Published in final edited form as:

Curr Opin Cell Biol. 2018 October ; 54: 1–8. doi:10.1016/j.ceb.2018.02.007.

ULTRASTRUCTURE OF THE ACTIN CYTOSKELETON

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Abstract

The actin cytoskeleton is the primary force-generating machinery in the cell, which can produce pushing (protrusive) forces using energy of actin polymerization and pulling (contractile) forces via sliding of bipolar filaments of myosin II along actin filaments, as well as perform other key functions. These functions are essential for whole cell migration, cell interaction with the environment, mechanical properties of the cell surface and other key aspects of cell physiology. The actin cytoskeleton is a highly complex and dynamic system of actin filaments organized into various superstructures by multiple accessory proteins. High resolution architecture of functionally distinct actin arrays provides key clues for understanding actin cytoskeleton functions. This review summarizes recent advance in our understanding of the actin cytoskeleton ultrastructure.

Introduction

The actin cytoskeleton is a complex system of actin filaments organized into diverse structural arrays by numerous accessory proteins. As major force-generating cellular machinery, the actin cytoskeleton can produce pushing (protrusive) forces through coordinated polymerization of multiple actin filaments, pulling (contractile) forces via sliding of bipolar filaments of myosin II along actin filaments, and resistance (shaping) forces by forming cross-linked membrane-associated filament arrays. Actin-dependent forces are essential for cell migration, interaction with the environment, shape and mechanical properties of the cell surface and trafficking and morphogenesis of membrane organelles (reviewed in [1]).

The importance of the actin cytoskeleton for cell physiology asks for deep understanding of how it works structurally and dynamically. However, the actin cytoskeleton is not readily amenable to high resolution structural studies due to a small diameter, tight packing, complex 3D organization, and vulnerability of actin filaments. Individual actin filaments are unresolvable by either diffraction-limited light microscopy or subdiffraction fluorescence microscopy (SFM), while proper preservation of actin structures and visualization of their 3D organization is a challenge for electron microscopy (EM). So far, platinum replica EM

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and negative-staining EM, especially in combination with electron tomography, have been most productive in revealing actin cytoskeleton ultrastructure (reviewed in [2]). However, SFM, cryoEM and atomic force microscopy (AFM) increasingly contribute to this research. This review focuses on recent advances in our understanding of ultrastructure of protrusive and contractile actin arrays.

Actin cytoskeleton in protrusion

Pushing force driving membrane protrusion is generated by polymerizing actin filaments organized either into branched networks or parallel bundles. Branched networks are assembled through Arp2/3 complex-dependent actin nucleation and drive protrusion of lamellipodia, some membrane trafficking events, and formation of diverse cell-cell junctions. Parallel bundles are mostly known to drive protrusion of leading edge filopodia. Whereas basic principles of actin-based protrusion are well-established (reviewed in [1,3]), recent efforts focused on understanding how specific geometry of polymerizing actin arrays is affected by biophysical and biochemical conditions.

Load dependence of branched network geometry

Structural changes in branched actin networks pushing against load were studied both in cells [4**] and in vitro [5**]. Previous computer modeling studies predicted the predominant angles of $\pm 35^\circ$ relative to the direction of protrusion for branched actin filaments in lamellipodia [6,7]. This pattern corresponds to canonical geometry of branched actin networks, in which the 70° branch angle produced by the Arp2/3 complex faces the leading edge [4**,6,8]. This “slingshot” modality, however, was predicted to change to the $+70^\circ/0^\circ/-70^\circ$ (“trident”-like) distribution (Figure 1a), if branched filaments grow under too high or too low load [9] or are allowed to become longer [7]. These predictions have been experimentally validated using electron tomography of negatively stained lamellipodia [4**]. Indeed, when plasma membrane tension was experimentally increased or decreased in migrating fish keratocytes, more filaments acquired orientations around 0° and $\pm 70^\circ$, as compared with two major peaks of $\pm 35^\circ$ at the steady state (Figure 1b). Since small-scale periodic compression and relaxation of the lamellipodial network also occur without experimental interventions [4**,10], periodic rearrangements between the slingshot and trident network geometry may constantly occur at the leading edge.

