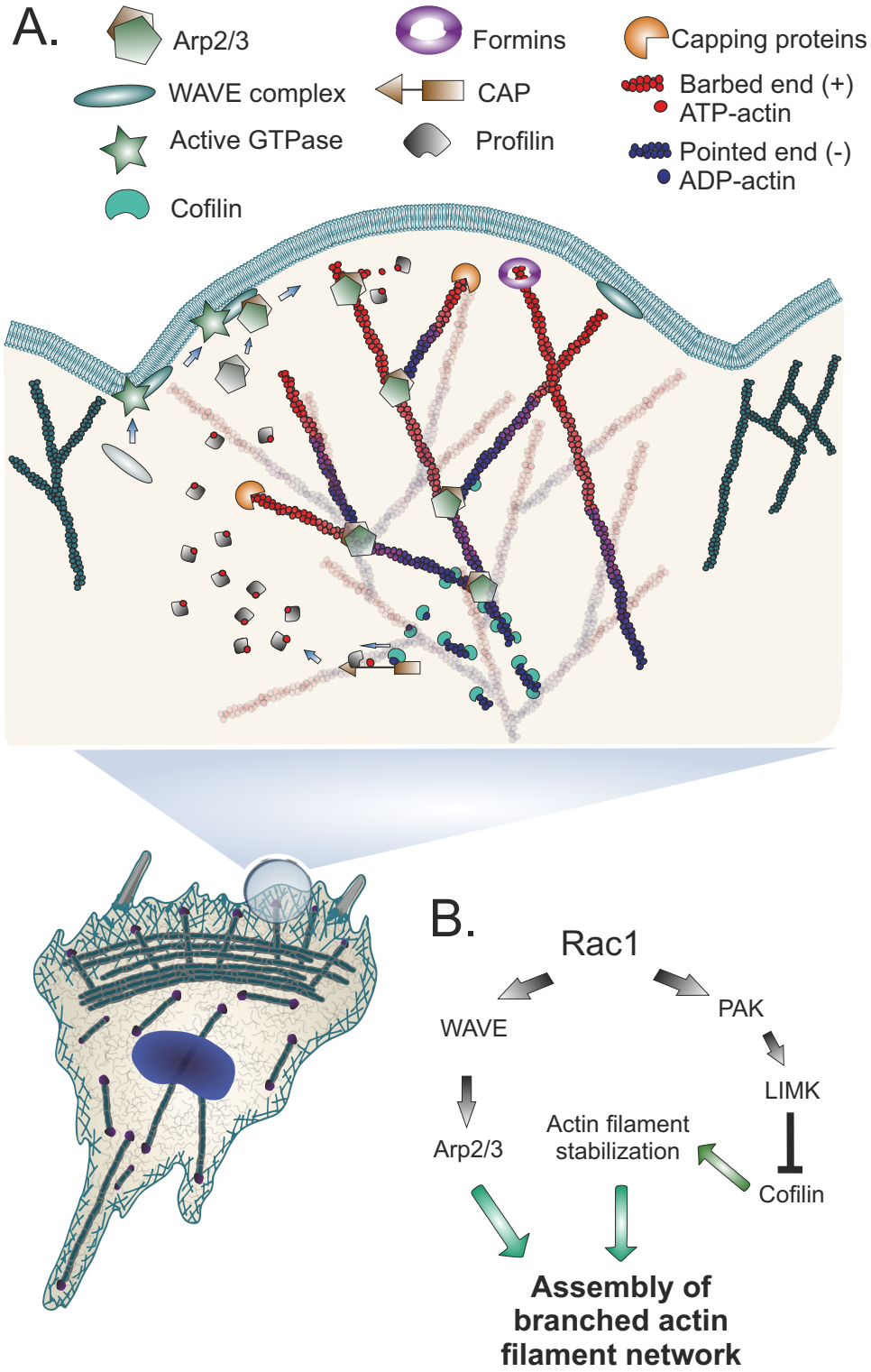

Figure. 1. Organization and assembly of the lamellipodial actin filament network. (A). Lamellipodium at the leading edge of a cell consists of a branched actin filament network, which is nucleated by the Arp2/3 complex. The rapidly growing actin filament barbed ends face the plasma membrane and their elongation generates the force for advancement of the leading edge. In addition to Arp2/3 complex, certain formins contribute to lamellipodial actin filament nucleation. Actin filament assembly and disassembly as well as monomer recycling in lamellipodium are controlled by a large array of actin-binding proteins including capping protein, cofilin, profilin, and cyclase-associated protein (CAP). **(B)**. Small GTPase Rac1 is the master regulator of lamellipodium assembly in many cell-types. It controls actin filament assembly by activating Arp2/3 through the WAVE complex, and prevents actin filament disassembly close to the plasma membrane by inhibiting cofilin through PAK and LIMK.

