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Building Distinct Actin Filament Networks in a Common Cytoplasm

Review

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Eukaryotic cells generate a diversity of actin filament networks in a common cytoplasm to optimally perform functions such as cell motility, cell adhesion, endocytosis and cytokinesis. Each of these networks maintains precise mechanical and dynamic properties by autonomously controlling the composition of its interacting proteins and spatial organization of its actin filaments. In this review, we discuss the chemical and physical mechanisms that target distinct sets of actin-binding proteins to distinct actin filament populations after nucleation, resulting in the assembly of actin filament networks that are optimized for specific functions.

Introduction

Actin is one of the most abundant, highly conserved and versatile proteins found in eukaryotic cells. *In vivo*, this 42 kDa globular protein undergoes cycles of polymerization and disassembly between its globular (G-actin) and filamentous (F-actin) forms [1,2]. This dynamic behavior allows cells to constantly remodel their actin cytoskeleton and to use it as a force-generating system in many different processes, such as cell motility, cell adhesion, endocytosis, cytokinesis, or more generally, for the control of cell morphology [1,3–6]. Actin filaments are not found in cells as disorganized meshworks, but rather as organized assemblies localized in precise areas of the cytoplasm where they can perform their functions in response to different stimuli. Numerous lines of evidence indicate that the geometrical, mechanical and dynamic properties of the actin networks are specifically adapted for each cellular function, and that these properties are tightly controlled by specific sets of interacting proteins [4,7,8].

In this review, we will not describe in detail what is known about the architectures and functions of these numerous actin-based structures. Nor will we address how all of the regulatory proteins work together to assemble and regulate these structures. Rather, we will view the problem from a different angle, by attempting to answer the following question: by which mechanism(s) do cells assemble different actin-filament-based structures of distinct composition in a common cytoplasm that contains a complex mixture of all the proteins? After a short description of some of the structures encountered in metazoan and yeast cells, we will review, from chemical and physical standpoints, the different concepts emerging from the literature that explain the segregation mechanisms. This discussion will lead us to propose a general working model that explains the formation of branched and linear networks of actin filaments in all eukaryotic cells. We will conclude with a discussion of the remaining unanswered questions and of the approaches that will help us overcome these challenges.

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Evidence for Generation of Actin Structures of Distinct Compositions *In Vivo*

The formation of F-actin from a pool of G-actin is kinetically unfavorable due to the extreme instability of the dimeric and trimeric forms. Moreover, spontaneous nucleation of actin filaments is potently inhibited *in vivo* by additional factors such as profilin and thymosin β_4. To overcome this inhibition and to be able to control the generation of new actin filaments spatially and temporally, cells need additional factors called actin filament nucleators [9,10]. Subsequent to the initiation step, actin filaments elongate and become organized into networks of distinct geometrical arrangements and protein compositions. As examples, we will describe a few structures in metazoan and yeast cells.

In metazoan cells, actin filaments assemble into at least 15 distinct structures [11], some examples of which are given in Figure 1. At the leading edge of migrating cells (called the lamellipodium), at sites of clathrin–actin-mediated endocytosis, and associated with several intracellular organelles, dense networks of actin filaments are nucleated and cross-linked in branched arrays by the Arp2/3 complex (Figure 1) [12–15]. The Arp2/3 complex is recruited and activated at membranes by proteins called nucleation-promoting factors (NPFs), which include WASP at sites of clathrin–actin-mediated endocytosis and WAVE at the lamellipodium [10]. In the presence of the Arp2/3 complex, pre-existing actin filaments and G-actin, NPFs catalyze actin filament nucleation and formation of branched filament networks. Subsequently, additional regulatory proteins are recruited (Figure 1). While some of these regulatory proteins bind directly or indirectly to the NPFs (e.g., verprolin binds to yeast WASP [16,17]) or to the Arp2/3 complex (e.g., coronin [18]) and regulate their activity, most of these proteins appear to be recruited by the actin filaments.

In an area more distal from the leading edge of migrating cells, the lamellum is another actin-based structure composed of linear arrays of actin filaments principally organized into longitudinal stress fibers and transversal arcs in association with adhesion sites (Figure 1) [11,19]. Recent studies indicate that filaments within the lamellum can directly emerge from the lamellipodium [19,20] or can be generated independently from focal adhesion sites by formins [20,21], a family of actin nucleators that generates unbranched actin filaments and elongates them by remaining processively attached at their barbed ends [22–24]. The lamellum is implicated in productive advance of migrating cells [20,25] and in the maintenance of cell plasticity, and it is composed of actin-binding proteins (ABPs) that are distinct from the ones interacting with Arp2/3-derived branched networks (Figure 1).