In vitro, application of resisting force to growing branched networks increased the network density, as estimated by fluorescence microscopy [5**]. However, the calculated average length of actin filaments did not change, suggesting network reorganization, possibly, analogous to the slingshot-to-trident switch observed in cells [4**] and predicted by computer modeling [9]. The load-induced changes in network architecture were accompanied by increased filament density, enhanced formation of barbed ends [4**,5**] and greater stiffness of the network [5**], which could allow the network to withstand and counteract the mechanical challenge.

Relative contribution of elongators and nucleators to protrusive actin arrays

Proteins controlling actin filament elongation are important regulators of branched network geometry [1,3]. In particular, the barbed end-binding elongation factors, formins and Ena/VASP proteins, increase filament lengths in lamellipodia by protecting barbed ends from capping, recruiting monomers for polymerization and anchoring barbed ends to the membrane. Formins additionally can nucleate unbranched actin filaments (Figure 2).

A role of Ena/VASP proteins in promoting filament elongation in lamellipodia is well established [11]. Formins, on the other hand, are thought to predominantly assemble actin bundles. However, accumulating data reveal a role of various formins in lamellipodial protrusion [12–16] and other branched networks [17,18], where formins can function as elongators and/or nucleators of actin filaments. Specifically, mDia2 can stimulate both elongation and nucleation of actin filaments in protrusions [16], FMNL2 seems to mostly promote elongation of Arp2/3-nucleated filaments, whereas FMNL3 and mDia1 appear to be largely implicated in nucleation [14,15]. In lamellipodia, formin-nucleated filaments either serve as mother filaments for Arp2/3-mediated nucleation or become intermingled with branched filaments (Figure 2).

Reorganization of protrusive actin arrays

Acting as elongators, formins and Ena/VASP can reorganize branched networks into protrusive bundles in filopodia or contractile bundles in stress fibers (Figure 2), the latter process also requiring contribution from nonmuscle myosin II (NMII). Generation of filopodial bundles from branched networks often occurs via elongation of Arp2/3-nucleated branched filaments, which can be either an obligatory or optional pathway [19,20]. However, formin-nucleated filaments become essential for filopodial formation when Arp2/3-mediated nucleation is experimentally downregulated [21*–23]. Formation of filopodia in such cases can be even increased due to a competition between Arp2/3 complex and formins for actin monomers (reviewed in [19,24]).

Although spiky protrusions in Arp2/3-deficient cells are usually likened to filopodia, they combine features of filopodia and stress fibers. Actin filaments in such protrusions are bundled and oriented with barbed ends to the tip of the bundle [21*], which is a shared feature of filopodia and stress fibers. However, the presence of NMII [23] and overall geometry of these bundles [25*] makes them more similar to stress fibers. Since filopodia can be reorganized into stress fibers [26–28], the spiky protrusions in Arp2/3-deficient cells likely correspond to various transitional states during this process.

Lamellipodial actin networks also can be reorganized into stress fibers oriented parallel to the cell edge, which are usually called transverse arcs [8,29,30]. SFM analysis of this process in spread T cells showed that formin-dependent actin filaments formed within the lamellipodial network are particularly important for the formation of these arcs [31**].

Actin cytoskeleton in contraction

Pulling forces driving contraction are generated by mutual sliding of actin and myosin II filaments, as originally established for skeletal muscle. Nonmuscle cells use a combination

of three NMII paralogs, NMIIA, NMIIB, and NMIIC, to generate contractile activity for migration, cytokinesis, and formation of cell-cell and cell-matrix junctions among other functions (reviewed in [32]). The most conspicuous contractile structures in nonmuscle cells are stress fibers – bundles of actin filaments interdigitating with bipolar NMII filaments. Cytokinetic contractile rings in dividing cells and non-aligned actin-NMII networks are other examples of contractile actin structures.

Contractile networks

Non-aligned actin-NMII networks represent most common contractile machinery in nonmuscle cells, especially in cells lacking stress fibers. Actin-NMII networks visualized with single filament resolution by platinum replica EM consist of relatively isotropic actin networks containing clusters of bipolar NMII filaments interacting with each other at their ends [8,33] (Figure 3a). Purified NMII filaments exhibit similar end-to-end interaction of unknown biochemical mechanism [34].

