Actin-binding proteins: the long road to understanding the dynamic landscape of cellular actin networks

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ABSTRACT **The actin cytoskeleton supports a vast number of cellular processes in nonmuscle cells. It is well established that the organization and dynamics of the actin cytoskeleton are controlled by a large array of actin-binding proteins. However, it was only 40 years ago that the first nonmuscle actin-binding protein, filamin, was identified and characterized. Filamin was shown to bind and cross-link actin filaments into higher-order structures and contribute to phagocytosis in macrophages. Subsequently many other nonmuscle actin-binding proteins were identified and characterized. These proteins regulate almost all steps of the actin filament assembly and disassembly cycles, as well as the arrangement of actin filaments into diverse three-dimensional structures. Although the individual biochemical activities of most actin-regulatory proteins are relatively well understood, knowledge of how these proteins function together in a common cytoplasm to control actin dynamics and architecture is only beginning to emerge. Furthermore, understanding how signaling pathways and mechanical cues control the activities of various actin-binding proteins in different cellular, developmental, and pathological processes will keep researchers busy for decades.**

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Many cell biological processes, such as migration, morphogenesis, cytokinesis, and endocytosis, rely on a dynamic actin cytoskeleton. Precisely controlled polymerization of actin filaments against membranes provides a force to drive membrane dynamics. Furthermore, ATP-driven movement of myosin motor proteins along actin filaments transports cargo, as well as moves actin filaments relative to each other, generating contractile forces. It is well established that the organization and dynamics of actin filaments in cells are regulated by a large array of actin-binding proteins. However, this has not been acknowledged for very long. In the beginning of 1970s, actin was mainly studied in the context of skeletal muscle contraction, where it interacts with myosin II, α-actinin, and tropomyosins. Although homologues of these proteins were also shown to be expressed in nonmuscle cells (Adelstein *et al.*, 1971; Cohen and Cohen, 1972, Pollard and Korn, 1973; Lazarides and Burridge, 1975), research on the role of actin in nonmuscle cells centered on the function of myofibril-like contractile structures in cell migration (e.g., Wessells *et al.*, 1971).

It was not until the mid-1970s that the first nonmuscle actin regulatory protein, filamin, was identified and characterized. Filamin was independently discovered as a protein that copurified with myosin II from chicken gizzard and as a "high–molecular weight actin filament binding protein" isolated from rabbit alveolar macrophages (Wang *et al.*, 1975; Hartwig and Stossel, 1975). Coincident with the identification of filamin, it was also demonstrated that spectrin, a high–molecular weight protein identified from erythrocytes, interacts with actin (Tilney and Detmers, 1975). Biochemical studies demonstrated that filamin caused actin to form a gel. This is indicative of actin filament cross-linking activity. Furthermore, cell biological studies by Stossel and Hartwig (1976) suggested that the activity of filamin can be up-regulated in ingesting macrophages compared with resting macrophages, suggesting that filamin may control the actin cytoskeleton during phagocytosis (Shizuta *et al.*, 1976). A wealth of research carried out during the following four decades revealed that filamins not only cross-link actin filaments but also interact with a large array of cytoskeletal, adhesion, and signaling proteins to control the organization of the cytoskeleton and its interactions with cell–extracellular matrix adhesions. Furthermore, filamins serve as

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Abbreviations used: ADF, actin depolymerizing factor; VASP, vasodilator-stimulated phosphoprotein.