The final actin-based metazoan structures covered in this review are filopodia — finger-like protrusions at the leading edge of cells involved in cell–cell signaling and guidance toward chemoattractants. Filopodia contain linear bundled arrays of actin filaments (Figure 1) [11,26,27]. Whether the actin filaments that comprise filopodia are nucleated independently by formins at the plasma membrane, or whether they directly emerge and elongate from the lamellipodium,

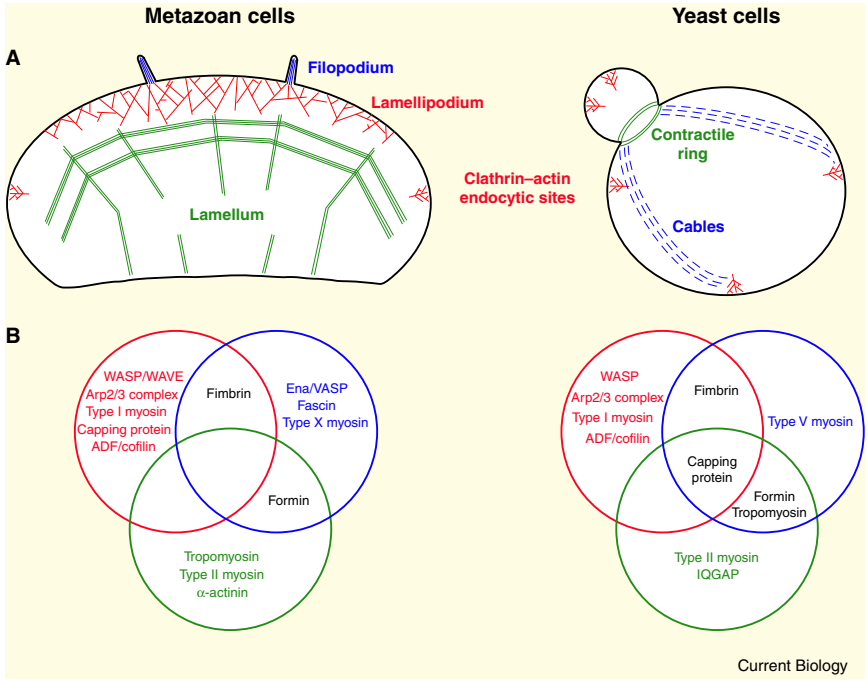

Figure 1. Actin-based structures and their key conserved regulators in metazoan and yeast cells.

(A) Schematic representation of actin-based structures in metazoan (left) and yeast (right) cells. Only a subset of actin-based structures in metazoan cells is presented. The color code distinguishes between branched (Arp2/3-derived) networks (in red) from linear (formin-derived) arrays of actin filaments (in blue and in green). (B) Comparison of the composition of actin-associated proteins in distinct cytoskeletal systems. For metazoan cells (left), red, blue and green circles contain proteins associated with the lamellipodium and/or clathrin–actin endocytic sites, the filopodium and the lamellum, respectively. For yeast cells (right), red, blue and green circles contain proteins associated with clathrin–actin endocytic sites, the cables and the contractile ring, respectively.

is not clear [26]. In any case, elongation factors such as VASP and formins play an important role in filopodia formation [26,28]. The protein composition of actin structures in filopodia is distinct from the composition of the other actin-based structures (Figure 1).

Yeast cells share many similarities with more complex organisms, but they are simpler to study in several ways. First, these cells have a smaller number of actin regulatory proteins than metazoan cells. For example, budding yeast has only three known nucleators of actin filaments (the Arp2/3 complex and the two formin isoforms, Bni1 and Bnr1) and four NPFs [8]. Yeast also have only one actin isoform: elimination of possible roles for actin isoforms facilitates our understanding of the differences between different actin-based structures. Second, only three actin networks are known to assemble in growing yeast: cortical actin patches, which are Arp2/3-branched actin filament networks involved in clathrin-mediated endocytosis; actin cables, which are linear bundles of short actin filaments nucleated by the formins, and are involved in vesicle trafficking and maintenance of cell polarity; and an actin contractile ring composed of short linear actin filaments nucleated by the formins (Figure 1) [8,29]. Similar to metazoan cells, these structures are all regulated by specific sets of actin regulators (Figure 1) [8]. Third, although there appear to be connections between actin patches and actin cables in the trafficking of vesicles after endocytosis [30,31], the actin networks in yeast are spatially distinct, which makes it easier to study these structures independently of one another. For these reasons, and as a result of the powerful genetic tools available, budding and fission yeast are among the most intensively studied cell types for actin biology.

It is interesting to note that homologous proteins often segregate to analogous actin structures across species (Figure 1). For example, in yeast and metazoan cells type I myosins only localize to sites where Arp2/3-derived branched actin networks are formed, while type II and type V myosins only localize to sites where formin-derived actin filament linear arrays are formed. Therefore, we now focus our discussion on Arp2/3-derived branched actin filament networks and formin-derived linear actin filament networks.

Possible Mechanisms for Segregation of Proteins to Different Networks

We start our discussion with a simple question: by what mechanisms could actin-associated proteins bind selectively to different types of networks?

