Published in final edited form as:

Annu Rev Biophys. 2015 ; 44: 285–310. doi:10.1146/annurev-biophys-060414-034308.

Specification of Architecture and Function of Actin Structures by Actin Nucleation Factors*

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Abstract

The actin cytoskeleton is essential for diverse processes in mammalian cells; these processes range from establishing cell polarity to powering cell migration to driving cytokinesis to positioning intracellular organelles. How these many functions are carried out in a spatiotemporally regulated manner in a single cytoplasm has been the subject of much study in the cytoskeleton field. Recent work has identified a host of actin nucleation factors that can build architecturally diverse actin structures. The biochemical properties of these factors, coupled with their cellular location, likely define the functional properties of actin structures. In this article, we describe how recent advances in cell biology and biochemistry have begun to elucidate the role of individual actin nucleation factors in generating distinct cellular structures. We also consider how the localization and orientation of actin nucleation factors, in addition to their kinetic properties, are critical to their ability to build a functional actin cytoskeleton.

Keywords

formin; Arp2/3; contractile ring; stress fiber; lamellipodium

INTRODUCTION

Actin assembly in motile cells is critical for several processes, from protruding the leading edge of a cell to regulating transcription to controlling organelle shape and size. To accomplish these functions, a cell builds several distinct actin structures, and coordination of actin polymerization among these structures presents a significant challenge for the cell. Although some regulation may be accomplished via compartmentalization or differential localization of actin-binding proteins, it is unlikely that these mechanisms alone are able to specify the variety of actin structures. Therefore, cells use a diverse group of actin nucleation factors to spatially and temporally construct different actin structures, and the organization and dynamics of these structures are defined by the nucleation factor. Many actin nucleation factors have been identified and biochemically characterized, although

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

much about their *in vivo* localization, roles, and regulation remains unknown. In this review, we explore the role of actin nucleation factors in constructing three different geometries of actin structures: dendritic networks, actin bundles, and isotropic networks (Figure 1). We first describe why cells require actin nucleation factors, along with the molecular mechanisms of the three main classes of actin nucleators. We then discuss the organization of actin structures in the cell and how the properties and orientation of each nucleator contribute to the different classes of structures.

THE BIOCHEMISTRY OF ACTIN IN CELLS

Why Are Actin Nucleation Factors Essential?

Actin nucleation is tightly controlled because (a) the critical concentration for assembly of actin monomers into filaments (F-actin) is low, and (b) actin filament assembly is energetically costly: the rate-limiting step for spontaneous actin assembly is the formation of the trimer. However, the vast majority of monomeric globular actin (G-actin) in the cell is bound by G-actin-sequestering proteins, such as profilin, that prohibit spontaneous nucleation (92, 132). To regulate the assembly of sequestered actin monomers, cells use actin nucleation factors, three main classes of which are found in most mammalian cells. These different classes form distinct actin structures that perform different functions in cells. They are the actin-related proteins 2 and 3 (Arp2/3) complex and its activating factors, tandem monomer binding proteins (TMBPs), and formins.

What Are the Mechanistic Differences Between the Three Main Classes of Actin Nucleation Factors?

The first actin nucleation factor to be discovered was the Arp2/3 complex (70, 124), which nucleates a new actin filament as a branch off of the side of an existing filament at an approximately 70° angle (81, 108). The core of this seven-member complex comprises Arp2 and Arp3, which are structurally similar to actin monomers (77, 94, 124), and the complex cannot function without a nucleation promoting factor (NPF) (for review, see Reference 96). The NPFs are grouped into related families described in Table 1: WASP/N-WASP, WAVE, WASH, and WHAMM (28, 33). After nucleation, the Arp2/3 complex does not affect the rate of assembly at the fast-growing barbed end of the actin filament; rather the complex remains associated with the pointed end (3, 81). Thus the Arp2/3 complex, activated by an NPF, nucleates the development of a branch from an existing actin filament.

The two other families of actin nucleation factors create unbranched filaments. The first family, TMBPs, includes Spire, Cordon-bleu, leiomodin, VopL/F, and JMY, and members of this family bind at least three actin monomers via WH2 domains, mimicking the stable actin trimer (91, 139). Similar to the Arp2/3 complex, TMBPs bind the pointed end of the filament and leave the barbed end free (91). Vasodilator-stimulated phosphoprotein (VASP) is related to this group, and this protein has been reported to have nucleation properties *in vitro* (43), although it likely functions as an elongation factor *in vivo* and, unlike other TMBPs, regulates assembly at the barbed end of filaments (13, 109, 126). Thus, diverse TMBPs utilize different mechanisms of monomer clustering to stabilize the formation of linear actin filaments with free barbed ends.

The second family of linear filament nucleation factors, formins, stabilizes the spontaneously formed actin trimer and remains processively associated with the fast-growing barbed end of a filament, regulating its elongation (61). Formins have a conserved actin polymerization core consisting of the FH1 (formin homology 1) and FH2 (formin homology 2) domains. The proline-rich coils of the FH1 domain bind profilin-actin complexes and promote addition of actin monomers to the barbed end of the filament, which is bound by the FH2 domain. The FH2 domain is a homo-dimer that binds like a ring around the outside of three interacting actin monomers, stabilizing the actin seed and regulating monomer addition at the barbed end (86, 131). Flexibility between the halves of the homodimer allows the formin to remain processively bound to the growing barbed end of the actin filament (62, 131). Formins are therefore unique as actin nucleators, which remain bound to the growing barbed end of linear actin filaments. The next sections describe how these diverse actin nucleators define distinct actin structures.

DENDRITIC NETWORKS

The following subsections discuss the structure, composition, and function of dendritic actin networks, along with how the Arp2/3 complex defines and controls them.

How Do the Properties of the Arp2/3 Complex Define the Functional Properties of Dendritic Networks?

Dendritic networks are used to generate pushing force against a membrane wherever they are found in the cell, and the properties of the Arp2/3 complex make it ideal for formation of structures that participate in this pushing process. Many NPFs are associated with the membrane and thus locally activate the Arp2/3 complex there (96). Filaments near the membrane push against it via a Brownian ratchet mechanism upon incorporation of new monomers at their barbed ends (138). Stochastic, rapid incorporation of capping protein onto the free barbed ends of these filaments distributes force along the entire edge of the membrane, allowing the dendritic network to act as an expanding gel (72). At the leading edge of a cell, compression from the membrane curves the actin filaments away from it, and as the Arp2/3 complex branches off of the convex side of a curved actin filament more frequently than off of the concave side (93), the Arp2/3 complex is biased toward generating productive branches that are oriented toward the leading edge of the cell (93). The dendritic network is therefore equipped to handle compressive forces from the membrane and to provide force over a large two-dimensional area (93). Thus, specific properties of the Arp2/3 complex tailor it to generate a force-producing dendritic network at the leading-edge membrane.