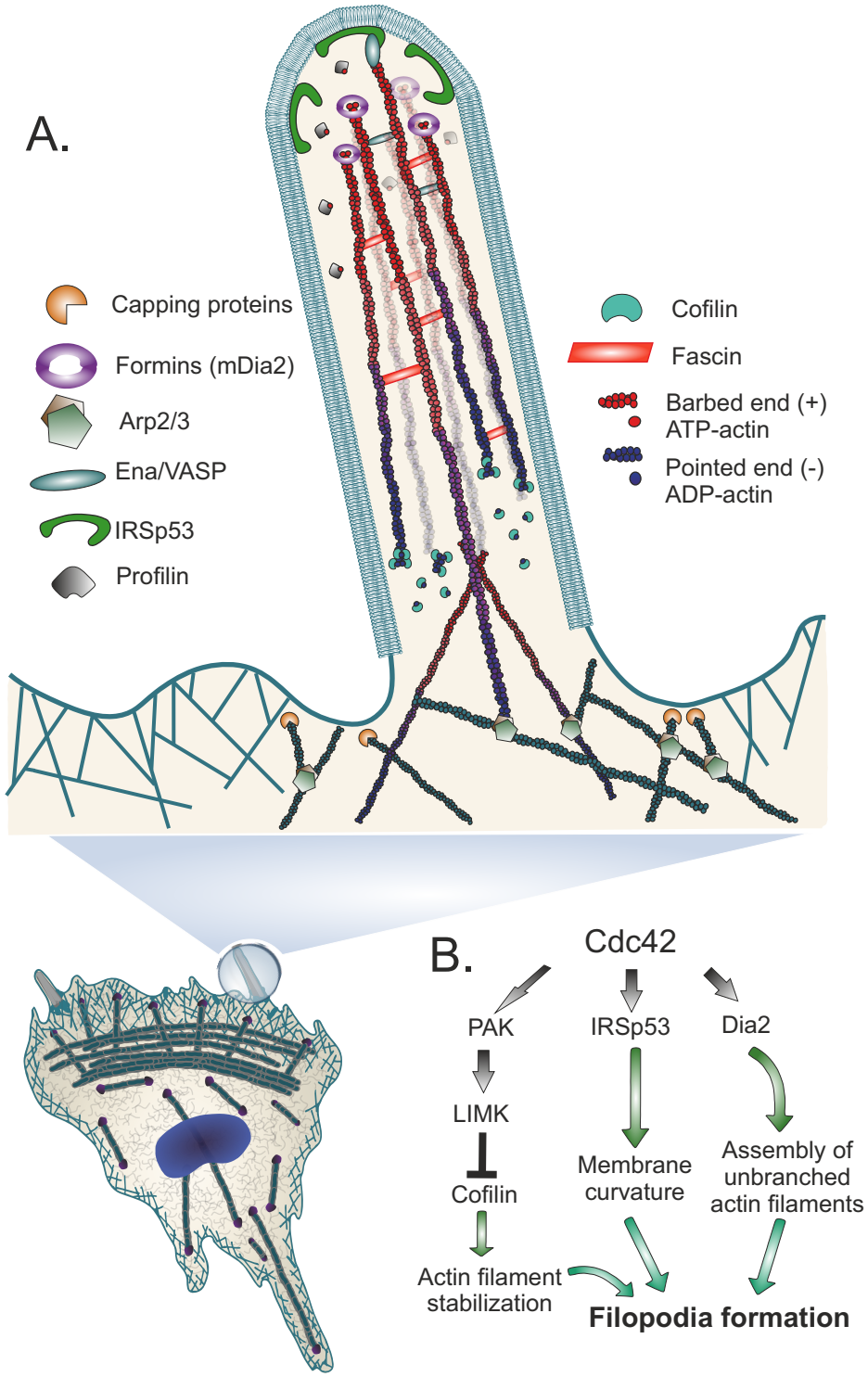

Figure 2. Organization and architecture of filopodial actin filament arrays. (A). Filopodium contains a bundle of unipolar actin filaments with their rapidly growing barbed ends facing the tip of filopodium. Fascin is the main actin filament cross-linking/bundling protein in filopodia. Actin filaments of filopodia can be either nucleated de novo by formins or generated from the pre-existing lamellipodial Arp2/3-nucleated network through Ena/VASP -mediated filament elongation and protection of filament barbed ends from capping protein. Ena/VASP also interacts with the I-BAR domain protein IRSp53, which senses or generates negative membrane curvature at the tip of filopodium. Cofilin -promoted filament severing is inhibited towards the tip of filopodum to stabilize the actin filament bundle. **(B)**. Small GTPase Cdc42 is the master regulator of filopodial actin filament dynamics in many cell-types. Cdc42 activates Dia2 formin to promote actin filament assembly, inactivates cofilin through LIMK and PAK kinases to inhibit actin filament disassembly, and interacts with IRSp53, which generates membrane curvature and functions as a scaffolding protein for other regulators of actin dynamics.

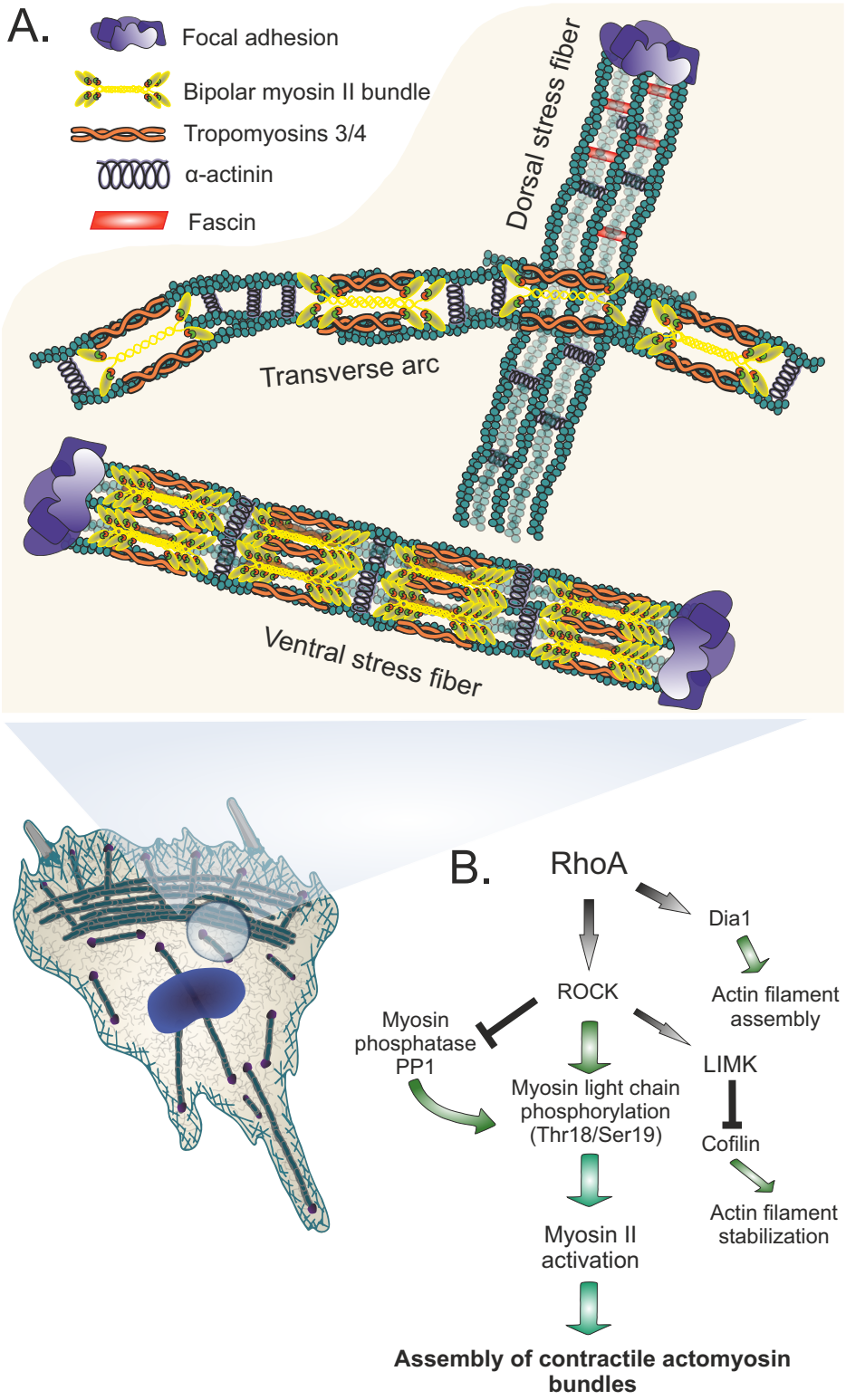

Figure 3. Assembly and organization of actin stress fibers. (A). Stress fibers are thick bundles of actin filaments, which are decorated by tropomyosins and cross-linked mainly by a-actinin. Stress fibers can be further divided into three different categories. Dorsal stress fibers are non-contractile actin filament bundles that associate with, and elongate through, actin polymerization at focal adhesions located at their distal ends. Transverse arcs are myosin II -containing contractile actin bundles that associate with dorsal stress fibers, but are not directly linked to focal adhesions. Transverse arcs are generated from the lamellipodial actin filament arrays. Ventral stress fibers are contractile, myosin II –containing actin filament bundles that can be generated from the pre-existing network of dorsal stress fibers and transverse arcs. Ventral stress fibers communicate with the extracellular matrix through focal adhesions located at their both ends. **(B)**. RhoA small GTPase is the master regulator stress fiber assembly and contractility in many animal cell-types. RhoA promotes actin filament assembly through Dia1 formin and inhibits actin filament disassembly through ROCK and LIMK kinase –mediated inactivation of cofilin. Additionally, RhoA promotes myosin II activation through ROCK kinase.