One potential mechanism relies on the fact that complex signaling networks in cells can target proteins to specific areas within cells. This mechanism allows cells to spatially regulate where different networks assemble by controlling the location and activity of NPFs or actin nucleators. Several elegant studies have shown that cell signaling can be rewired, or that key signaling components can be mistargeted, to artificially form specific actin filament networks in precise locations within cells [32,33]. A second possibility is that the local chemical environment (such as pH or ionic concentrations) within areas of cells could provide a mechanism to control protein activities, such as the binding affinity of proteins for actin filaments. In fact, the activities of numerous ABPs are regulated by such factors. For example, the activity of actin depolymerization factor (ADF)/cofilin is pH dependent and the activities of most proteins of the villin/gelsolin superfamily are regulated by Ca^{2+} concentration [34,35]. The last possibility, which we will further develop later in this article, is that different actin filament structures are preferred as substrates for selective binding by ABPs.

Actin Nucleators Are Sufficient to Reconstitute Actin Filament Networks of Biologically Relevant Composition *In Vitro*

Tremendous progress has been made in the last two decades in understanding how complex networks of actin filaments assemble. A key innovation driving this research was the use of bacterial pathogens, such as *Listeria* and *Shigella*, as basic tools to understand Arp2/3-based motility.

These pathogens hijack the actin cytoskeleton of host cells to drive their movement [36–38]. As a first step, motility of these bacteria was reconstituted in cell extracts, demonstrating that factors present at their cell surfaces are sufficient to stimulate the assembly of productive actin filament networks for propulsion of the pathogens during host cell infection [39]. The surface proteins responsible for the generation of the branched actin networks were later identified, purified and characterized as NPFs of the Arp2/3 complex [40,41]. These purified NPFs, when symmetrically conjugated to polystyrene microbeads, trigger motility of these artificial objects in cell extracts, proving that NPFs are sufficient to induce the recruitment and organized assembly of all the factors required for actin-based motility [42]. Another crucial discovery was that, when formins are used in similar bead experiments, they induce the assembly of a different kind of network that is instead composed of parallel assemblies of actin filaments, similar to those observed in filopodia or actin cables [43,44] (Figure 1).

Bead/pathogen-based motility and cytoplasmic extracts remain important tools for studying actin-based motile processes. In a recent study, we demonstrated that the NPF WASP (Las17 in yeast) is sufficient to assemble cortical endocytic patch-like structures of biologically relevant protein composition in a yeast protein extract [45]. While some of the proteins were recruited by WASP independent of actin assembly, many others depended on actin filament assembly. Unexpectedly, among all of the proteins that are known to bind to actin filaments with a high affinity *in vitro*, only the subset of proteins that colocalize with endocytic patches *in vivo* were associated with the reconstituted structures. Since there is only one actin gene in yeast, these results demonstrate the existence of an actin-isoform-independent mechanism for the segregation of ABPs to specific subpopulations of actin filaments so that the final structure has the composition appropriate for WASP–Arp2/3-nucleated filaments *in vivo*. This result also rules out the possibilities raised in the previous section that ABPs (other than the NPF) are recruited to specific actin structures by independent signaling networks, or that their recruitment is due to a specific and local cellular context, such as pH or salt concentration.

In another study, Brawley and Rock followed the recruitment of three classes of myosins to triton-extracted actin cytoskeletons [46]. This treatment preserves the architecture of the cell's actin networks and retains their ability to interact with other components. The authors found that each class of myosin was recruited to the appropriate actin-based structure, demonstrating in a different system that actin networks have specific binding properties depending on the structure to which they belong.

In the following sections of this review, we will focus on the mechanisms that can explain the recruitment of ABPs to specific populations of actin filaments.

Cooperativity and Competition for Actin Filament Binding

Many studies have concluded that binding of ABPs to actin filaments can be strongly influenced by the presence of the other molecules already bound along the filament, via cooperative or competitive binding effects. The first studies to report such observations date almost to the time of the discoveries and purifications of the first ABPs. Since then, many examples of cooperativity and competition have been reported *in vitro* and *in vivo*. Here we will focus on several examples relevant to our discussion — in particular, tropomyosins and their crosstalk with other ABPs — as described below and summarized in Table 1.

Tropomyosins

Tropomyosins exist as rod-shaped, coiled-coil dimers that form head-to-tail polymers wrapped around actin filaments and stabilize actin filaments *in vitro* (for a complete review of these proteins, we refer the readers to a collection of excellent reviews and book chapters [47–49]). Most eukaryotic cells express tropomyosins from multiple genes and produce multiple isoforms from each gene by alternative splicing or by acetylation of the amino-terminal methionine; moreover, tropomyosins can form homodimers or heterodimers. All of these processes result in the expression of a large number of tropomyosins. While certain tropomyosin isoforms show cooperative binding to actin filaments, other isoforms compete for binding to actin filaments, likely explaining the segregation of different isoforms to different actin filament populations *in vivo*. The existence of multiple isoforms and the fact that tropomyosins regulate the binding of many other accessory proteins (see below and [50]) suggest that this family of proteins plays a central role in regulating the protein composition of actin structures. Interestingly, tropomyosins usually compete for binding to actin filaments with protein components of Arp2/3-derived branched networks, but cooperate for binding with proteins present in formin-derived linear networks (see below and Table 1).