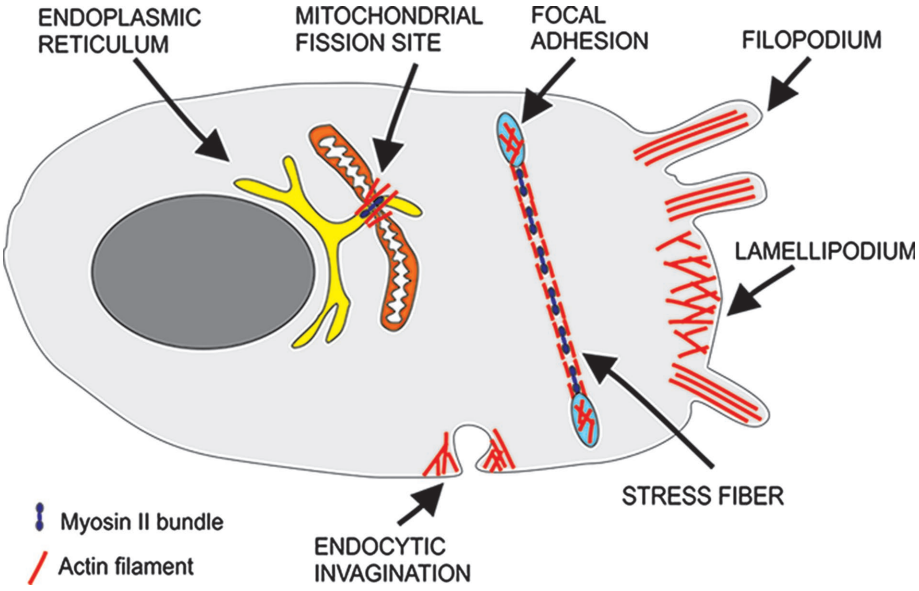

FIGURE 1: Examples of actin filament structures of motile animal cells. Cell migration is driven by the branched lamellipodial actin filament network, which pushes the leading edge forward. Thin, parallel actin filament bundles—filopodia—that extend from the lamellipodial network sense the extracellular environment during cell migration. Many animal cells also harbor stress fibers, which are thick, bipolar actin filament bundles that also contain myosin II filaments and are thus able to contract. Stress fibers are often linked to focal adhesions, actin-rich structures mediating cytoskeleton–extracellular matrix interplay. In addition, the dynamics of many membrane organelles in cells, such as endosomes and mitochondria (at the endoplasmic reticulum–mitochondria contact sites), are controlled by specific actin filament structures.

central mechanosensory proteins in cells by undergoing conformational changes and partial unfolding in response to extrinsic or intrinsic mechanical forces (Razinia *et al.*, 2012).

The discovery and initial characterization of filamin were soon followed by identification of a large array of proteins that regulate different aspects of actin filament turnover in nonmuscle cells. These include, among others, the actin monomer–binding protein profilin (Carlsson *et al.*, 1977), actin filament–severing proteins gelsolin and actin depolymerizing factor (ADF)/cofilin (Yin and Stossel, 1979; Bamburg *et al.*, 1980), and the heterodimeric capping protein, which blocks actin filament assembly at their rapidly growing barbed ends (Isenberg *et al.*, 1980; Cooper *et al.*, 1984). Finally, in the mid-1990s the first actin filament–nucleating protein, the Arp2/3 complex, was discovered (Machesky *et al.*, 1994; Welch *et al.*, 1997; Mullins *et al.*, 1998). These and other studies established that actin can assemble into both protrusive and contractile structures in cells and that the dynamics of actin filaments is precisely controlled by a plethora of actin-binding proteins. Even organisms of lower complexity, such as yeasts, express ~100 proteins that either directly or indirectly regulate the assembly or organization of the actin cytoskeleton (Mishra *et al.*, 2014). In multicellular organisms, including mammals, this number is significantly higher.

Why do we need such a large and diverse repertoire of actin-binding proteins? There are a number of reasons. First, actin filaments contribute to a vast number of different cellular processes, many of which require specific three-dimensional (3D) organization of actin filaments with distinct dynamic properties (Figure 1). During adhesion-dependent migration, cells extend thin, actin-rich membrane protrusions—filopodia—at their leading edge to probe the extracellular environment. Filopodia contain compact, unipolar actin filament bundles. Actual advancement of the cell edge in migration is driven by a branched actin filament network called the lamellipodium, which pushes the plasma membrane forward through coordinated polymerization of actin filaments against the membrane. Many animal cells also contain bipolar, contractile actomyosin bundles, often called stress fibers, which contribute to cell adhesion, mechanosensing, and tail retraction during cell migration (Blanchoin *et al.*, 2014). Moreover, cells harbor several additional actin filament arrays that generate force for other processes involving membrane dynamics, such as endocytosis and mitochondrial fission (Kaksonen *et al.*, 2006; Hatch *et al.*, 2014). Finally, it has become evident that actin not only functions in the cytoplasm, but also executes specific functions inside the nucleus (Grosse and Vartiainen, 2013). Because different actin filament networks are uniquely designed to execute specific cellular processes, it is not surprising that partially different sets of proteins control their organization and dynamics. Furthermore, some actin filaments must be connected to other relevant protein machineries or specific cellular membrane compartments, increasing the number of actin-binding proteins required for these functions. Thus actin filaments appear to exhibit a much larger diversity of 3D structures and contribute to a wider range of cellular processes than microtubules. This might at least partially explain why cells have a larger number of functionally distinct actin-binding proteins than microtubule-binding proteins.