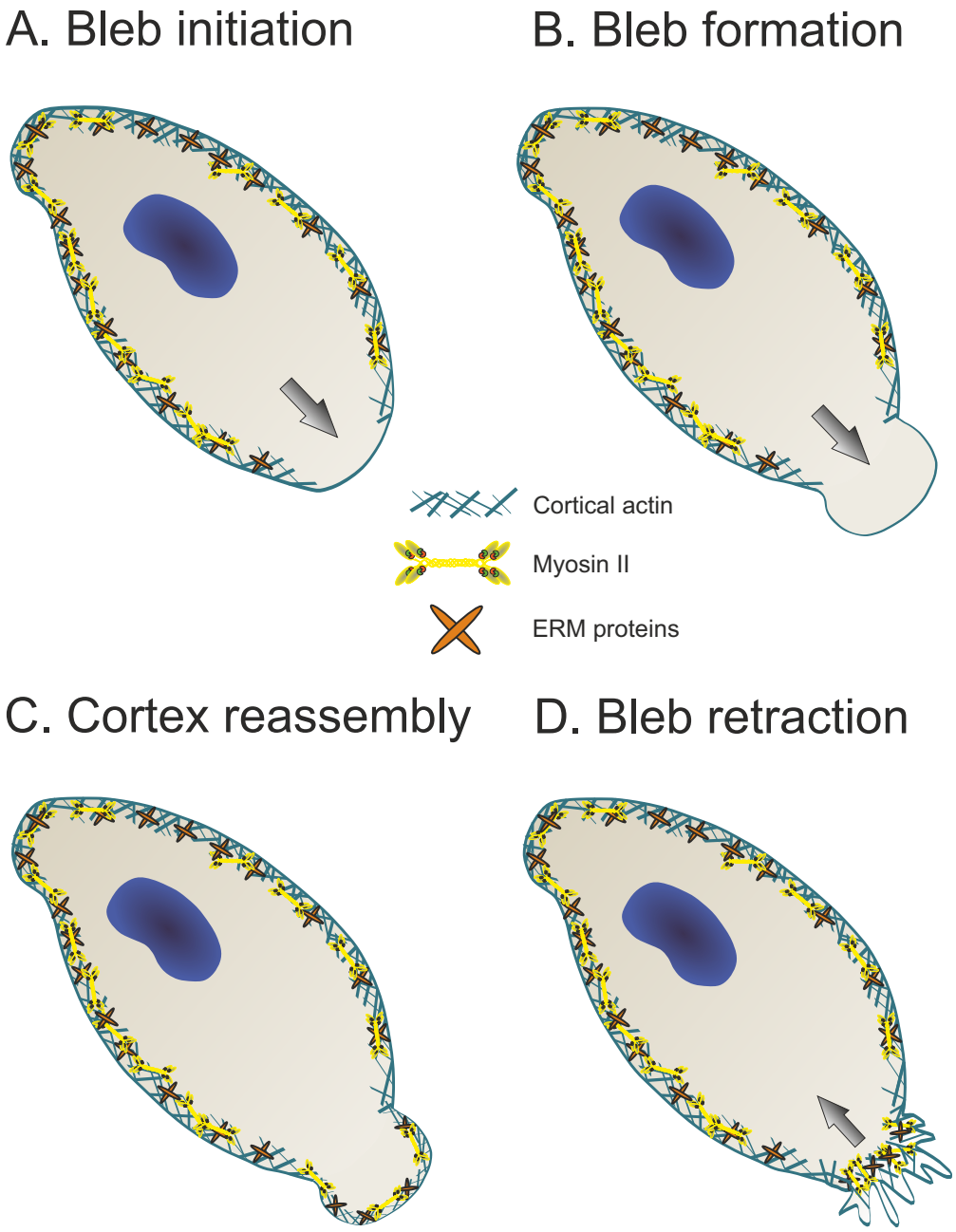

Figure 4. The life cycle of a bleb. (A). Many cells contain an actomyosin cortex, which can be associated to the plasma membrane via ezrin/radixin/moesin (ERM) proteins. The initiation of a bleb can result from a local disassembly of the actomyosin cortex. **(B)**. Hydrostatic pressure of the cytoplasm drives the expansion of the bleb. **(C)**. New actin cortex assembles to the bleb via Arp2/3 and formin -mediated actin filament nucleation, and subsequent recruitment of myosin II. **(D)**. Assembly of actomyosin cortex is followed by myosin II -driven bleb retraction (modified from Charras and Paluch, 2008).

5. REFERENCES

Abercrombie M, Heaysman JEM, Pegrum SM (1970a) The locomotion of fibroblasts in culture I. Movements of the leading edge. Exp Cell Res 59:393–398. doi: 10.1016/0014-4827(70)90646-4

Abercrombie M, Heaysman JEM, Pegrum SM (1970b) The locomotion of fibroblasts in culture. II. "Ruffling." Exp Cell Res 60:437–444. doi: 10.1016/0014-4827(70)90537-9

Abraham VC, Krishnamurthi V, Taylor DL, Lanni F (1999) The actin-based nanomachine at the leading edge of migrating cells. Biophys J 77:1721–32. doi: 10.1016/S0006-3495(99)77018-9

Al-Alwan MM, Rowden G, Lee TD, West KA (2001) Fascin is involved in the antigen presentation activity of mature dendritic cells. J Immunol 166:338–45. doi:10.4049/jimmunol.166.1.338

Anderson TW, Vaughan AN, Cramer LP (2008) Retrograde flow and myosin II activity within the leading cell edge deliver F-actin to the lamella to seed the formation of graded polarity actomyosin II filament bundles in migrating fibroblasts. Mol Biol Cell 19:5006–18. doi: 10.1091/mbc.E08-01-0034

Andrianantoandro E, Pollard TD (2006) Mechanism of actin filament turnover by severing and nucleation at different concentrations of ADF/cofilin. Mol Cell 24:13–23. doi: 10.1016/j.molcel.2006.08.006

Beach JR, Shao L, Remmert K, et al (2014) Nonmuscle myosin II isoforms coassemble in living cells. Curr Biol 24:1160–6. doi: 10.1016/j.cub.2014.03.071

Bear JE, Rawls JF, Saxe CL (1998) SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late Dictyostelium development. J Cell Biol 142:1325–1335. doi: 10.1083/jcb.142.5.1325

Bear JE, Svitkina TM, Krause M, et al (2002) Antagonism between Ena/VASP Proteins and Actin Filament Capping Regulates Fibroblast Motility. Cell 109:509–521. doi: 10.1016/S0092-8674(02)00731-6

Bentley D, Toroian-Raymond A (1986) Disoriented pathfinding by pioneer neurone growth cones deprived of filopodia by cytochalasin treatment. Nature 323:712–5. doi: 10.1038/323712a0

Bergert M, Chandradoss SD, Desai RA, Paluch E (2012) Cell mechanics control rapid transitions between blebs and lamellipodia during migration. Proc Natl Acad Sci U S A 109:14434–9. doi: 10.1073/pnas.1207968109

Bertling E, Hotulainen P, Mattila PK, et al (2004) Cyclase-associated protein 1 (CAP1) promotes cofilin-induced actin dynamics in mammalian nonmuscle cells. Mol Biol Cell 15:2324–34. doi: 10.1091/mbc.E04-01-0048

Bertling E, Quintero-Monzon O, Mattila PK, et al (2007) Mechanism and biological role of profilin-Srv2/CAP interaction. J Cell Sci 120:1225–34. doi: 10.1242/jcs.000158

Bhuwania R, Cornfine S, Fang Z, et al (2012) Supervillin couples myosin-dependent contractility to podosomes and enables their turnover. J Cell Sci 125:2300–14. doi: 10.1242/jcs.100032