Tropomyosins and Myosins

It has long been known that tropomyosins are master regulators of myosin motor activities in muscle and non-muscle cells [51]. Tropomyosins affect myosin activities on actin filaments in different ways, including binding to the motor domain and regulation of ATPase kinetics. These interactions are dependent on the particular isoform of tropomyosin and myosin. For example, single-headed type I myosins are unable to bind to tropomyosin-decorated actin filaments in various organisms [52,53]. On the other hand, type II and type V myosins bind to tropomyosin-decorated actin filaments and even have their motor activity enhanced by this binding [51,53,54].

Tropomyosin and the Arp2/3 Complex

Because in many eukaryotic cells the Arp2/3 complex and tropomyosins localize to very different areas, whether this segregation might be due to a competitive effect was investigated soon after the discovery of the Arp2/3 complex. It was in fact demonstrated that tropomyosin inhibits actin filament branching and nucleation by the Arp2/3 complex *in vitro* [55] and competitively inhibits Arp2/3-dependent nucleation in a reconstituted motility assay [56]. Overexpression of tropomyosin *in vivo* depletes the Arp2/3 complex from the leading edge of epithelial cells, therefore inhibiting the formation of the lamellipodium [57]. In contrast, tropomyosin depletion expands the lamellipodium at the expense of the lamellum in *Drosophila* S2 cells [58].

ADF/Cofilin

Proteins of the ADF/cofilin family are found in all eukaryotic cells. ADF/cofilin mediates disassembly of aged actin networks by binding with a high affinity to ADP–actin filaments and inducing their fragmentation [44,59–62]. In most cases, ADF/cofilin molecules bind cooperatively to actin

Table 1. F-actin binding proteins in eukaryotes: examples of cooperative/competitive effects and their impact on F-actin's structure and properties.

Protein family	Activities/functions	Cooperativity/competition	Impact on F-actin structure and properties (+ method of detection)	Key references
Formin-derived networks (linear networks)				
Formin	• Nucleates filaments • Processively caps barbed ends	• Cooperative effect with tropomyosin: barbed-end bound formin recruits tropomyosin to filaments and formins elongate tropomyosin-decorated filaments faster • Tropomyosin competes with formins for binding to sides of actin filaments • Competes with capping protein to bind actin filament barbed ends	• Changes the conformation of actin filaments when bound at the barbed ends through long-range allosteric interactions (fluorescence lifetime, anisotropy decay and FRET experiments)	[92–94,99,100,110–112]
Tropomyosin	• Binds filament sides • Stabilizes filaments *in vitro*	• Tropomyosin molecules bind cooperatively along actin filaments; different isoforms can show cooperative or competitive binding • Many cooperative and competitive effects with other proteins (see corresponding proteins)	• Tropomyosin increases filament stiffness (light scattering)	[47–50]
Type II myosin	• F-actin motor; low duty ratio • Double-headed • Involved in contractile processes	• ATPase and binding activities increased by specific tropomyosin isoforms	• Binding to actin filaments induces structural and mechanical modifications (phosphorescence anisotropy, electron microscopy)	[47,48,50,51,54,84–86]
Type V myosin	• F-actin motor; high duty ratio • Double-headed • Involved in vesicle and cargo trafficking	• Affinity and ATPase activity increased by tropomyosin	• Binding to actin filaments induces structural and mechanical modifications (time-resolved phosphorescence anisotropy)	[51,53,86]
Arp2/3 complex-derived networks (branched networks)				
Arp2/3 complex	• Nucleates filaments • Induces actin filament branching	• Skeletal and non-skeletal tropomyosins compete with Arp2/3 complex to prevent nucleation of daughter filaments	• Modifies the structure of the mother filament (3D reconstruction for electron tomography)	[55,56,98]
Fimbrin	• Binds filament sides	• CH domains present in fimbrin bind cooperatively to actin filaments • Competes with tropomyosin in fission yeast	• Binding to actin filaments induces structural modifications that propagate along actin filaments (electron microscopy)	[66,113]
ADF/cofilin	• Binds filament sides • Disassembles aged networks by fragmentation of ADP–F-actin	• ADF/cofilin molecules bind cooperatively to actin filaments • Competes with most tropomyosin isoforms; Cooperative binding with several isoforms of tropomyosin	• Binding to actin filaments induces structural, mechanical and energetic modifications (electron microscopy, FRET, time-resolved phosphorescence anisotropy, calorimetry, fluorescence microscopy)	[59,61,63–65,79–83,90,91]
Type I myosin	• F-actin motor; low duty ratio • Single-headed	• Does not bind tropomyosin-decorated actin filaments	• None determined	[51–53]

filaments, leading to the formation of stretches of actin filaments decorated by ADF/cofilin [59,62].