The leading edge of a cell is not the only structure that employs a network polymerized by Arp2/3, however. Endocytosis, bacterial pathogens, and cell-cell junctions also use Arp2/3-created actin networks to push against membranes. The organization and role of this actin network are less well established for the case of endocytosis than for the other two examples. Endocytic actin may be arranged similarly to the lamellipodial network. Alternatively, growth at the inner surface of an object such as a vesicle may exert squeezing forces as the Arp2/3 gel expands around it (93). The bacterium *Listeria* takes advantage of

squeezing forces generated by Arp2/3-mediated actin polymerization to rocket around inside cells and spread to neighboring cells (66), and endocytic vesicles may use a similar structure. The Arp2/3 complex is also critical in formation of adherens junctions (AJs) at cell–cell contacts (1, 47). Initial contacts may be formed using Arp2/3-mediated lamellipodia similar to those seen at the protruding edge of a cell, although cells forming AJs are not motile. As it does for lamellipodia, the Arp2/3 complex produces a force-generating network used for pushing a membrane in both endocytic vesicles and AJs. We now discuss the organization of Arp2/3-generated structures in greater detail.

Lamellipodia.—Nucleation of the branched actin network that makes up the lamellipodium depends on the Arp2/3 complex and, in fact, this structure was the first for which the importance of the Arp2/3 complex was characterized. The following sections describe current knowledge regarding the organization and dynamics of the lamellipodium as generated by the Arp2/3 complex and other actin nucleation factors.

What is the architecture of the lamellipodium?—The lamellipodium is a thin, veil-like region of cytoplasm that dynamically protrudes and retracts along the front edge of migrating cells based on the directed polymerization of actin filaments (46). Lamellipodia in migrating cells contain a dendritic meshwork of actin filaments and extend laterally up to tens of micrometers along the cell edge, although they are typically less than two micrometers wide (105). When membrane tension is low, actin polymerization drives the membrane forward to protrude the leading edge. When tension is high, however, polymerization drives retrograde flow, which, coupled to extracellular matrix adhesion, generates traction to drive cell movement (121, 123). Thus, polymerization of the actin network at the lamellipodium is the motor that drives the cell forward.

What proteins are necessary and sufficient for generation of a lamellipodium?

—The proteins essential for generating a functional Arp2/3 network are actin; Arp2/3 with an NPF; cofilin; capping protein; and an actin monomer-binding protein, profilin (66). Because the Arp2/3 complex is intrinsically inactive, the NPF localization is critical for determining where Arp2/3 polymerizes actin (28, 70, 125). Thus, association of NPFs with the membrane at the leading edge of the cell is essential for the Arp2/3 complex to generate a lamellipodium. The actin-severing protein actin depolymerizing factor (ADF)/cofilin is also important for generating a lamellipodium via actin turnover (14, 83). In motile cells, ADF/cofilin is localized behind the lamellipodial network, where it severs older actin filaments (7, 71, 108). This severing promotes monomer recycling and thereby supplies the Arp2/3 at the leading edge of the cell with enough G-actin to drive rapid membrane protrusion (90). Capping protein also increases branching by blocking the assembly of filaments and thus directs monomers toward new nucleation by the Arp2/3 complex (2). Additional proteins that regulate the Arp2/3 complex, including cortactin and coronin, are also found at the edge, and new Arp2/3-regulatory proteins such as WASP-interacting protein (WIP), capping protein, Arp2/3 and myosin I linker (CARMIL), and glial maturation factor (GMF) are under active study (for review, see Reference 22). Together, Arp2/3 and its NPFs directly drive the polymerization of a dendritic actin mesh, and this process critically

depends on actin monomers being released by ADF/cofilin-based severing and funneled to the Arp2/3 complex by capping protein.

How do other actin nucleation factors contribute to formation of the lamellipodium?—Recent work indicates that other actin nucleation factors besides Arp2/3 play a role in generating lamellipodia. The formin FMNL2 (also called FRL3) accumulates in filopodia and at the lamellipodium (15), where it works with the Arp2/3 complex to regulate the rate of protrusion (15). Other formins have also been proposed to contribute to the formation of lamellipodia, including the Diaphanous formin mDia2 (134) and inverted formin-2 (INF2) (103a), although details about their roles in that process remain unclear (Table 2) (for review, see Reference 63). The dendritic network generated by Arp2/3 is critical for lamellipodial protrusion; other actin nucleation factors at the leading edge of the cell likely make a secondary contribution (66).

Endocytosis and vesicles.—Extensive biochemical and cell biological work in yeast has clearly delineated an essential role for actin polymerization by the Arp2/3 complex in endocytosis. In fact, the steps of the endocytic pathway in yeast are spatially and temporally well characterized. However, the role of actin polymerization in mammalian cell endocytosis is much less well studied, and the geometries of the actin structures in endocytosis remain poorly understood.

Is actin essential for mammalian endocytosis?—Although actin is essential for endocytosis in yeast, the role of actin assembly in clathrin-mediated endocytosis (CME) in mammalian cells is debated (for review, see Reference 79). Initial studies indicated that the role of actin varied depending on the cell type and on whether the cells were adherent or in suspension (36). Thus, actin was thought to play an accessory role in endocytosis. Several studies also indicated that the importance of actin depended on local membrane tension and on the ability of clathrin to assemble on a membrane under tension (9, 17, 65). Taken together, these studies suggest that actin has a supporting role in mammalian endocytosis.

What is the source of actin for endocytosis?—Other studies have identified an essential role for actin in CME (73, 75, 136), suggesting that it likely generates force at the membrane. Both the Arp2/3 complex and N-WASP localize to sites of endocytosis (74). Electron microscopy has shown short, branched filaments and the Arp2/3 complex at sites of clathrin-coated pit invagination, where the barbed ends of actin filaments were directed toward internalizing clathrin structures (29). We therefore propose that actin generated by Arp2/3 is critical for the later stages of endocytosis and plays a role in pushing the plasma membrane around a forming vesicle, similar to its role in pushing the plasma membrane forward at the leading edge of a cell. However, how actin generated by Arp2/3 is organizationally related to lamellipodial actin is unknown.

Adherens junctions.—AJs are dynamic complexes of proteins that connect neighboring cells to each other. Extracellular cadherin molecules connect to each other and span the plasma membrane, where they interact with catenin molecules inside the cell. Catenins can bind directly to the actin cytoskeleton, and they can also interact with other actin-binding

proteins (for review, see Reference 76). However, the organization of actin at AJs remains somewhat unknown.

What is the role of the Arp2/3 complex in adherens junctions?—Arp2/3-mediated dendritic actin networks are also critical for structures that connect cells. Adherens junctions rely on the homotypic interaction between cadherins in adjacent cells and catenins that link cadherins to the cortical actin cytoskeleton (for review, see Reference 76). The Arp2/3 complex can bind to E-cadherin directly and localize to AJs, where it can regulate actin polymerization (60). The Arp2/3 complex, in conjunction with its activators (60, 76), is required to establish and maintain AJs (1, 47, 118). Similar to a lamellipodium, a dendritic actin network at the cell edge can push the membrane outward, allowing extensive contact with neighboring cells and the formation of junctions.