Blanchoin L, Amann KJ, Higgs HN, et al (2000) Direct observation of dendritic actin filament networks nucleated by Arp2/3 complex and WASP/Scar proteins. Nature 404:1007–11. doi: 10.1038/35010008

Block J, Breitsprecher D, Kühn S, et al (2012) FMNL2 drives actin-based protrusion and migration downstream of Cdc42. Curr Biol 22:1005–12. doi: 10.1016/j.cub.2012.03.064

Bornschlögl T (2013) How filopodia pull: what we know about the mechanics and dynamics of filopodia. Cytoskeleton (Hoboken) 70:590–603. doi: 10.1002/cm.21130

Bovellan M, Romeo Y, Biro M, et al (2014) Cellular control of cortical actin nucleation. Curr Biol 24:1628–35. doi: 10.1016/j.cub.2014.05.069

Bray D, White J (1988) Cortical flow in animal cells. Science 239:883–888. doi: 10.1126/science.3277283

Breitsprecher D, Kiesewetter AK, Linkner J, et al (2011a) Molecular mechanism of Ena/VASP-mediated actin-filament elongation. EMBO J 30:456–67. doi: 10.1038/emboj.2010.348

Breitsprecher D, Koestler SA, Chizhov I, et al (2011b) Cofilin cooperates with fascin to disassemble filopodial actin filaments. J Cell Sci 124:3305–18. doi: 10.1242/jcs.086934

Brieher WM, Coughlin M, Mitchison TJ (2004) Fascin-mediated propulsion of Listeria monocytogenes independent of frequent nucleation by the Arp2/3 complex. J Cell Biol 165:233–42. doi: 10.1083/jcb.200311040

Broschat KO, Weber A, Burgess DR (1989) Tropomyosin stabilizes the pointed end of actin filaments by slowing depolymerization. Biochemistry 28:8501–6. doi: 10.1021/bi00447a035

Burnette DT, Manley S, Sengupta P, et al (2011) A role for actin arcs in the leading-edge advance of migrating cells. Nat Cell Biol 13:371–81. doi: 10.1038/ncb2205

Burnette DT, Shao L, Ott C, et al (2014) A contractile and counterbalancing adhesion system controls the 3D shape of crawling cells. J Cell Biol 205:83–96. doi: 10.1083/jcb.201311104

Campellone KG, Welch MD (2010) A nucleator arms race: cellular control of actin assembly. Nat Rev Mol Cell Biol 11:237–51. doi: 10.1038/nrm2867

Carlier MF, Laurent V, Santolini J, et al (1997) Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. J Cell Biol

136:1307–22. doi:10.1083/jcb.136.6.1307

Charras G, Paluch E (2008) Blebs lead the way: how to migrate without lamellipodia. Nat Rev Mol Cell Biol 9:730–6. doi: 10.1038/nrm2453

Charras GT, Hu C-KC-K, Coughlin M, Mitchison TJ (2006) Reassembly of contractile actin cortex in cell blebs. J Cell Biol 175:477–90. doi: 10.1083/jcb.200602085

Chaudhry F, Breitsprecher D, et al (2013) Srv2/cyclase-associated protein forms hexameric shurikens that directly catalyze actin filament severing by cofilin. Mol Biol Cell 24:31-41. doi: 10.1091/mbc.E12-08-0589.

Costa KD, Hucker WJ, Yin FCP (2002) Buckling of actin stress fibers: A new wrinkle in the cytoskeletal tapestry. Cell Motil Cytoskeleton 52:266–274. doi: 10.1002/cm.10056

Cox S, Rosten E, Monypenny J, et al (2012) Bayesian localization microscopy reveals nanoscale podosome dynamics. Nat Methods 9:195–200. doi: 10.1038/nmeth.1812

Cramer LP, Siebert M, Mitchison TJ (1997) Identification of novel graded polarity actin filament bundles in locomoting heart fibroblasts: Implications for the generation of motile force. J Cell Biol 136:1287–1305. doi: 10.1083/jcb.136.6.1287

Cunningham CC (1995) Actin polymerization and intracellular solvent flow in cell surface blebbing. J Cell Biol 129:1589–99. doi: 0021-9525/95/06/1589/11

DeRosier DJ, Edds KT (1980) Evidence for fascin cross-links between the actin filaments in coelomocyte filopodia. Exp Cell Res 126:490–494. doi: 10.1016/0014-4827(80)90295-5

Deryugina EI, Ratnikov B, Monosov E, et al (2001) MT1-MMP initiates activation of pro-MMP-2 and integrin alphavbeta3 promotes maturation of MMP-2 in breast carcinoma cells. Exp Cell Res 263:209–23. doi: 10.1006/excr.2000.5118

Destaing O, Saltel F, Géminard J-C, et al (2003) Podosomes display actin turnover and dynamic self-organization in osteoclasts expressing actin-green fluorescent protein. Mol Biol Cell 14:407–16. doi: 10.1091/mbc.E02-07-0389

Disanza A, Mantoani S, Hertzog M, et al (2006) Regulation of cell shape by Cdc42 is mediated by the synergic actin-bundling activity of the Eps8-IRSp53 complex. Nat Cell Biol 8:1337–47. doi: 10.1038/ncb1502

Discher DE, Janmey P, Wang Y-L (2005) Tissue cells feel and respond to the stiffness of their substrate. Science 310:1139–1143. doi: 10.1126/science.1116995

Doyle AD, Petrie RJ, Kutys ML, Yamada KM (2013) Dimensions in cell migration. Curr Opin Cell Biol 25:642–9. doi: 10.1016/j.ceb.2013.06.004

Edlund M, Lotano MA, Otey CA (2001) Dynamics of alpha-actinin in focal adhesions and stress fibers visualized with alpha-actinin-green fluorescent protein. Cell Motil Cytoskeleton 48:190–200. doi: 10.1002/1097-0169(200103)48:3<190::AID-CM1008>3.0.CO;2-C

Elkhatib N, Neu MB, Zensen C, et al (2014) Fascin plays a role in stress fiber organization and focal adhesion disassembly. Curr Biol 24:1492–9. doi: 10.1016/j.cub.2014.05.023

Euteneuer U, Schliwa M (1984) Persistent, directional motility of cells and cytoplasmic fragments in the absence of microtubules. Nature 310:58–61. doi: 10.1038/310058a0

Franke RP, Gräfe M, Schnittler H, et al (1984) Induction of human vascular endothelial stress fibres by fluid shear stress. Nature 307:648–9. doi: 10.1038/307648a0

Friedl P, Alexander S (2011) Cancer invasion and the microenvironment: plasticity and reciprocity. Cell 147:992–1009. doi: 10.1016/j.cell.2011.11.016

Galbraith CG, Yamada KM, Galbraith JA (2007) Polymerizing actin fibers position integrins primed to probe for adhesion sites. Science 315:992–5. doi: 10.1126/science.1137904

Gallo G, Letourneau PC (2004) Regulation of growth cone actin filaments by guidance cues. J Neurobiol 58:92–102. doi: 10.1002/neu.10282

Gandhi M, Smith BA, Bovellan M, et al (2010) GMF is a cofilin homolog that binds Arp2/3 complex to stimulate filament debranching and inhibit actin nucleation. Curr Biol 20:861–7. doi: 10.1016/j.cub.2010.03.026