Tropomyosin and ADF/Cofilin

Many isoforms of tropomyosin compete with ADF/cofilin for binding to actin filaments *in vitro* [63–65]. As a consequence, tropomyosins and ADF/cofilin have antagonistic effects in actin-based motile processes [56–58].

Tropomyosin and Fimbrin

A recent study showed similar competitive interactions between tropomyosins and fimbrin in fission yeast. Using

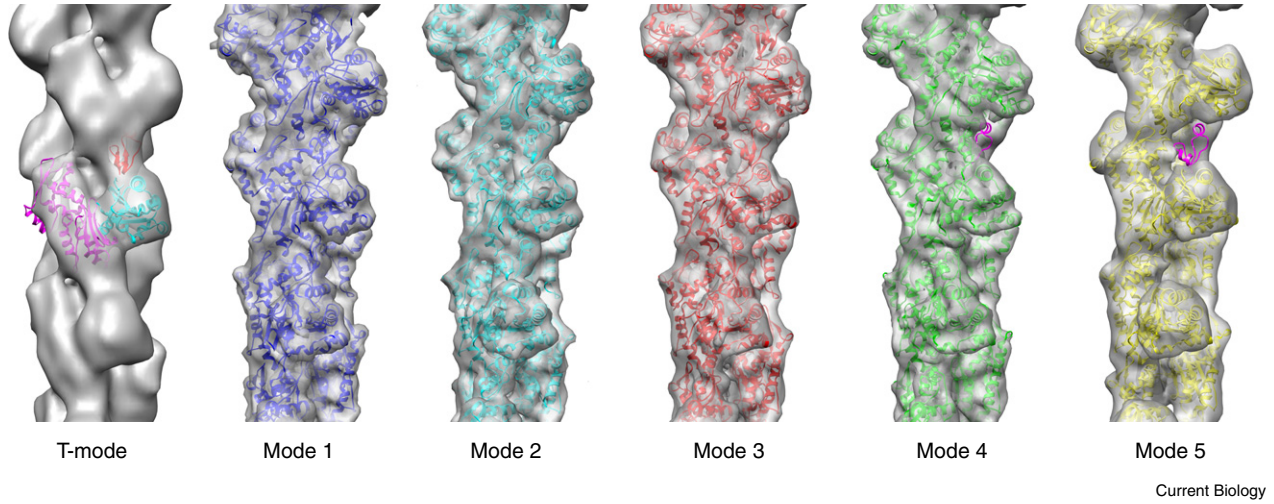

Figure 2. Examples of multiple conformations that can be adopted by actin filaments.

This image represents three-dimensional reconstructions of six structural modes observed by electron cryomicroscopy of frozen-hydrated actin filaments. T-mode represents a tilted state of the subunits within the filament (modified from [74]).

pelleting assays, the authors demonstrated that these two proteins compete for binding to F-actin *in vitro* [66]. As a consequence, maintenance of the proper physiological ratio of these proteins *in vivo* is important for their localization to the correct actin-based structures. When these proteins are mistargeted in cells, other ABPs such as ADF/cofilin and myosins also fail to localize properly and cannot perform their functions optimally [53,66].

The Different Conformations of an Actin Filament

We now discuss physical explanations for these cooperative and competitive effects. Competitive binding of different ABPs to actin filaments due to steric effects along the actin filament certainly occurs. However, such effects are not sufficient to account for the formation of actin networks of different protein compositions. If we suppose that all of the cytoskeletal proteins obey the law of mass action and if their binding to the same substrate (the actin filaments) results from a chemical equilibrium, then all of the actin filaments inside a cell should be decorated with a similar ratio of all of the ABPs available. Since this is not what is observed, an alternative explanation is suggested in which the properties of the substrate (the actin filaments) are modified at the time they are being decorated by a reactant (an ABP), with long-range effects that subsequently affect the binding of other ABPs.

Evidence for such effects can be found by looking at the structure and mechanical properties of actin filaments at the molecular scale. Actin filaments assemble as double-stranded, right-handed helices, with a pitch for a single strand of about 75 nm, which corresponds to approximately 13 subunits along one strand. Actin filaments are structurally polarized, with one dynamic end called the barbed end and the other end called the pointed end [1]. Importantly, actin filaments are flexible. First, they can bend along their long axis with a persistence length (a mechanical parameter that quantifies stiffness) of about 17 μm [67]. Second, actin subunits in a filament can also twist and tilt [68–70], leading to long-range rearrangements of actin subunits within filaments along the filament long axis. Lastly, several actin subdomains, such as the DNAse-I-binding loop, are highly motile. Thus, actin filaments can adopt multiple conformations (Figure 2). This is the case for actin filaments in different nucleotide states (ATP, ADP-Pi or ADP) [71,72], but also for actin filaments with identical bound nucleotides [73–76]. How energetically favorable each conformation is relative to the others is not known, but it seems probable that Brownian motion helps actin filaments switch from one state to another, an effect referred to as actin filament 'breathing' [77]. Actin filament conformations vary in terms of stability, and this may explain the variations observed in filament disassembly rates *in vitro* [78].