NMII clusters are usually formed behind cellular protrusions. Subsequently, they either reorganize into larger structures in parallel with retrograde flow [8,33] or, in case of NMIIA, disassemble after producing local contraction [35]. SFM studies [36*–38**] revealed that formation of NMII clusters begins with spontaneous nucleation of individual (at SFM resolution) bipolar NMII filaments, which then appear to duplicate. The “sister” NMII filaments then usually move apart along actin tracks and seed additional filaments, thus forming a growing NMII cluster. The biochemical mechanism of NMII filament duplication remains puzzling, but likely represents templated nucleation rather than actual splitting of NMII filaments [36*]. NMII filament amplification could potentially be stimulated by local actin filaments, which become stretched by the initial NMII filament, gain higher affinity for NMII due to a conformational change [39] and attract more NMII subunits for further nucleation.

Stress fibers

Structure of stress fibers is still incompletely understood despite long history of their investigation (reviewed in [1]). Stress fibers share some features with muscle sarcomeres, such as discontinuous distribution of NMII, formation of aligned stacks of NMII filaments, and accumulation of α -actinin between the NMII stacks, but with reduced regularity (Figure 3c). Typically, NMII stacks are more widely separated in distal regions of stress fibers and can completely merge in their middle in the course of contraction.

Actin filaments in stress fibers, in contrast to sarcomeres, have variable lengths and exhibit continuous distribution with extensive overlaps between filaments of opposite orientation. Such overlaps allow stress fibers to be stretched by external force up to twice of their original length without formation of gaps in actin distribution [40]. Nonetheless, visualization of reporters of actin filament ends by SFM revealed that pointed ends in stress fibers were largely colocalized with NMII clusters, whereas barbed ends were enriched in-between [38**], similar to actin organization in sarcomeres (Figure 3c). Together with direct observations by EM showing that actin filaments in stress fibers are long enough to pass

through several NMII-rich regions, this finding suggests existence of a sorting mechanism that promotes the specific distribution of actin filament ends.

Because of their polymorphism, stress fibers are usually categorized into transverse, radial (or dorsal) and ventral types (reviewed in [41]). Transverse stress fibers (or arcs) appear to form through reorganization of lamellipodial actin filaments [8,30] during lamellipodium retraction [29]. The formin-nucleated subpopulation of lamellipodial filaments is particularly important for this process [31**]. This reorganization process is described by the network contraction model (Figure 3). It is driven by clusters of NMII filaments, which contract the network, become coaligned with actin filaments and form registered stacks separated by α -actinin-containing spaces [8]. Stack formation occurs by movement of NMII filaments toward each other along actin tracks, while their proper registration likely relies on head-to-head affinity between NMII filaments [38**].

Radial stress fibers, in contrast to transverse arcs, are anchored to focal adhesions at their distal tips. They can arise from filopodial bundles [26,27], which recruit NMII filaments to their shaft [28] (Figure 3). Alternatively, NMII molecules can be recruited to focal adhesions at the tips of nascent radial stress fibers for subsequent polymerization and incorporation into the actin bundle [42]. Radial stress fibers can grow via actin filament elongation at focal adhesions, which is assisted by VASP [43] and formins [44], and because they are pulled centripetally by contracting transverse arcs [45]. Contraction-driven straightening of an interlinked radial-transverse-radial stress fiber set leads to the formation of ventral stress fibers attached to focal adhesions at both ends. This process requires inhibition of actin polymerization at focal adhesions achieved by phosphorylation of VASP by AMPK [43].