Gateva G, Tojkander S, Koho S, et al (2014) Palladin promotes assembly of non-contractile dorsal stress fibers through VASP recruitment. J Cell Sci 127:1887–98. doi: 10.1242/jcs.135780

Geiger B, Spatz JP, Bershadsky AD (2009) Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol 10:21–33. doi: 10.1038/nrm2593

Geiger B, Yamada KM (2011) Molecular architecture and function of matrix adhesions. Cold Spring Harb Perspect Biol 3:a005033. doi: 10.1101/cshperspect.a005033

Giannone G, Dubin-Thaler BJ, Rossier O, et al (2007) Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell 128:561–75. doi: 10.1016/j.cell.2006.12.039

Golomb E, Ma X, Jana SS, et al (2004) Identification and characterization of nonmuscle myosin II-C, a new member of the myosin II family. J Biol Chem 279:2800–8. doi: 10.1074/jbc.M309981200

Gressin L, Guillotin A, Guérin C, et al (2015) Architecture dependence of actin filament network disassembly. Curr Biol 25:1437–47. doi: 10.1016/j.cub.2015.04.011

Gunning P, O'Neill G, Hardeman E (2008) Tropomyosin-based regulation of the actin cytoskeleton in time and space. Physiol Rev 88:1–35. doi: 10.1152/physrev.00001.2007

Gunning PW, Hardeman EC, Lappalainen P, Mulvihill DP (2015) Tropomyosin - master regulator of actin filament function in the cytoskeleton. J Cell Sci 128:2965–74. doi: 10.1242/jcs.172502

Gupton SL, Anderson KL, Kole TP, et al (2005) Cell migration without a lamellipodium: translation of actin dynamics into cell movement mediated by tropomyosin. J Cell Biol 168:619–31. doi: 10.1083/jcb.200406063

Haeger A, Wolf K, Zegers MM, Friedl P (2015) Collective cell migration: guidance principles and hierarchies. Trends Cell Biol 25:556–566. doi: 10.1016/j.tcb.2015.06.003

Hawkins RJ, Piel M, Faure-Andre G, et al (2009) Pushing off the Walls: A Mechanism of Cell Motility in Confinement. Phys Rev Lett 102:058103. doi: 10.1103/PhysRevLett.102.058103

Haynes EM, Asokan SB, King SJ, et al (2015) GMFβ controls branched actin content and lamellipodial retraction in fibroblasts. J Cell Biol 209:803–812. doi: 10.1083/jcb.201501094

Heasman SJ, Ridley AJ (2008) Mammalian Rho GTPases: new insights into their functions from in vivo studies. Nat Rev Mol Cell Biol 9:690–701. doi: 10.1038/nrm2476

Heath JP (1983) Behaviour and structure of the leading lamella in moving fibroblasts. I. Occurrence and centripetal movement of arc-shaped microfilament bundles beneath the dorsal cell surface. J Cell Sci 60:331–354. doi: 10.1016/0309-1651(81)90079-5

Hinz B, Mastrangelo D, Iselin CE, et al (2001) Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol 159:1009–20. doi: 10.1016/S0002-9440(10)61776-2

Hitchcock-DeGregori SE, Sampath P, Pollard TD (1988) Tropomyosin inhibits the rate of actin polymerization by stabilizing actin filaments. Biochemistry 27:9182–5. doi: 10.1021/bi00426a016

Hoshino D, Branch KM, Weaver AM (2013) Signaling inputs to invadopodia and podosomes. J Cell Sci 126:2979–89. doi: 10.1242/jcs.079475

Hotulainen P, Lappalainen P (2006) Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. J Cell Biol 173:383–394. doi: 10.1083/jcb.200511093

Hotulainen P, Paunola E, Vartiainen MK, Lappalainen P (2005) Actin-depolymerizing factor and cofilin-1 play overlapping roles in promoting rapid F-actin depolymerization in mammalian nonmuscle cells. Mol Biol Cell 16:649–64. doi: 10.1091/mbc.E04-07-0555

Isenberg G, Aebi U, Pollard TD (1980) An actin-binding protein from Acanthamoeba regulates actin filament polymerization and interactions. Nature 288:455–9. doi: 10.1038/288455a0

Iwasa JH, Mullins RD (2007) Spatial and temporal relationships between actin-filament nucleation, capping, and disassembly. Curr Biol 17:395–406. doi: 10.1016/j.cub.2007.02.012

Jaiswal R, Breitsprecher D, Collins A, et al (2013) The formin Daam1 and fascin directly

collaborate to promote filopodia formation. Curr Biol 23:1373–9. doi: 10.1016/j.cub.2013.06.013

Jansen S, Collins A, Chin SM, et al (2015) Single-molecule imaging of a three-component ordered actin disassembly mechanism. Nat Commun 6:7202. doi: 10.1038/ncomms8202

Jiu Y, Lehtimäki J, Tojkander S, et al (2015) Bidirectional Interplay between Vimentin Intermediate Filaments and Contractile Actin Stress Fibers. Cell Rep 11:1511–8. doi: 10.1016/j.celrep.2015.05.008

Johnston AB, Collins A, Goode BL (2015) High-speed depolymerization at actin filament ends jointly catalysed by Twinfilin and Srv2/CAP. Nat Cell Biol. *In press.* doi: 10.1038/ncb3252

Kardash E, Reichman-Fried M, Maître J-L, et al (2010) A role for Rho GTPases and cell-cell adhesion in single-cell motility in vivo. Nat Cell Biol 12:47–53. doi: 10.1038/ncb2003

Katsuragawa Y, Yanagisawa M, Inoue a, Masaki T (1989) Two distinct nonmuscle myosin-heavy-chain mRNAs are differentially expressed in various chicken tissues. Identification of a novel gene family of vertebrate non-sarcomeric myosin heavy chains. Eur J Biochem 184:611–616. doi: 10.1111/j.1432-1033.1989.tb15057.x

Kaverina I, Stradal TEB, Gimona M (2003) Podosome formation in cultured A7r5 vascular smooth muscle cells requires Arp2/3-dependent de-novo actin polymerization at discrete microdomains. J Cell Sci 116:4915–4924. doi: 10.1242/jcs.00818

Kawamoto S, Adelstein R (1991) Chicken Nonmuscle Myosin Heavy Chains : Differential Expression. 112:915–924. doi: 0021-9525/91/03/915/10

Kiuchi T, Ohashi K, Kurita S, Mizuno K (2007) Cofilin promotes stimulus-induced lamellipodium formation by generating an abundant supply of actin monomers. J Cell Biol 177:465–76. doi: 10.1083/jcb.200610005

Kolega J (2003) Asymmetric distribution of IIB in migrating endothelial cells is regulated by a Rho-dependent kinase and contributes to tail retraction. Mol Biol Cell 14:4745–4757. doi: 10.1091/mbc.E03

Korobova F, Svitkina T (2008) Arp2/3 complex is important for filopodia formation, growth cone motility, and neuritogenesis in neuronal cells. Mol Biol Cell 19:1561–74. doi: 10.1091/mbc.E07-09-0964

Kovac B, Teo JL, Mäkelä TP, Vallenius T (2013) Assembly of non-contractile dorsal stress fibers requires α-actinin-1 and Rac1 in migrating and spreading cells. J Cell Sci 126:263–73. doi: 10.1242/jcs.115063

Kovar DR, Kuhn JR, Tichy AL, Pollard TD (2003) The fission yeast cytokinesis formin Cdc12p is a barbed end actin filament capping protein gated by profilin. J Cell Biol 161:875–87. doi: 10.1083/jcb.200211078

Kress H, Stelzer EHK, Holzer D, et al (2007) Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity. Proc Natl Acad Sci U S A 104:11633–8. doi: 10.1073/pnas.0702449104

Krugmann S, Jordens I, Gevaert K, et al (2001) Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. Curr Biol 11:1645–55. doi: 10.1016/S0960-9822(01)00506-1

Lai FPL, Szczodrak M, Block J, et al (2008) Arp2/3 complex interactions and actin network turnover in lamellipodia. EMBO J 27:982–92. doi: 10.1038/emboj.2008.34

Lewis WH, Lewis MR 1924. Behavior of cells in tissue cultures. *In* General Cytology. E.V. Cowdry, editor. The University of Chicago Press, Chicago, IL. 385–447.