All of these results take on another dimension when considered in the context of the observation that many ABPs exhibit a binding preference for specific actin filament conformations *in vitro* (Table 1) [75]. These effects imply on the one hand that ABPs might stabilize specific actin filament conformations with long-range effects, and on the other hand that ABPs might selectively bind to specific actin filament populations that are in their preferred conformation. This is the case for ADF/cofilin, which has been shown to interact with and stabilize a specific conformational state of ADP–F-actin [59,61,79,80], modifying the mechanical properties of the filaments [81–83]. Structural modification of F-actin induced by an ABP has also been reported for other side-binding proteins, such as myosins [84–86], and for barbed-end binding proteins, such as gelsolin [87–89].

Another important point is that allosteric interactions can have long-range effects on individual actin filaments, maintaining the filaments in a specific conformation over biologically relevant distances [75,87], although not necessarily over the entire length of the actin filaments [74]. Thus, binding a single ABP may be sufficient to modify the structure of the filament over a long distance [83,90], explaining why additional ABPs would subsequently bind cooperatively to the filament to form regions of actin filaments saturated

with this ABP. This is true for binding of ADF/cofilin, and it was originally proposed that the severing of the filaments happens because the conformation adopted by ADF/cofilin-decorated filaments is unstable [91]. However, this model is contradicted by the observations that actin filaments fully decorated by ADF/cofilin are stable and that fragmentation events occur mainly at low ADF/cofilin concentration [60,90]. Therefore, a competing model proposes that the fragmentation events necessary for the disassembly of actin networks happen at the interface between ADF/cofilin-decorated and non-decorated regions of filaments, where actin filaments are forced into an unstable conformation [62,81–83,90].

A variety of studies suggest that upon nucleation, actin filaments adopt a certain conformation, which favors binding by a specific subset of ABPs. In other words, actin filaments acquire an identity at birth. Long-range structural effects can be imparted on actin filaments mediated by nucleation by formins and the Arp2/3 complex. In a succession of papers, the Nyitrai group used temperature-dependent fluorescence resonance energy transfer (FRET) and fluorescence anisotropy to demonstrate that the binding of formin at the barbed end of actin filaments induces modifications to the mechanical and structural properties of the actin filament [75,92–94]. These modifications could be due in part to the nucleotide state of these formin-derived filaments, since formins have been shown to accelerate the hydrolysis of the ATP-bound nucleotide during elongation [43,95], although the latter conclusion remains controversial [96,97]. Also, cryo-electron microscopy images of Arp2/3–F-actin branches along with tomographic reconstruction showed that the Arp2/3 complex induces conformational modifications in the mother filament to which they are bound [98]. Interestingly, these Arp2/3-induced modifications in the mother filament seem conserved across species. These related studies demonstrate that conformational modifications of an actin filament are induced by nucleators, with potential long-range effects, suggesting that actin filaments acquire a specific conformation at the time that they are nucleated.

These observations are important for understanding how multiple ABPs collaborate to optimally regulate actin filaments. For example, interesting *in vitro* studies reveal that formins interact with and recruit tropomyosins to actin filaments, while tropomyosins have major effects on actin dynamics at the barbed end of the filament [99,100]. In fact, while *Schizosaccharomyces pombe* tropomyosin (SpTm) on its own inhibits elongation from the barbed end of the actin filament, it induces a twofold increase in the rate of elongation at the barbed ends of actin filaments nucleated by the formin Cdc12p [100]. Also, SpTm facilitates the annealing of actin filaments capped by Cdc12p, suggesting that tropomyosin may help to maintain actin filaments in a similar conformation all along their lengths and therefore favor end-to-end annealing of formin-capped actin filaments. These observations were corroborated *in vivo* by the observation that tropomyosin prefers to re-localize to the formin-nucleated contractile ring during cytokinesis in a fimbrin-null yeast strain in which tropomyosin inappropriately associates with endocytic actin patches during interphase [66]. It would be interesting in the future to determine whether collaborative effects between proteins bound to actin filaments are isoform dependent in more complex eukaryotes.

Influence of the Geometry of the Actin Filament Network

Another physical aspect of actin networks that could also influence the binding properties or the activities of ABPs is the geometry of the networks, which is governed by the nature and the geometrical organization of the proteins that initiate the network [101]. For example, Nagy *et al.* [102,103] showed that myosin X, as an obligate dimer, selectively walks in a processive manner along actin bundles while avoiding single filaments. In contrast, other studies show that severing factors, such as villin and ADF/cofilin, are principally active in severing single actin filaments while having less activity on actin filament bundles *in vitro* [44,104].