Are other actin assembly factors associated with adherens junctions?—Some evidence also suggests that the formins Diaphanous 1 (Dia1) and Formin 1 (FMN1) may be important at AJs (23, 57), but inhibition of formins in general does not disrupt cell–cell junctions (110). Interestingly, however, in some cell types, the cortical actin cytoskeleton underlying the plasma membrane at the site of AJs is composed of linear actin bundles rather than a dendritic meshwork (47). Thus actin at AJs may comprise either two distinct networks, one made by a formin and the other by the Arp2/3 complex, or a single network resulting from the cooperation between formins and Arp2/3. We postulate that the network created by the Arp2/3 complex is more important for the initial generation of AJs by pushing the membrane forward (and thus promoting contact with neighboring cells) using a mechanism common to all dendritic networks.

ACTIN BUNDLES

What Are the Functional Differences Between Dendritic Networks and Linear Bundles?

In contrast to the relatively uniform dendritic networks that generate pushing force over a large area, linear actin bundles are heterogeneous: some of these structures are composed of filaments of uniform polarity, whereas others are bundles of filaments with mixed polarity. The roles of linear actin bundles are also heterogeneous. Bundles with uniform polarity can push a membrane forward at a very local site or can act as a noncontractile tether that connects one organelle in the cell to a contractile actin network. Once they are connected to the contractile networks, uniform polarity bundles can be used to pull on targets. Conversely, mixed polarity bundles are intrinsically contractile and can therefore provide constriction forces to deform substrates or generate force by shortening. Thus, owing to organizational differences generated by actin nucleators, linear bundles of actin filaments play roles that are distinct from those played by dendritic networks and by each other.

How Do the Molecular Properties of Formins Contribute to Generation of Diverse Linear Bundle Structures?

Formins generate linear actin structures with specific properties. Different formins have different biochemical properties, and these properties define organizational and functional differences among linear structures (52). Formins can define an actin structure both directly

and indirectly. The variation among kinetic properties of formins allows them to act directly on actin structure formation by nucleating and assembling actin at different rates and by modifying actin filaments by bundling or severing them. Additionally, recent evidence shows that application of force to formins can modulate the rate of actin polymerization (51). These parameters define how a structure grows and disassembles. Formins can also act indirectly by modifying the flexibility and twist of actin filaments (88), and these changes can alter which actin-binding proteins associate with which actin bundles (103, 112). Thus, the biochemical properties of formins make them well-suited to generating both uniform and mixed polarity bundles that have a variety of kinetic properties (Table 2).

The orientation of formins within a structure is critical to defining its geometry (Figure 2). Formins can polymerize actin structures with either uniform or mixed polarity because in contrast to the Arp2/3 complex, formins do not have an intrinsic nucleation bias. Therefore, the polarity of a bundle must be defined by physical tethering. To create a bundle of uniform polarity, for example, formins localized to a defined spot in the cell would all be oriented in the same direction, and the barbed ends of the actin filaments would be retained at a single location (**Figure 2a**). To create a bundle of mixed polarity, however, formins would have to be spaced along the bundle track and oriented away from each other. This orientation would result in polymerization of filaments with barbed ends grouped at the location of the tethered formin but with pointed ends moving in opposite directions (**Figure 2b**). Interestingly, some formins associate with microtubules, and this association may provide an actin-independent mechanism for localization (37). Thus, formin orientation is critical to the ability of formins to generate bundles of both uniform and mixed polarity, although the mechanisms of orienting and tethering these proteins are unknown. We now describe each type of bundle, providing specific examples of distinct bundle structures and what is known about how nucleators define these structures.

UNIFORM POLARITY ACTIN BUNDLES

What Are the Roles of Uniform Polarity Actin Bundles?

Actin bundles with uniform polarity have two main roles. They can push the membrane forward, as filopodia do, or they can act as noncontractile tethers. Bundles of uniform polarity are not effective substrates for myosin II; however, they can be connected to contractile actin networks, from which the tethered bundles can transmit force to other structures. Bundles with uniform polarity can be generated in multiple ways. Formins localized at a single site could orient in the same direction relative to each other and thus polymerize filaments in the same direction. Alternatively, bundling proteins, such as fascin, or motor proteins, such as myosin, could align filaments into a bundle. At least two structures in the cell are made up of actin filaments with uniform polarity: filopodia and dorsal stress fibers (SF). We also postulate that, given their function and organization, transmembrane actin-associated nuclear (TAN) lines may be composed of bundles of uniform polarity. We now discuss the architecture and role of each structure, along with what is known about how each is polymerized.

Filopodia.—One of the most obvious structures on many motile cells is a series of thin spikes extending from the plasma membrane at intervals around the cell. Although these structures were originally identified as microvilli, they have since been recognized as being a separate kind of dynamic protrusion with their own actin organization and function: the filopodia. Furthermore, recent work has begun to delineate different roles for different types of filopodia in a single cell.

What are filopodia?—Filopodia are slender, finger-like projections that protrude from the edge of a cell and are made up of a plasma membrane–enclosed array of actin filaments bundled together by fascin (for review, see Reference 16). Individual actin filaments within a bundle span the entire length of the filopodium and are oriented such that their fast-growing barbed ends are at the distal tip (82). Similar to the dendritic network, actin polymerization at the tip of a filopodium pushes the membrane forward; in contrast to the dendritic network, however, the main role of most filopodia is not to drive cell motility. Filopodia are critical for early cell spreading by functioning as local sensors, termed “sticky fingers,” that allow integrins to detect properties of the extracellular matrix and find permissive attachment sites (38). The ability of filopodia to protrude dynamically is critical for their ability to explore space, and the mechanism underlying this dynamic protrusion is in contrast to that of the dendritic actin network, which provides more force but cannot be fully remodeled as rapidly.

How are filopodial bundles generated by actin nucleation factors?—Filopodia are embedded in the dendritic actin mesh at the leading edge of the cell (90, 98), although it is still unclear whether initiation of filopodia formation depends on an actin nucleation factor. Original models propose that long filaments form spontaneously within the dendritic network, whereupon VASP prevents capping and promotes elongation at the filopodial tip (109, 135). Subsequent work, however, has shown that the formin mDia2 plays an important role in filopodia formation (100, 134). VASP and Dia2 interact at filopodia (32, 99). mDia2 may therefore protect filaments in the lamellipodium from capping, promoting their elongation, and may also promote the association of lamellipodial filaments with VASP through its interaction with mDia2 (134, 135). The details of this interaction are still under study (8, 13, 126), but it now seems clear that the formin Dia2 cooperates with VASP to promote the assembly of filopodia.

mDia2 is not the only formin thought to play a role in filopodia assembly. Recent work has shown that the formin DAAM1 (disheveled-associated activator of morphogenesis 1) localizes along the length of the filopodium in a manner dependent on its physical interaction with fascin (50). Depletion of DAAM1 leads to losses of filopodial integrity and number (50). DAAM1 appears to function primarily as a bundling protein, rather than as a polymerase (50). Along the length of filopodia, DAAM1 may work with mDia2 as part of a hand-off mechanism to help elongate filaments, but this has not been demonstrated. Additionally, the formin FRL2 (also called FMNL3) localizes to the tips of filopodia and can induce filopodia formation, although the mechanisms underlying these behaviors are not entirely clear (44). Thus, multiple actin nucleation factors may function together to generate a filopodial bundle. Critically, filopodial actin nucleators must be gathered at the tip of the

filopodium and must all be oriented in the same direction to ensure that actin polymerization drives membrane protrusion, rather than filament polymerization back into the cell.