Li R, Li G, Deng L, et al (2010) IL-6 augments the invasiveness of U87MG human glioblastoma multiforme cells via up-regulation of MMP-2 and fascin-1. Oncol Rep 23:1553–9. doi: 10.3892/or_00000795

Lim FY, Koon YL, Chiam K-H (2013) A computational model of amoeboid cell migration. Comput Methods Biomech Biomed Engin 16:1085–1095. doi: 10.1080/10255842.2012.757598

Lim KB, Bu W, Goh WI, et al (2008) The Cdc42 effector IRSp53 generates filopodia by coupling membrane protrusion with actin dynamics. J Biol Chem 283:20454–72. doi: 10.1074/jbc.M710185200

Linder S, Wiesner C (2015) Tools of the trade: podosomes as multipurpose organelles of monocytic cells. Cell Mol Life Sci 72:121–35. doi: 10.1007/s00018-014-1731-z

Linder S, Wiesner C, Himmel M (2011) Degrading Devices: Invadosomes in Proteolytic Cell Invasion. Annu Rev Cell Dev Biol 27:185–211. doi: 10.1146/annurev-cellbio-092910-154216

Lizárraga F, Poincloux R, Romão M, et al (2009) Diaphanous-related formins are required for invadopodia formation and invasion of breast tumor cells. Cancer Res 69:2792–2800. doi: 10.1158/0008-5472.CAN-08-3709

Logue JS, Cartagena-Rivera AX, Baird MA, et al (2015) Erk regulation of actin capping and bundling by Eps8 promotes cortex tension and leader bleb-based migration. Elife 4:e08314. doi: 10.7554/eLife.08314

Loisel TP, Boujemaa R, Pantaloni D, Carlier MF (1999) Reconstitution of actin-based motility of Listeria and Shigella using pure proteins . Nature 401:613–616. doi: 10.1038/44183

Lämmermann T, Bader BL, Monkley SJ, et al (2008) Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature 453:51–55. doi: 10.1038/nature06887

Machesky LM, Atkinson SJ, Ampe C, Vandekercldaove J (1994) Purification of a Cortical

Complex Containing Two Unconventional Actins from Acanthamoeba by affinity chromatography on profilin-agarose. J Cell Biol 127:107–115. doi: 10.1083/jcb.127.1.107

Machesky LM, Insall RH (1998) Scar1 and the related Wiskott-Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. Curr Biol 8:1347–56. doi: http://dx.doi.org/10.1016/S0960-9822(98)00015-3

Machesky LM, Mullins RD, Higgs HN, et al (1999) Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. Proc Natl Acad Sci U S A 96:3739–44. doi: 10.1073/pnas.96.7.3739

Majumdar R, Sixt M, Parent CA (2014) New paradigms in the establishment and maintenance of gradients during directed cell migration. Curr Opin Cell Biol 30:33–40. doi: 10.1016/j.ceb.2014.05.010

Mallavarapu A, Mitchison T (1999) Regulated actin cytoskeleton assembly at filopodium tips controls their extension and retraction. J Cell Biol 146:1097–106. doi: 10.1083/jcb.146.5.1097

Mattila PK, Lappalainen P (2008) Filopodia: molecular architecture and cellular functions. Nat Rev Mol Cell Biol 9:446–54. doi: 10.1038/nrm2406

Mattila PK, Pykäläinen A, Saarikangas J, et al (2007) Missing-in-metastasis and IRSp53 deform PI(4,5)P2-rich membranes by an inverse BAR domain-like mechanism. J Cell Biol 176:953–64. doi: 10.1083/jcb.200609176

Mattila PK, Quintero-Monzon O, Kugler J, et al (2004) A high-affinity interaction with ADP-actin monomers underlies the mechanism and in vivo function of Srv2/cyclase-associated protein. Mol Biol Cell 15:5158–71. doi: 10.1091/mbc.E04-06-0444

Maupin P, Phillips C, Adelstein R, Pollard T (1994) Differential localization of myosin-II isozymes in human cultured cells and blood cells. J Cell Sci 107:3077–3090.

Medalia O, Beck M, Ecke M, et al (2007) Organization of actin networks in intact filopodia. Curr Biol 17:79–84. doi: 10.1016/j.cub.2006.11.022

Mejillano MR, Kojima S, Applewhite DA, et al (2004) Lamellipodial Versus Filopodial Mode of the Actin Nanomachinery. Cell 118:363–373. doi: 10.1016/j.cell.2004.07.019

Miller J, Fraser SE, McClay D (1995) Dynamics of thin filopodia during sea urchin gastrulation. Development 121:2501–11.

Mogilner A, Rubinstein B (2005) The physics of filopodial protrusion. Biophys J 89:782–95. doi: 10.1529/biophysj.104.056515

Moulding DA, Record J, Malinova D, Thrasher AJ (2013) Actin cytoskeletal defects in immunodeficiency. Immunol Rev 256:282–99. doi: 10.1111/imr.12114

Mueller SC, Chen WT (1991) Cellular invasion into matrix beads: localization of beta 1

integrins and fibronectin to the invadopodia. J Cell Sci 99:213–25.

Muller WA (2015) The regulation of transendothelial migration: new knowledge and new questions. Cardiovasc Res 107:310–320. doi: 10.1093/cvr/cvv145

Mullins RD, Heuser J a, Pollard TD (1998) 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 95:6181–6. doi: 0027-8424y98y956181-6

Nemethova M, Auinger S, Small JV (2008) Building the actin cytoskeleton: Filopodia contribute to the construction of contractile bundles in the lamella. J Cell Biol 180:1233–1244. doi: 10.1083/jcb.200709134

Norris AD, Dyer JO, Lundquist EA (2009) The Arp2/3 complex, UNC-115/abLIM, and UNC-34/Enabled regulate axon guidance and growth cone filopodia formation in Caenorhabditis elegans. Neural Dev 4:38. doi: 10.1186/1749-8104-4-38

Nourshargh S, Hordijk PL, Sixt M (2010) Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. Nat Rev Mol Cell Biol 11:366–78. doi: 10.1038/nrm2889

Osiak AE, Zenner G, Linder S (2005) Subconfluent endothelial cells form podosomes downstream of cytokine and RhoGTPase signaling. Exp Cell Res 307:342–353. doi: 10.1016/j.yexcr.2005.03.035

Otto JJ, Kane RE, Bryan J (1979) Formation of filopodia in coelomocytes: Localization of Fascin, a 58,000 Dalton actin cross-linking protein. Cell 17:285–293. doi: 10.1016/0092-8674(79)90154-5

Paluch E, Piel M, Prost J, et al (2005) Cortical actomyosin breakage triggers shape oscillations in cells and cell fragments. Biophys J 89:724–33. doi: 10.1529/biophysj.105.060590