Recent evidence indicates that several biochemically and functionally distinct actin filament populations can be generated in cells from a single actin isoform (Michelot and Drubin, 2011). Those diverse actin filament populations can result from the presence of different types of actin filament–nucleating proteins, which generate filaments that subsequently are selectively decorated by distinct sets of actin-binding proteins (Michelot *et al.*, 2010; Johnson *et al.*, 2014). The most extensively studied actin filament–decorating proteins that can alter the biochemical properties of actin are tropomyosins. Tropomyosins are a diverse family of long α-helical proteins that bind to the main groove of an actin filament and form continuous head-to-tail oligomers along filaments. In animals, >30 isoforms can be generated through alternative splicing from four tropomyosin genes. When bound to filaments, tropomyosin isoforms specify the physicochemical properties of actin, and it has been proposed that different tropomyosin isoforms can recruit specific downstream proteins to filaments and hence specify different actin filament populations in cells (Gunning *et al.*, 2015). In addition, application of external force to actin filaments can affect their structural properties and thus regulate the binding of proteins to filaments. This phenomenon was recently demonstrated for ADF/cofilins, which selectively bind and sever only flexible actin filaments while being unable to interact with actin filaments under tension (Hayakawa *et al.*, 2011). Moreover, studies on experimentally defined actin structures in vitro revealed that the architecture and connectivity of the actin filament network can affect activities of actin-associating proteins, including myosins (Reymann *et al.*, 2012; Ennomani *et al.*, 2016). Thus actin-dependent cellular processes may require several functionally distinct actin filament populations,

whose interactions with various actin-binding proteins can be further controlled by mechanical force and architecture of the filament network.

Finally, in multicellular organisms, actin filaments often display tissue- or cell-specific functions. Consequently, distinct isoforms of actin-binding proteins with biochemical activities fine-tuned to control filament dynamics or organize specific networks are expressed in a cell-specific manner. For example, unicellular organisms typically express only one actin filament–severing ADF/cofilin protein, but three biochemically distinct ADF/cofilin isoforms are found in mammals, each of which displays specific biochemical properties fine-tuned to control actin dynamics in the specific tissue or cell type where they are highly expressed (Vartiainen *et al.*, 2002; Kremneva *et al.*, 2014).

What lies ahead in actin-binding protein research for the next 40 years? Although during the past few decades we have certainly learned a great deal about the basic principles underlying actin dynamics, there remain several important unanswered questions. The majority of biochemical and cell biological studies on actin-binding proteins have focused on analyzing the functions of individual proteins. However, there is already evidence that, when examined in combinations with each other, actin-binding proteins can display striking new activities relating to the dynamics or organization of actin filaments. For example, binding of ADF/cofilin to actin filaments triggers a change in filament conformation that is critical for their rapid disassembly via the action of another actin-binding protein, Aip1 (McGough *et al.*, 1997; Galkin *et al.*, 2011; Gressin *et al.*, 2015; Jansen *et al.*, 2015). Furthermore, two actin filament barbed-end–binding proteins with opposing activities—heterodimeric capping protein and formins—were shown to interact with each other as a complex that controls actin filament elongation at rapidly growing barbed ends (Bombardier *et al.*, 2015; Shekhar *et al.*, 2015). Finally, it was demonstrated that different actin filament nucleation machineries compete for a limited pool of actin monomers in cells and that the actin monomer–binding protein profilin functions as a "gate keeper" between these nucleators. Profilin delivers actin monomers to power formin- and vasodilator-stimulated phosphoprotein (VASP)–driven filament elongation (Reinhard *et al.*, 1995; Sagot *et al.*, 2002; Evangelista *et al.*, 2002; Romero *et al.*, 2004). Of importance, recent studies revealed that a decrease in the abundance or activity of profilin in cells can lead to an increase in Arp2/3-induced actin filament assembly at the expense of formin- and VASP-polymerized actin filament structures, demonstrating that these different actin assembly machineries indeed compete with each other for actin monomers (Suarez *et al.*, 2015; Rotty *et al.*, 2015).

In the future, it will be important to examine the activities of actin-binding proteins in combination with each other and with other interacting proteins, as well as reconstitute different cellular actin-driven machineries in vitro to reveal the combined effects of various actin regulators in modulating network properties and functions. The latter was already achieved, for example, for the most crucial actin-binding proteins that are required for actin-based motility and for a membrane-associated actomyosin cortex (Loisel *et al.*, 1999; Murrell and Gardel, 2012). However, reconstituting more complex cellular processes remains to be done. Finally, studies focusing on regulation of actin-binding proteins by different biochemical signals and mechanical cues are likely to lead to many surprising findings concerning the principles by which the actin cytoskeleton communicates with cellular signaling pathways and responds to changes in mechanical properties of the extracellular environment of cells.

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