Interestingly, some cell types can produce multiple kinds of filopodia, depending on biochemical differences among the actin nucleation factors that create the protrusions (13, 126). In Schneider 2 (S2) cells from *Drosophila*, filopodia formed by Ena, a member of the VASP family, are more dynamic but shorter than those produced by Dia (13). Similarly, mammalian fibroblasts expressing either VASP or mDia2 generate filopodia that have different dynamics, morphology, and number but similar molecular compositions (8). Thus, a single cell type can express multiple types of filopodial protrusions that may have different *in vivo* roles.

Dorsal stress fibers.—Both electron microscopy and fluorescence microscopy allow visualization of an intricate network of apparently parallel actin bundles that span the length of many cell types. Even before any molecular mechanism for generating these fibers was proposed, two distinct networks could be identified in cells spread on a solid substrate: one near the dorsal side of the cell, and one at the ventral side near the substrate. These fibers were coincident with the long-identified stress fibers or tension striae, and further examination revealed that actin was providing this tension. The following sections describe different types of actin stress fibers.

What are the organization and function of dorsal stress fibers?—Actin SF can be divided into three categories on the basis of their cellular location and assembly mechanisms: dorsal SF, ventral SF, and transverse arcs (48). Of these, only dorsal SF bundles have uniform polarity; transverse arcs and ventral stress fibers have nonuniform polarity. Therefore, we discuss only dorsal SF in this subsection; transverse arcs and ventral SF are considered with other nonuniform bundles.

In culture, dorsal SF run roughly perpendicular to the leading edge of migrating cells and terminate with their barbed ends at a focal adhesion on the ventral side of adherent cells (48). Because the filaments in the dorsal SF have a uniform orientation, they largely lack myosin II (48, 111). However, the ends of the bundles not associated with focal adhesions connect either to the contractile actin mesh on the dorsal side of the cell or to rearward-flowing transverse arcs (48). Force transmitted to focal adhesions from the dorsal cortex through dorsal SF is critical for the elongation and maturation of these structures (27). We further postulate that the uniform orientation of filaments in dorsal SF is critical for their ability to direct this elongation; pulling on noncontractile dorsal SF by the cortical actin mesh generates a unidirectional force for focal adhesion elongation. If dorsal SF were mixed polarity bundles, this pulling could result in bundle stretching and less transmission of force to the focal adhesions. Recent work (84, 103a) has indicated that dorsal SF play a critical role in generating the elongated fibrillar adhesions used by fibroblasts to remodel and build the ECM. Thus, despite being localized near the leading edge of cells, dorsal SF are not used for pushing the membrane forward; rather, these structures appear to be involved in rearrangement of proteins at focal adhesions via their connection to a contractile actin network.

How are uniformly oriented dorsal stress fibers generated?—The formation of dorsal SF relies on the uniform orientation of formins at focal adhesions; the uniformity of formin orientation tethers the barbed ends of all actin filaments at these adhesions. Hotulainen & Lappalainen (48) proposed that the formin mDia1 contributes to elongation of stress fibers from the distal side of focal adhesions; however, neither mDia1 nor mDia2, which may also play a role in SF formation (41), has been localized to dorsal SF. We have now shown (103a) that the formin INF2 localizes to the proximal end of dorsal SF at the junction with focal adhesions, and INF2 nucleates and polymerizes actin at that location. In fact, polymerization of actin at the proximal end of dorsal SF is critical for the transition of focal adhesions into fibrillar adhesions. Thus dorsal SF are generated by actin filament polymerization in a uniform direction from a discrete spot, the focal adhesion, and these fibers function as a tether to transmit force from the contractile actin network to focal adhesions in a defined direction.

TAN lines and the nucleus.—The term dorsal SF has largely been applied to the actin bundles terminating in a focal adhesion near the edge of the cell and reaching toward the dorsal side of the cell to connect to the actin cortex there. However, some stress fibers terminate in focal adhesions and run all the way across the dorsal side of the nucleus. The following section considers these fibers to be distinct from dorsal SF despite their somewhat similar locations.

Why does a cell need actin around the nucleus?—Positioning the nucleus in migrating cells is a significant challenge given the size and relative inflexibility of the nucleus compared with those of the cytoplasm. Cells migrating into a scratch wound use an actin-based system to position their nuclei toward the rear of the cell and behind the microtubule organizing center (MTOC), which is oriented toward the leading edge (39, 40). As cell migration into the wound is impaired in the absence of active nuclear positioning systems (69), actin-based positioning of the large, rigid nucleus is a key to efficient cell migration.

How are actin structures that control nuclear position organized, and how are they formed?—The major structures shown to control nuclear position are TAN lines, which are composed of aligned actin bundles that run along the dorsal side of the nucleus parallel to the axis of migration, and linear arrays of the LINC (linker of nucleus and cytoskeleton) complex that connects the nucleus to these actin cables (69). Linear organization of LINC proteins on the dorsal side of the nucleus depends on the actin cables (64, 69). The organization of these actin cables has not been conclusively shown, although we hypothesize that they are uniformly oriented actin bundles, given their orientation along the nucleus and their ability to align other protein complexes. In fact, we suggest that TAN lines could function similarly to dorsal SF; that is, they transmit force generated by the contractile actin mesh to proteins integral to the nuclear envelope, and the nuclear envelope proteins in turn reorganize in the direction of applied force. However, it is unclear whether these cables contain myosin II; they may in fact be contractile bundles of nonuniform orientation.

What does this mechanism suggest about the orientation of formins that might nucleate actin cables for TAN lines? Similar to creation of dorsal SF by formins at focal adhesions, formins for TAN lines must all have the same fixed orientation. In contrast to the case of dorsal SF, however, there is no evidence to suggest that the barbed ends of TAN lines are localized to a single location. Instead, we suggest that rearward contraction of stress fibers from the edges of cells could form cables of actin with formins scattered throughout them. Bundling by a protein specific for filaments oriented in the same direction could orient the formins. Alternatively, recent work shows that the formin FHOD1 is critical for TAN line function, although this protein does not nucleate the dorsal actin cables directly (64). Thus, this formin could possibly direct actin cable orientation. The mechanism by which actin cables with oriented polarity on the dorsal side of the nucleus are generated remains to be elucidated.