Paluch EK, Raz E (2013) The role and regulation of blebs in cell migration. Curr Opin Cell Biol 25:582–90. doi: 10.1016/j.ceb.2013.05.005

Pantaloni D, Carlier M-F (1993) How profilin promotes actin filament assembly in the presence of thymosin β4. Cell 75:1007–1014. doi: 10.1016/0092-8674(93)90544-Z

Parry DA, Squire JM (1973) Structural role of tropomyosin in muscle regulation: analysis of the x-ray diffraction patterns from relaxed and contracting muscles. J Mol Biol 75:33–55. doi: 10.1016/0022-2836(73)90527-5

Pellegrin S, Mellor H (2007) Actin stress fibres. J Cell Sci 120:3491–3499. doi: 10.1242/jcs.018473

Peng J, Wallar BJ, Flanders A, et al (2003) Disruption of the Diaphanous-related formin Drf1 gene encoding mDia1 reveals a role for Drf3 as an effector for Cdc42. Curr Biol 13:534–45. doi: http://dx.doi.org/10.1016/S0960-9822(03)00170-2

Peter BJ, Kent HM, Mills IG, et al (2004) BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. Science 303:495–9. doi: 10.1126/science.1092586

Petrie RJ, Yamada KM (2012) At the leading edge of three-dimensional cell migration. J Cell Sci 125:5917–26. doi: 10.1242/jcs.093732

Pfaff M, Jurdic P (2001) Podosomes in osteoclast-like cells: structural analysis and cooperative roles of paxillin, proline-rich tyrosine kinase 2 (Pyk2) and integrin alphaVbeta3. J Cell Sci 114:2775–2786.

Pinner S, Sahai E (2008) PDK1 regulates cancer cell motility by antagonising inhibition of ROCK1 by RhoE. Nat Cell Biol 10:127–37. doi: 10.1038/ncb1675

Poincloux R, Collin O, Lizárraga F, et al (2011) Contractility of the cell rear drives invasion of breast tumor cells in 3D Matrigel. Proc Natl Acad Sci U S A 108:1943–8. doi: 10.1073/pnas.1010396108

Pollard TD (1986) Rate constants for the reactions of ATP- and ADP-actin with the ends of actin filaments. J Cell Biol 103:2747–54. doi: 0021-9525/86/12/2747/8

Pollard TD, Borisy GG (2003) Cellular motility driven by assembly and disassembly of actin filaments. Cell 112:453–465. doi: http://dx.doi.org/10.1016/S0092-8674(03)00120-X

Pollard TD, Cooper JA (2009) Actin, a central player in cell shape and movement. Science 326:1208–12. doi: 10.1126/science.1175862

Pollard TD, Cooper JA (1984) Quantitative analysis of the effect of Acanthamoeba profilin on actin filament nucleation and elongation. Biochemistry 23:6631–6641. doi: 10.1021/bi00321a054

Poukkula M, Hakala M, Pentinmikko N, et al (2014) GMF promotes leading-edge dynamics and collective cell migration in vivo. Curr Biol 24:2533–40. doi: 10.1016/j.cub.2014.08.066

Prévost C, Zhao H, Manzi J, et al (2015) IRSp53 senses negative membrane curvature and phase separates along membrane tubules. Nat Commun 6:8529. doi: 10.1038/ncomms9529

Pruyne D, Evangelista M, Yang C, et al (2002) Role of formins in actin assembly: nucleation and barbed-end association. Science (80-) 297:612–5. doi: 10.1126/science.1072309

Puius Y a., Mahoney NM, Almo SC (1998) The modular structure of actin-regulatory proteins. Curr Opin Cell Biol 10:23–34. doi: 10.1016/S0955-0674(98)80083-5

Quintero-Monzon O, Jonasson EM, Bertling E, et al (2009) Reconstitution and dissection of the 600-kDa Srv2/CAP complex: roles for oligomerization and cofilin-actin binding in driving actin turnover. J Biol Chem 284:10923–34. doi: 10.1074/jbc.M808760200

Reinhard M, Halbrügge M, Scheer U, et al (1992) The 46/50 kDa phosphoprotein VASP purified from human platelets is a novel protein associated with actin filaments and focal

contacts. EMBO J 11:2063–70.

Ridley AJ (2011) Life at the leading edge. Cell 145:1012–22. doi: 10.1016/j.cell.2011.06.010

Ridley AJ (2015) Rho GTPase signalling in cell migration. Curr Opin Cell Biol 36:103–112. doi: 10.1016/j.ceb.2015.08.005

Ridley AJ, Hall A (1992) The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell 70:389–399. doi: http://dx.doi.org/10.1016/0092-8674(92)90163-7

Riveline D, Zamir E, Balaban NQ, et al (2001) Focal contacts as mechanosensors: Externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J Cell Biol 153:1175–1185. doi: 10.1083/jcb.153.6.1175

Rogers SL, Wiedemann U, Stuurman N, Vale RD (2003) Molecular requirements for actin-based lamella formation in Drosophila S2 cells. J Cell Biol 162:1079–88. doi: 10.1083/jcb.200303023

Romero S, Le Clainche C, Didry D, et al (2004) Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis. Cell 119:419–29. doi: 10.1016/j.cell.2004.09.039

Rotty JD, Wu C, Haynes EM, et al (2015) Profilin-1 serves as a gatekeeper for actin assembly by Arp2/3-dependent and -independent pathways. Dev Cell 32:54–67. doi: 10.1016/j.devcel.2014.10.026

Saarikangas J, Zhao H, Lappalainen P (2010) Regulation of the actin cytoskeleton-plasma membrane interplay by phosphoinositides. Physiol Rev 90:259–89. doi: 10.1152/physrev.00036.2009

Saarikangas J, Zhao H, Pykäläinen A, et al (2009) Molecular mechanisms of membrane deformation by I-BAR domain proteins. Curr Biol 19:95–107. doi: 10.1016/j.cub.2008.12.029

Sagot I, Klee SK, Pellman D (2002) Yeast formins regulate cell polarity by controlling the assembly of actin cables. Nat Cell Biol 4:42–50. doi: 10.1038/ncb719

Sandquist JC, Means AR (2008) The C-terminal tail region of nonmuscle myosin II directs isoform-specific distribution in migrating cells. Mol Biol Cell 19:5156–67. doi: 10.1091/mbc.E08-05-0533

Sanger JW, Sanger JM, Jockusch BM (1983). Differences in the stress fibers between fibroblasts and epithelial cells. J Cell Biol. 96:961-9.