Actin Filament Identities Dictated by ABPs

The results described above indicate that actin filaments have specific properties depending on the cellular structures to which they belong. Recent research suggests that many proteins characterized previously as actin-filament bundling proteins may have an important function in maintaining filament identity. In fact, since many proteins were shown to have crosslinking, decorating or stabilizing effects on actin filaments *in vitro*, it has generally been accepted that their principal *in vivo* functions are to modify the organization and/or mechanical properties of the actin networks. However, as Kovar *et al.* [5] discuss in their recent review, it is important to consider whether the observed *in vivo* phenotypes that result from mutations in genes encoding these proteins are the result of direct or indirect effects. For example, Skau and Kovar [66] demonstrated elegantly that some phenotypes observed in a fimbrin-null yeast strain are not due to the absence of fimbrin, but rather are due to resulting ectopic mislocalization of tropomyosin to actin patches, where fimbrin normally resides. In fact, in this study, the authors showed that the fimbrin loss-of-function phenotype can be partially rescued by a loss-of-function mutation of tropomyosin. Thus, this study demonstrated that fimbrin's most important function in actin patches is not to provide crosslinking between filaments as previously thought, but rather to maintain the specific biochemical identity of the actin patches. It will be important in the future to experimentally challenge at a cellular level currently held views of the functions of other ABPs.

Model for Assembly of Linear and Branched Networks of Appropriate Protein Composition

Here we attempt to synthesize the results presented in the previous sections and to formulate a general working model for assembly of branched and linear networks of actin filaments of appropriate protein composition (Figure 3). This model is based on the observations detailed above that: a specific nucleator can by itself create an actin filament network of a defined organization and composition; actin filaments acquire specific identities depending on the structures into which they are assembled; and the affinity of ABPs for actin filaments is dependent upon the identity of the actin filament.

The first point for this model, that the composition of a specific actin filament network is mainly determined by the type of actin nucleator that generates it, was demonstrated *in vitro* for Arp2/3-derived networks in yeast [45]. In other words, activation of one kind of nucleator in a spatially limited area of its cytoplasm is sufficient to specify which proteins will associate with the resulting network and will dictate the network's properties (Figure 3).

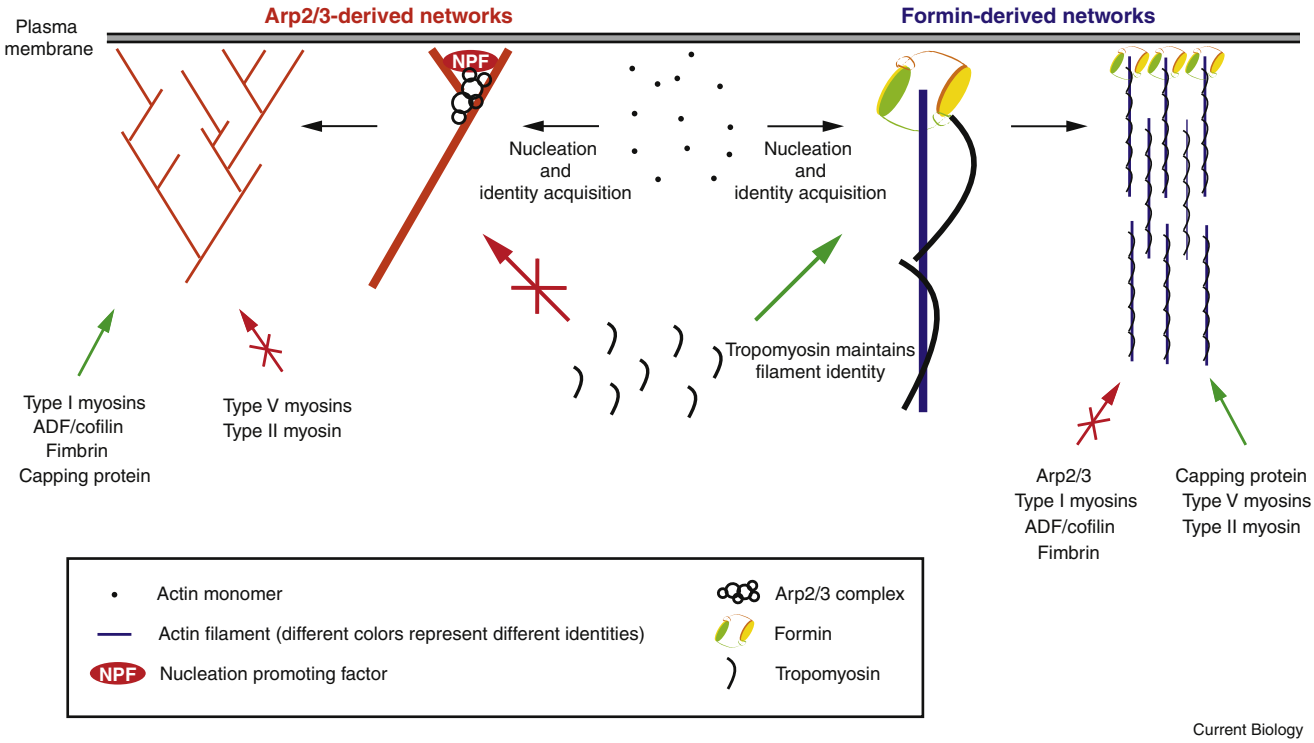

Figure 3. Model for the formation of Arp2/3-nucleated (branched) and formin-nucleated (linear) networks of actin filaments of distinct protein composition in eukaryotic cells.