NONUNIFORM POLARITY ACTIN BUNDLES

What Are the Roles of Nonuniform Actin Bundles?

Many linear actin structures in the cell are composed of mixed polarity filaments. We propose that generation of bundles with nonuniform polarity relies on nucleating factors having a fixed orientation such that the barbed ends of filaments are clustered at a particular location while the pointed ends protrude away; many such locations oriented in a linear fashion would generate a mixed polarity bundle (**Figure 2b**). Alternatively, mixed polarity bundles could be generated by rearrangement of uniform polarity filaments in the dendritic network, as the network contraction model for contractile arcs suggests (117).

Nonuniform polarity bundles are a substrate for myosin II, and the action of myosin II on these bundles results in one of two actions: constriction if the filaments are in a ring and bundle shortening if the filaments are in a linear bundle. For instance, the contractile ring constricts as filaments of alternating polarity slide past each other owing to myosin II activity (78). A similar mechanism may drive mitochondrial fission: actin assembles around the mitochondrion and aids in constricting it via a myosin II-dependent mechanism (45). Additionally, as transverse arcs move from the cell edge to the cell center, they shorten as a result of the action of myosin and control the shape of the cell by doing so (20, 21, 48). Thus, nonuniform polarity bundles are critical for constriction or shortening mechanisms that exert the forces needed to control shape.

Cytokinesis and the contractile ring.—A ring of filamentous structures has been seen at the ingressing furrows of cells, particularly those of invertebrate eggs, for almost 50 years (1). Whether this ring pulls the plasma membrane in as the cell divides or merely guides membrane deposition has been a subject of much debate, as has the mechanism of filament constriction. However, studies on a variety of organisms have begun to reach a consensus regarding the mechanism of contractile ring assembly. The following subsections describe current knowledge regarding the role of actin in mammalian cell division.

What structures are responsible for dividing a cell?—Assembly of the contractile ring in mammalian cells begins at anaphase onset. MgcRacGAP activity results in localization of RhoA to the midzone of the cell (for review, see Reference 78), where local

stimulation of RhoA activity promotes actin polymerization and myosin II activity (89). Myosin II activity causes membrane-linked actin filaments to slide past each other, providing the force needed for ingression of the cleavage furrow (19), so it is critical that actin filaments in the ring are mixed in polarity. The scaffold protein anillin also localizes to the cleavage furrow, where it links the actomyosin ring to the septins that deform the membrane (85). In comparison with the detailed genetic and biochemical work describing the actions of cytokinesis in yeast, however, assembly and organization of the contractile ring in mammalian cells are not well understood.

How is the contractile ring formed in mammalian cells?—RhoA promotes actin polymerization through formin activation (for review, see Reference 140). Whether there is a specific formin at contractile rings remains unclear, although evidence has shown that Arp2/3 has only a minor role at best. Recent work has suggested that mDia2 may be involved in contractile ring assembly, as loss of mDia2 results in contractile ring defects (122). However, data regarding the role of the closely related formin mDia1 present conflicting accounts. Examination of formins belonging to the Dia family found increased binucleate cells when mDia2 was lost, but not when mDia1 was lost (122). In contrast, recent work on cortical actin polymerization identified mDia1 as a critical component of cell division (18). The orientation of formins that generate the contractile ring is another open issue. To create bundles with mixed polarity, formins would have to be fixed relative to each other in a back to back configuration. This could be accomplished by two formin spots on both sides of the contractile ring, each of which polymerizes filaments in both directions around it. However, studies in fission yeast have shown that formin in the contractile ring localizes to a series of spots around the cell and polymerizes filaments radially in all directions from each spot (128). Only filaments that are in the correct orientation to be captured by myosin and that are in nearby spots are incorporated into the contractile ring; off-target filaments are destroyed by cofilin (116, 128). It is unknown whether similar mechanisms are utilized by the Dia formins in vertebrate cells.

Mitochondrial fission.—Although cell division by a constricting ring of actin has been studied for decades, the idea that rings of actin could be used for constriction elsewhere in the cell has been shown only recently. Specifically, mechanisms similar to those involved in cell division may also be at play in mitochondrial division.

How are specific actin structures involved in mitochondrial fission?—Cell division requires assembly of an actin ring that is tens of microns in diameter. However, smaller actin rings can be used to divide organelles within the cell. Depletion of F-actin leads to a loss of mitochondrial fission and to reduced accumulation of the dynamin-related protein Drp1, which is also critical for mitochondrial fission (11, 31). Recent evidence has also suggested that myosin II plays an important role at sites of mitochondrial fission (58). As with the contractile ring, this myosin-mediated constriction mechanism strongly indicates that the actin filaments at the site of mitochondrial division are oriented in an antiparallel arrangement.

How does actin polymerize at sites of mitochondrial fission?—INF2 is essential for the actin polymerization necessary for recruitment of Drp1 (59). INF2-generated actin filaments appear at sites of close contact between the endoplasmic reticulum (ER) and mitochondria (59). A model of mitochondrial fission, termed mitokinesis, has been proposed, in which INF2-generated actin filaments localize to the mitochondria via the ER and “preconstrict” the mitochondria using a mechanism similar to the constriction of a contractile ring (45). Drp1 is then able to localize and complete fission via ring formation of a ring, similar to the behavior of dynamin (45). It is interesting to consider how the ER would be able to position INF2 molecules to generate filaments with mixed polarity if mitokinesis is in fact similar to cytokinesis.

Transverse arcs.—In addition to dorsal SF that run along the length of the cell, motile cells contain other types of linear actin bundles. The following subsections discuss dynamic transverse arcs, which assemble and flow rearward from the leading edge of the cell. Transverse arcs from different cell types vary in thickness; some cell types, such as osteosarcoma cells, show prominent transverse arcs, whereas others, such as fibroblasts, have thinner arcs. We focus specifically on the common assembly mechanism and roles of transverse arcs that are likely the same in many cell types (48).

What are the characteristics of transverse arcs?—In contrast to dorsal SF, transverse arcs run parallel to the leading edge of the cell and do not terminate in focal adhesions (48). Transverse arcs flow toward the center of the cell at the same speed as retrograde flow from the leading edge and disappear in front of the nucleus (104). Both α -actinin and myosin are found in sarcomeric patterns along transverse arcs, indicating that they are mixed polarity bundles (48). In fact, transverse arcs can be seen condensing from short actin bundles in live cells (48).