Schirenbeck A, Arasada R, Bretschneider T, et al (2006) The bundling activity of vasodilator-stimulated phosphoprotein is required for filopodium formation. Proc Natl Acad Sci U S A 103:7694–9. doi: 10.1073/pnas.0511243103

Schirenbeck A, Bretschneider T, Arasada R, et al (2005) The Diaphanous-related formin dDia2 is required for the formation and maintenance of filopodia. Nat Cell Biol 7:619–25. doi: 10.1038/ncb1266

Shimada A, Niwa H, Tsujita K, et al (2007) Curved EFC/F-BAR-Domain Dimers Are Joined End to End into a Filament for Membrane Invagination in Endocytosis. Cell 129:761–772. doi: 10.1016/j.cell.2007.03.040

Shutova MS, Spessott WA, Giraudo CG, Svitkina T (2014) Endogenous species of mammalian nonmuscle myosin IIA and IIB include activated monomers and heteropolymers. Curr Biol 24:1958–68. doi: 10.1016/j.cub.2014.07.070

Sinnar SA, Antoku S, Saffin J-M, et al (2014) Capping protein is essential for cell migration in vivo and for filopodial morphology and dynamics. Mol Biol Cell 25:2152–60. doi: 10.1091/mbc.E13-12-0749

Skau CT, Plotnikov S V., Doyle AD, Waterman CM (2015) Inverted formin 2 in focal adhesions promotes dorsal stress fiber and fibrillar adhesion formation to drive extracellular matrix assembly. Proc Natl Acad Sci U S A 112:2447-56. doi: 10.1073/pnas.1505035112

Skoble J, Auerbuch V, Goley ED, et al (2001) Pivotal role of VASP in Arp2/3 complex-mediated actin nucleation, actin branch-formation, and Listeria monocytogenes motility. J Cell Biol 155:89–100. doi: 10.1083/jcb.200106061

Small JV, Rottner K, Kaverina I, Anderson KI (1998) Assembling an actin cytoskeleton for cell attachment and movement. Biochim Biophys Acta - Mol Cell Res 1404:271–281. doi: 10.1016/S0167-4889(98)00080-9

Small J V, Celis JE (1978) Filament arrangements in negatively stained cultured cells: the organization of actin. Cytobiologie 16:308–25.

Suarez C, Carroll RT, Burke TA, et al (2015) Profilin regulates F-actin network homeostasis by favoring formin over Arp2/3 complex. Dev Cell 32:43–53. doi: 10.1016/j.devcel.2014.10.027

Suetsugu S, Murayama K, Sakamoto A, et al (2006) The RAC binding domain/IRSp53-MIM homology domain of IRSp53 induces RAC-dependent membrane deformation. J Biol Chem 281:35347–58. doi: 10.1074/jbc.M606814200

Suraneni P, Rubinstein B, Unruh JR, et al (2012) The Arp2/3 complex is required for lamellipodia extension and directional fibroblast cell migration. J Cell Biol 197:239–51. doi: 10.1083/jcb.201112113

Svitkina TM, Borisy GG (1999) Arp2/3 Complex and Actin Depolymerizing Factor/Cofilin in Dendritic Organization and Treadmilling of Actin Filament Array in Lamellipodia. J Cell Biol 145:1009–1026. doi: 10.1083/jcb.145.5.1009

Svitkina TM, Bulanova EA, Chaga OY, et al (2003) Mechanism of filopodia initiation by

reorganization of a dendritic network. J Cell Biol 160:409–21. doi: 10.1083/jcb.200210174

Svitkina TM, Verkhovsky AB, McQuade KM, Borisy GG (1997) Analysis of the Actin-Myosin II System in Fish Epidermal Keratocytes: Mechanism of Cell Body Translocation. J Cell Biol 139:397–415. doi: 10.1083/jcb.139.2.397

Taylor AC, Robbins E (1963) Observations on microextensions from the surface of isolated vertebrate cells. Dev Biol 7:660–673. doi: 10.1016/0012-1606(63)90150-7

Tee YH, Shemesh T, Thiagarajan V, et al (2015) Cellular chirality arising from the self-organization of the actin cytoskeleton. Nat Cell Biol 17:445–457. doi: 10.1038/ncb3137

Tilney LG, Bonder EM, Coluccio LM, Mooseker MS (1983) Actin from Thyone sperm assembles on only one end of an actin filament: a behavior regulated by profilin. J Cell Biol 97:112–24. doi: 0021-9525/83/07/0112/13

Tojkander S, Gateva G, Husain A, et al (2015) Generation of contractile actomyosin bundles depends on mechanosensitive actin filament assembly and disassembly. Elife *In press.*

Tojkander S, Gateva G, Lappalainen P (2012) Actin stress fibers - assembly, dynamics and biological roles. J Cell Sci 125:1855–1864. doi: 10.1242/jcs.098087

Tojkander S, Gateva G, Schevzov G, et al (2011) A molecular pathway for myosin II recruitment to stress fibers. Curr Biol 21:539–550. doi: 10.1016/j.cub.2011.03.007

Tozluoğlu M, Tournier AL, Jenkins RP, et al (2013) Matrix geometry determines optimal cancer cell migration strategy and modulates response to interventions. Nat Cell Biol 15:751–62. doi: 10.1038/ncb2775

Tseng Y, Fedorov E, McCaffery JM, et al (2001) Micromechanics and ultrastructure of actin filament networks crosslinked by human fascin: a comparison with alpha-actinin. J Mol Biol 310:351–366. doi: 10.1006/jmbi.2001.4716

Türmer K, Orbán J, Gróf P, Nyitrai M (2015) FASCIN and alpha-actinin can regulate the conformation of actin filaments. Biochim Biophys Acta 1850:1855–61. doi: 10.1016/j.bbagen.2015.05.018

van den Dries K, Schwartz SL, Byars J, et al (2013) Dual-color superresolution microscopy reveals nanoscale organization of mechanosensory podosomes. Mol Biol Cell 24:2112–23. doi: 10.1091/mbc.E12-12-0856

Vasioukhin V, Bauer C, Yin M, Fuchs E (2000) Directed actin polymerization is the driving force for epithelial cell-cell adhesion. Cell 100:209–19. doi: http://dx.doi.org/10.1016/S0092-8674(00)81559-7

Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz a. R (2009) Non-muscle myosin II takes centre stage in cell adhesion and migration. Nat Rev Mol Cell Biol 10:778–790. doi: 10.1038/nrm2786.Non-muscle

Vignjevic D, Kojima S, Aratyn Y, et al (2006) Role of fascin in filopodial protrusion. J Cell Biol 174:863–75. doi: 10.1083/jcb.200603013

Wang F, Harvey E V., Conti MA, et al (2000) A Conserved Negatively Charged Amino Acid Modulates Function in Human Nonmuscle Myosin IIA. Biochemistry 39:5555–5560. doi: 10.1021/bi000133x

Wang F, Kovacs M, Hu A, et al (2003) Kinetic mechanism of non-muscle myosin IIB: functional adaptations for tension generation and maintenance. J Biol Chem 278:27439–48. doi: 10.1074/jbc.M302510200

Watanabe N, Mitchison TJ (2002) Single-molecule speckle analysis of actin filament turnover in lamellipodia. Science 295:1083–6. doi: 10.1126/science.1067470

Wiesner C, Faix J, Himmel M, et al (2010) KIF5B and KIF3A/KIF3B kinesins drive MT1-MMP surface exposure, CD44 shedding, and extracellular matrix degradation in primary macrophages. Blood 116:1559–69. doi: 10.1182/blood-2009-12-257089

Wolf K, Mazo I, Leung H, et al (2003) Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. J Cell Biol 160:267–77. doi: 10.1083/jcb.200209006

Wu C, Asokan SB, Berginski ME, et al (2012) Arp2/3 is critical for lamellipodia and response to extracellular matrix cues but is dispensable for chemotaxis. Cell 148:973–87. doi: 10.1016/j.cell.2011.12.034

Yang C, Czech L, Gerboth S, et al (2007) Novel roles of formin mDia2 in lamellipodia and filopodia formation in motile cells. PLoS Biol 5:e317. doi: 10.1371/journal.pbio.0050317

Yang C, Svitkina T (2011) Filopodia initiation: focus on the Arp2/3 complex and formins. Cell Adh Migr 5:402–8. doi: 10.4161/cam.5.5.16971