In this model, actin filaments nucleated by formins (right) and the Apr2/3 complex (left) are generated with distinct conformations, therefore acquiring specific identities at birth (represented by the different colors of the filaments). Tropomyosin specifically decorates formin-nucleated actin filaments and maintains their identity all along their lengths. Arp2/3- and formin/tropomyosin-derived networks subsequently become accessible to specific subsets of ABPs.

In areas of cells in which formins are activated, linear arrays of actin filaments are nucleated [23,24]. Because formins establish the local conformation of actin filaments [75,92–94], the filaments acquire their specific identity at the moment they are nucleated (Figure 3, blue filaments). As a consequence, proteins that bind to actin filaments with this identity will interact with these structures, while those that do not bind, such as the proteins that localize with branched networks, will not interact with these structures. Since formins actively cooperate with tropomyosins *in vitro* [99,100], we propose that formins are key factors that target tropomyosins to linear arrays of actin filaments in order to maintain filaments with this identity. In areas of eukaryotic cells in which NPFs activate the Arp2/3 complex, actin filaments are nucleated and organized into branched arrays [12,13]. Since the Arp2/3 complex has also been shown to modify the structure of the actin filaments [98], we propose that these filaments also acquire a specific identity at the time they are nucleated, distinct from the identity of actin filaments in linear networks (Figure 3, red filaments). This identity allows a different set of ABPs to associate with actin filaments in branched networks, and to antagonize binding of other ABPs, such as the tropomyosins [55,56].

This model is well adapted for yeast, wherein actin filaments acquire a specific identity upon nucleation and maintain this identity until they age or disassemble. While the model also seems to fit well with more complex cell types, it has been proposed that in some cases one kind of actin filament network might evolve from a pre-existing different kind of network, as seen, for example, with lamella and lamellipodia [11,19,20]. Recent work demonstrates that, after a period of lamellipodium protrusion, a period of retraction follows, wherein filaments localizing at the leading edge of cells suddenly become substrates for type II myosins and condense rearward as transverse arcs in the lamella [19,20,54]. It seems that these arcs could arise from an existing sub-population of unbranched actin filaments nucleated by formins within the lamellipodium [54,105], or be derived from branched networks [19]. In the latter case, our model predicts that branched filaments of the lamellipodium need to be progressively debranched in order to become progressively accessible to tropomyosins and to change their identities, therefore becoming accessible to a different set of ABPs, such as type II myosins for contraction of the network, and to crosslinkers, such as α-actinin. What might trigger the de-branching of the network is still unclear, but one hypothesis is that ATP hydrolysis by Arp2 and possibly Arp3 could be involved [56]. Another hypothesis is that additional factors, such as the cofilin homolog GMF, might bind to the Arp2/3 complex and destabilize the branches [106].

Conclusions and Future Perspectives

In this review, we attempted to highlight the fact that the properties of actin filaments within distinct networks are not identical and that this may be due to different actin filament conformations adopted at the time of nucleation and then stabilized by certain binding partners. So far, most available information about biochemical properties of actin

filaments and their associated proteins has been obtained using simple systems containing a limited number of factors. Modifications to filament conformation and emergent properties resulting from combined effects of multiple different ABPs have not been considered. Also, how actin filament assembly and disassembly rates are affected by their structural conformation is not known. Therefore, complex models involving multiple proteins but based on biochemical parameters measured in simple systems should be interpreted with care. To build more relevant models in the future it will be important to develop approaches to assay key system parameters, such as filament conformation and assembly and disassembly rates resulting from interactions with the full panoply of ABPs present in cells.

Three approaches seem to be particularly important in order to reach this goal. First, more structural and mechanical information about actin filaments when they are associated with binding proteins is needed. Presently, obtaining structural information about actin filaments in cells is probably not possible. However, obtaining more information *in vitro*, for example with the use of cryo-electron microscopy techniques, seems attainable. Such an effort should help us to better understand cooperative and competitive interactions among ABPs. Second, powerful reconstituted systems mimicking actin-based cellular processes are needed. Many important findings during the past 20 years have come from such systems, using a bottom-up approach with purified proteins or a top-down approach with cytoplasmic extracts, wherein many if not all the binding partners are present in solution [107–109]. Third, more genetic and RNAi studies, like the one described above involving tropomyosin and fimbrin [66], to test functional relationships between different ABPs *in vivo*, are required. These three approaches will provide powerful tests of current models and will yield new insights into the mechanisms that establish actin filament identities.

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