How are transverse arcs formed and what is their role?—Multiple mechanisms for formation of transverse arcs have been proposed. These arcs may assemble from short pieces of the lamellipodium that are annealed end-to-end (48, 104). This mechanism does not require a specific arc-associated actin nucleator, as the actin in the annealed pieces is from the Arp2/3-generated lamellipodial network. The formin FHOD1 (FH1/FH2 domain-containing protein 1) has been shown to be necessary for arc-driven cell spreading (49), however, and in the absence of FHOD1, both dorsal SF and transverse arcs are lost (102). Interestingly, FHOD1 does not appear to polymerize actin; rather, FHOD1 appears to bundle it (101). Therefore, FHOD1 may play a critical role in knitting arcs together through its ability to specifically localize to areas of antiparallel actin organization (101, 102). The contribution of bundling by FHOD1 versus that by α -actinin or myosin II remains unclear, however. If actin nucleation by a formin is required for assembly of transverse arcs, the formin in question would have to be distributed throughout the bundle in spots in which molecules were oriented back to back, as described for the contractile ring. This fixed orientation of formins would then ensure the presence of overlapping stretches of filaments with opposite polarity needed to form a mixed polarity bundle.

Myosin II acts on transverse arcs in two directions: shortening them from side to side as the sarcomeres contract, and thereby limiting the width of the cell (20), and contracting them

rearward, which exerts force on the dorsal SF to flatten the lamella and to drive elongation of focal adhesions (see the subsection titled “Dorsal stress fibers”) (12, 84). Thus, in addition to promoting cell spreading (49), arcs may help maintain the shape of cells (21), and may help slow retrograde flow to promote focal adhesion assembly in some cell types (20). Because these actions rely on the shortening of arcs, the nonuniform polarity of filaments in the arcs is essential for their activity. However, the mechanism by which these functions are regulated is unknown.

Ventral stress fibers and graded polarity bundles.—In addition to the dorsal SF, which rise from the leading edge of a motile cell toward the dorsal cell cortex, cells contain actin bundles along their ventral surfaces that often display a sarcomere-like pattern of organization. Initial observations largely differentiated these stress fibers from dorsal SF solely on the basis of their location; recent work, however, has begun to show that dorsal and ventral SF have different mechanisms of assembly and likely play different roles in the cell.

What are ventral stress fibers?—Ventral SF are the third major type of stress fiber in motile cells (48, 104). In contrast to dorsal SF, ventral SF are essentially parallel to the bottom of the cell and are found underneath the cell body, often terminating in a focal adhesion at each end (48, 104). One type of ventral SF is the graded polarity bundle (30). These long, overlapping bundles of actin span the ventral side of the cell and exhibit unusual filament orientation; as their name suggests, the polarity of the filaments changes depending on their position in the cell (30). Filaments within a graded polarity bundle that are located near the leading edge of the cell are uniform in orientation, whereas those in the center of the cell have mixed polarities (30). It is unclear whether all SF on the ventral side of the cell have a graded polarity organization. Studies have shown that ventral SF in some cells are limited to the area under the cell body and do not extend all the way to the cell edge as graded polarity bundles do (105). Therefore, graded polarity bundles are likely a specific type of ventral SF.

How are ventral stress fibers formed and what is their role?—The formation of graded polarity bundles is a challenging problem, as their dynamics appear to vary along the length of the bundle (30). One mechanism suggests that these bundles are formed from filopodia that become disconnected from the lamellipodium (4), but additional filament growth would be necessary for the new graded polarity bundle to span the length of the cell. No specific actin nucleation factor has been shown to be essential for or localized to graded polarity bundles or to any kind of ventral SF. Interestingly, however, the myosin IIB isoform concentrates toward the rear of migrating cells, and recent work (5) has shown that the formin DAAM1 colocalizes with myosin IIB and specifically regulates the myosin IIB-containing actin network. This finding raises the following question: How might DAAM1 polymerize actin for graded polarity bundles and/or ventral SF? As with other types of mixed polarity bundles, generation of the filament arrangement needed for formation of graded polarity bundles would rely on spots of DAAM1 being oriented back to back along the track of the ventral SF. Perhaps myosin IIB, which acts more as a tensile cross-linker than as a stepping motor, serves to localize DAAM1 to spots along the rear of the cell.

The role played by ventral SF is also unknown; they may help regulate contraction in the rear of a migrating cell (113) or movement of the cell body. As it is difficult to specifically eliminate ventral stress fibers, however, their contribution to motility is as yet undefined. Ventral SF also associate with adhesions under the center of the cell where fibrillogenesis of fibronectin occurs in fibroblasts, so they may also regulate this process. Further work that allows independent inhibition of ventral SF is necessary to conclusively establish their role.

The perinuclear actin cap.—Although a great deal of work in the field of actin in cell biology has focused on its role at the leading edge of the cell, specifically concentrating on SF and the lamellipodium, cells also contain many other actin structures. In recent years, particular interest has developed in understanding how the actin cytoskeleton can mechanically influence the nucleus. In addition to playing a role in nuclear positioning (see the subsection titled “TAN lines and the nucleus”), new evidence suggests that actin can also control other aspects of the nucleus.

What is the perinuclear actin cap?—The shape of nuclei in most cells correlates with the overall shape of the cell, thus suggesting that the two are connected (10). In fact, a dense cap of actin bundles on the dorsal side of the nucleus orients along the long axis of the cell and coordinates cell and nuclear shape in a manner dependent on interaction with lamins (54). The actin cap appears to differ from TAN lines, as loss of it leads specifically to the misregulation of nuclear shape that has been implicated in laminopathies, rather than to nuclear positioning defects (55, 56). Therefore, the perinuclear cap is a distinct actin structure found particularly in cells under shear stress that regulates the shape of the nucleus (54, 56).

How is the actin cap generated?—Actin cap fibers terminate in a specific subset of focal adhesions that are located near the periphery of the cell and that are critical for mechanotransduction to the nucleus (54, 56). As with ventral SF, termination at both ends in focal adhesions indicates that these bundles are likely composed of mixed polarity filaments and may be generated by similar mechanisms. The actin at the actin cap is significantly more dynamic than that in the dorsal and ventral SF, suggesting specific nucleation by a formin, although the formin involved has not yet been identified (55). Perhaps a formin could interact with proteins in the outer nuclear membrane to polymerize actin there, or some factor at the nuclear membrane could locally activate a nearby formin. Alternatively, the actin cap-associated adhesions could have their own formin, which gathers barbed ends and polymerizes actin out of the adhesions, promoting generation of mixed polarity bundles when the two populations meet over the nucleus. How the actin cap is polymerized remains an open issue.

ISOTROPIC NETWORKS

How Are Isotropic Networks Formed in Cells, and How Are They Used?

Although the most apparent structures in motile cells are linear actin bundles and the dendritic network at the lamellipodium, other actin networks exist, some of which are essentially isotropic in nature. Isotropic networks can be used to stabilize structures in cell;

for instance, the cell cortex helps control cell shape and integrity and regulates pressure release in the form of blebs. The isotropic nature of the network provides stability without concentrating force generation at any single point. The actin polymerized inside the nucleus appears to be an isotropic network that plays a role in helping to regulate transcription, rather than to control shape, although nuclear actin may also have an unappreciated structural role. Therefore isotropic networks serve a variety of incompletely understood functions in the cell.

How isotropic networks are generated is also somewhat unclear. We postulate that they result from the interaction between two classes of nucleators, Arp 2/3 and formins, both of which have been implicated in nuclear actin and play a role in the functioning of the cortex. How would a cell generate filaments with uniform polarity, but arranged in a network rather than linearly? The orientation of the filaments is likely controlled by the orientation of the formins. As described above, (see the section titled “Nonuniform Polarity Actin Bundles”) orienting formins back to back generates filaments with pointed ends projecting in opposite directions. In the case of isotropic networks, formins are radially organized with uniform polarity with respect to each other, as in the center of a pom-pom (**Figure 2c**), rather than simply aligning back to back along a linear track. The filaments these formins generate will therefore have barbed ends gathered at the center of the pompom and pointed ends extending away from it. How Arp2/3 plays into this network is unknown. As a dendritic network is ideal for generating force at a membrane, perhaps these radiating forminnucleated filaments act as a scaffold for the dendritic network that in turn supports the membrane. How interaction between classes of nucleators generates isotropic networks remains a fascinating area of research.

The mesenchymal cell cortex.—In contrast to the highly organized cortex of erythrocytes, the cortex of mesenchymal cells is poorly understood and likely lacks the rigid structure of red blood cells. However, recent work has begun to show the importance of the actin cortex in providing structure for the cell and in regulating migration.

What is the organization of the actin cortex?—The surface of the cell proximal to the plasma membrane is lined with an actin mesh known as the actin cortex (80, 130). This contractile actin network is critical for maintaining cell shape and cytoplasmic coherence (95), but the organization and dynamics of this actin mesh are only beginning to be elucidated. Several actin-binding proteins are localized to the actin cortex (for review, see Reference 97), but the roles of only a few of these proteins have been defined. For example, the ERM (ezrin, radixin, moesin) proteins link the cortex to the membrane (35). Additionally, the cross-linking protein filamin is known to localize to the cortex and link it to the membrane (107, 120). The precise organization of this mesh has not been elucidated.

How is actin at the cortex polymerized?—Recent studies have highlighted the complex dynamics of the actin cortex. Loss of the Arp2/3 complex reduces the expression of actin under the membrane and impairs cell motility (127). However, a recent study also shows that the cortex is organized into dense motile actin nodes from which pom-poms of actin filaments emanate, dependent on myosin IIA, the cross-linking protein filamin A, and the formin DAAM1 (68). Interestingly, this organization resembles the well-characterized

assembly of a contractile ring in fission yeast; in fission yeast, the ring comprises a series of cross-linked formin-containing nodes that contract toward each other (116, 128). How DAAM1 and Arp2/3 work together or with other actin nucleation factors to generate the submembranous actin cortex remains unclear.

Blebbing cells.—The role of blebs in cell migration is a developing frontier in motility research. Fascinatingly, in vivo imaging has revealed cell migration via bleb-based motility in live mice. Although this migration mechanism is by no means as thoroughly studied as lamellipodium-based migration, ongoing work from multiple labs not only has shown that cells can migrate by blebbing but also has begun to describe the physical properties behind this motility.

What is the role of actin in the dynamics of membrane blebs?—Dynamic blebs are pressure-driven, non-actin-dependent membrane protrusions that are generated spontaneously at the edges of cells. Recent work has begun to elucidate the role of blebbing as a novel mode of migration for cells in vivo (for review, see Reference 24). Blebs are formed by disruption of the membrane–cortex connection and by bulging of the membrane (26, 87). In their earliest stages, protruding blebs are devoid of actin (87), and as expansion halts, ERM proteins are recruited to the bleb membrane prior to actin recruitment (25). The actin cortex is then rebuilt within the bleb, and myosin localizes to the bleb neck, where it acts on the newly built cortex to retract the bleb (25). In contrast to lamellipodia, blebs do not rely on actin polymerization for protrusion and do not have specific attachment points to the substrate; thus, the role of actin in bleb-driven cell migration is less clear. The cortical actin network may be responsible for generating force perpendicular to the surface to allow cells to “chimney” forward via a bleb (106), but the molecular details of this mechanism are not yet known.

How is actin rebuilt on the membrane of a bleb?—The molecular mechanisms involved in rebuilding the actin cortex in a bleb are a topic of much research. One spliceoform of the formin FMNL1, which is restricted to hematopoietic-derived cells and certain cancers, is involved in the formation of dynamic blebs in blood cells, and other formins and the Arp2/3 complex do not appear to be involved (42). In migrating melanoma cells, however, the situation appears quite different. Proteomics analyses of blebs have indicated roles for several formins and for the Arp2/3 complex at the actin cortex (18). Subsequent work identified distinct, critical contributions of mDia1 and the Arp2/3 complex to the formation of a blebbing cell cortex (18). Furthermore, Arp2/3 can potentiate the effects of mDia1 at the cortex (18). Perhaps rapid branching by the Arp2/3 complex provides the barbed ends needed for mDia1 to bind and assemble. This behavior suggests a different mechanism of isotropic network formation than the pom-pom model described above. Here, the Arp2/3 complex establishes the geometry of a dendritic network, and a formin acts on that network to rapidly expand it. A major difference between these two models is the localization of the formins; in the pom-pom model, multiple formins cluster at discrete spots, whereas in the Arp2/3-potential model, individual formins assemble actin filaments away from Arp2/3 nodes. Careful examination of actin and actin nucleation factors at the cortex will reveal the assembly mechanisms. Together these data reveal novel roles for

formins working with the Arp2/3 complex in polymerizing the cortical actin mesh underlying the membrane.

Actin in the nucleus.—Among controversial topics in actin research, the presence of actin in the nucleus has been second only to perhaps the presence of actin in bacteria. Although earlier studies suggested that actin was absent from the nucleus, recent careful biochemical and microscopy studies have shown that actin does exist in the nucleus, either as short filaments or as monomers.

Why does a cell need actin in the nucleus?—In addition to cytoplasmic actin, which helps control the shape and position of the nucleus, actin assembles within the nucleus itself to control transcription through the megakaryocytic acute leukemia protein (MAL) (115). In the absence of nuclear actin polymerization, MAL shuttles rapidly between the nucleus and the cytoplasm (115). Polymerized nuclear actin binds to MAL and retains it in the nucleus, where low levels of G-actin also inhibit actin-dependent nuclear export (115). Thus, although the presence of F-actin in the nucleus has been controversial, nuclear actin may play a role in regulating transcription in somatic cells.

How does actin polymerize in the nucleus?—How nuclear actin polymerizes is not fully understood. Transcriptional studies indicate that WASP is expressed inside the nucleus and that Arp2/3 specifically may have a role in regulating transcription (137). Furthermore, blocking nuclear export leads to actin polymerization via mDia2 accumulation in the nucleus (6). Activation of mDia2 in the nucleus increases F-actin, nuclear accumulation of MAL, and transcription (6). As both Arp2/3 and a formin have been found in the nucleus, multiple nuclear actin networks may exist, or the actin nucleation factors may cooperate.

CONCLUSIONS AND FUTURE DIRECTIONS

In this article, we have described many of the known actin structures in migratory cells, along with their mechanisms of assembly. The problem of how so many different actin structures can assemble in a temporally and spatially regulated way within one cytoplasm remains a fascinating one in cell biology, and its answer no doubt relies on the actin nucleating proteins, namely, the Arp2/3 complex, formins, and the TMBPs. Although it is tempting to speculate that a distinct nucleator is associated with each structure, there are now several examples of actin assembly factors working together, such as the cell cortex and filopodia, and many nucleators that localize to more than one structure. Of particular interest in this latter group is the formin mDia1, which appears to be required by many cellular structures, but the localization of which remains undefined. mDia1 may represent a transient nucleator presence at these structures, perhaps nucleating the first filaments then handing off further assembly to other factors. As new actin structures are frequently being described, however, assigning a single nucleation factor to each structure becomes ever more complicated, and understanding how nucleation factors synergize becomes ever more important. Furthermore, many actin nucleators, particularly those in the TMBP family and some divergent formins, do not yet have any known role in cells. Therefore much remains to be understood about the construction of a spatially organized, dynamically coordinated actin cytoskeleton in motile cells.

One tantalizing prospect for the future of understanding how the actin cytoskeleton is connected involves the use of cutting-edge superresolution microscopy techniques. Because the diameter of an actin filament is far below the resolution limit of a light microscope, visualizing the fine details of actin structures has been difficult and has generally been confined to complicated electron microscopy studies with limited molecular specificity (69, 128). New superresolution microscopy techniques, such as structured illumination (SIM), photoactivated localization microscopy (PALM), and stochastic optical reconstruction microscopy (STORM), and variations of these techniques allow for accurate description of actin structures with high molecular detail (34, 53, 114, 129). So far, these techniques have produced stunningly detailed characterization of several actin structures in cells, including focal adhesions, junctions, axons, and TAN lines (53, 67, 119, 129), but the characterizations have remained more descriptive than predictive. Other localization techniques such as single-molecule tracking of individual formins offer further promise for understanding the role of actin nucleators in living cells (133). A challenge for the field going forward is how to apply the power of superresolution microscopy to gain mechanistic insight into the mechanical integration and dynamic organization of the actin cytoskeleton in living cells.

ACKNOWLEDGMENTS

This work was supported by the National Heart, Lung, and Blood Institute Division of Intramural Research.

Glossary

TMBP	tandem monomer binding protein
VASP	vasodilator-stimulated phosphoprotein
AJ	adherens junction
ADF	actin depolymerizing factor
CME	clathrin-mediated endocytosis
SF	stress fibers
TAN lines	transmembrane actin-associated lines
MTOC	microtubule organizing center
LINC	linker of nucleus and cytoskeleton
ER	endoplasmic reticulum
ERM	ezrin, radixin, moesin
MAL	megakaryocytic acute leukemia protein

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SUMMARY POINTS

1. Different actin nucleation factors generate different geometries among actin structures: Arp2/3 creates branched structures, whereas formins and TMBPs create linear filaments.
2. Several actin structures rely prominently on branched actin filaments: lamellipodia, endocytic vesicles, and AJs.
3. Other actin structures are composed primarily of linear actin filaments created by formins: actin bundles found in filopodia, TAN lines, and various types of stress fibers.
4. Contractile actin structures such as the contractile ring are also linear filaments generated by formins, as are structures involved in mitochondrial division.
5. Isotropic networks such as those found at the cell cortex or in the nucleus may involve cooperation between formins and the Arp2/3 complex.
6. Both the localization and the specific orientation of formin molecules are critical for determining the molecular properties of organizationally and functionally diverse actin bundles.

Figure 1.

Different actin nucleators specify architecturally and functionally distinct actin structures. (Center) Diagram showing actin organization in a motile cell. Actin is shown in green, the Arp2/3 complex at the branch point of two filaments is shown in purple, and the FH2 homodimers of formins are shown in orange. Insets highlight organelles with associated actin structures, including vesicles, focal adhesions, mitochondria, and the nucleus, and show these structures in greater detail. Note the different geometries of actin associated with different organelles. For clarity, only the best-characterized actin nucleator is shown for each actin structure, and only some of the described actin structures are shown. Some actin structures are not depicted; these include cell–cell junctions, the contractile ring, ventral stress fibers, and blebs. Abbreviations: Arp2/3, actin-related proteins 2 and 3; DAAM1, disheveled-associated activator of morphogenesis 1; FHOD1, FH1/FH2 domain-containing protein 1; FMNL3, formin-like protein 3; INF2, inverted formin-2; mDia2, mouse diaphanous-related formin 2; TAN lines, transmembrane actin-associated nuclear lines; VASP, vasodilator-stimulated phosphoprotein.

Figure 2.

Orientation of formin actin nucleators may define the geometry of linear actin structures. Formin molecules are shown in orange, and actin filaments are shown in green; the fast-growing barbed end of each filament is marked by an arrowhead. (a) Formins oriented in the same direction generate a uniform polarity bundle, for example, at the focal adhesion. A cross-linking protein specific for filaments oriented in the same direction is also shown. (b). Formins oriented back to back in linear arrays generate bundles with mixed polarity. A cross-linking protein specific for filaments oriented in the antiparallel direction is also shown, as are myosin II minifilaments. (c) Formins oriented back to back in discrete location, called pom-poms, generate isotropic networks. A cross-linking protein specific for filaments oriented in the antiparallel direction is also shown, as are myosin II minifilaments.

Table 1

Proteins associated with the actin-related protein 2 and 3 (Arp2/3) complex

Abbreviation	Description
NPF	Nucleation-promoting factor
WASP/N-WASP	Wiskott-Aldrich syndrome protein/neural-WASP
WAVE	WASP family verprolin-homologous protein
WASH	WASP and Scar homology protein
WHAMM	WASP homolog associated with actin, membranes, and microtubules
JMY	Junction-mediating and -regulatory protein
GMF	Glia maturation factor
WIP	WASP-interacting protein
CARMIL	Capping protein, Arp2/3, and myosin I linker

Table 2

Formins described in the text, grouped by family

Protein name	Structural affiliation(s)
FH1/FH2	Formin homology domains 1/2
mDia1	Adherens junctions Dorsal stress fibers Cortex and/or blebs
mDia2	Lamellipodia Filopodia Contractile ring Nucleus
FHOD1	TAN lines Transverse arcs
DAAM1	Filopodia Ventral stress fibers (?) Cortical nodes
FMNL1	Cortex/blebs
FMNL2/FRL3	Filopodia Lamellipodial protrusion
FMNL3/FRL2	Filopodia
FMN1	Adherens junctions
INF2	Mitochondrial fission Stress fiber assembly