Cytokinetic contractile rings

Structure of cytokinetic contractile rings has long been debated, even though they closely resemble stress fibers, especially transverse arcs. This similarity has been recently validated by SFM and platinum replica EM of contractile rings in mechanically opened dividing sea urchin embryos [46**]. Similar to stress fibers, mature contractile rings at late stages of cytokinesis consisted of mutually aligned NMII filaments and unbranched actin filaments with mixed polarity, both oriented along the axis of contraction. At earlier stages of cytokinesis, the assembly of the contractile ring appeared to follow the network contraction mechanism (Figure 3). It started from isolated NMII clusters and eventually developed into aligned bundles. Although NMII filaments in mature cytokinetic rings were tightly packed into continuous arrays [46**], a periodic NMII pattern was observed at the onset of constriction in cytokinetic rings of mammalian cells by conventional fluorescence microscopy [47]. Progressive alignment of actin filaments during cytokinesis was detected in live cells by advanced fluorescence polarization microscopy [48**]. Importantly, significant equatorial constriction occurred even before actin filaments were grossly aligned, suggesting that force is generated by network contraction at early stages of cytokinesis, but may switch to sarcomere-like contraction at later stages.

Cortex

Actin cortex is a rather ambiguous part of the actin cytoskeleton. It was discovered in large cells, such as amoeba and animal eggs, as a thick gelatinous layer under the plasma membrane with apparent contractile properties. Subsequently, the concept of cortex, defined as a contiguous uniform membrane-associated actin-NMII network, has been extrapolated to virtually all animal cells despite serious limitations [49]. The classically defined cortex appears to exist in mitotic and other unattached cells and, possibly, at some surface regions of attached cells, especially cells migrating in an amoeboid manner. However, for cells with a more differentiated actin cytoskeleton, the concept of cortex becomes confusing. On one hand, all actin-NMII structures in the cell are associated with the plasma membrane and thus fit the definition of the cortex. However, being sufficiently well characterized they could exist under their own names, such as “stress fibers” or “actin-NMII networks”. The same is true for protrusive actin structures, which are also often referred to as cortical actin. On the other hand, some surface areas in spread cells lack detectable NMII structures, thus challenging the assumed uniformity of the cortex. The term cortex, in an undefined form, is also applied to actin structures that are not yet characterized, or if their identity is not important. In such contexts, “cortex” becomes synonymous with “actin cytoskeleton”, which could be convenient, but not very specific.

The classically defined cortex, when present, appears to be structurally related to disordered actin-NMII networks. Scanning EM images of the mitotic cortex showed a relatively homogenous cytoskeletal meshwork with small pores [50]. Similar organization was reported for the cytoskeleton of cell surface blebs [51], which are often used for studies of cortex properties, because of simplicity of bleb’s cytoskeleton. Meshwork-like organization, with cell type-specific pore sizes, was also observed at higher resolution by platinum replica EM in dorsal cortices associated with mechanically detached apical membranes of cultured epithelial cells [52*]. Time lapse imaging by high-speed high-resolution AFM showed that the dorsal cortex in fibroblasts consisted of a mixture of filament bundles and meshworks in different proportions that could change over time [53**]. Cortical filament bundles in these cells likely correspond to dorsally located stress fibers integrated into more isotropic actin networks.

A combination of bundles and meshworks in the cortex is consistent with functional contribution of both Arp2/3 complex and formins to cortex assembly [50,51,53**], although formins may play a dominant role for the assembly of the mitotic cortex [54] and stabilization of the rear surface in migrating cells [55]. Among different NMII paralogs, NMIIA has greater contribution to cortex stiffness and contractility than NMIIB or NMIIC, whereas NMIIB is more important for cortex stabilization [56].

Conclusions

Actin cytoskeleton is a remarkably multifunctional system in eukaryotic cells. It supports a constantly growing list of cellular activities, thus motivating more and more scientists to understand intricacies of this system. The molecular mechanisms underlying actin cytoskeleton activities are immensely complex due to a large number of accessory proteins that support formation of diverse actin filament arrays with different dynamics, spatial

organizations and interactions with other structures. This complexity is necessary for the actin cytoskeleton to perform its functions, but poses significant challenges to researchers aiming to understand how the actin cytoskeleton works. Structural studies help reaching this goal in a most efficient way. At present, we have decent understanding of the structure, functions and underlying mechanisms of the most common actin-based processes, such as muscle contraction and lamellipodial protrusion. On the other hand, it is much less clear how the actin cytoskeleton works in the context of other functions, such as trafficking and morphogenesis of various membrane organelles, formation and remodeling of cell-cell junctions, organization of the submembrane cytoskeleton in different cells and conditions. In part, these deficiencies result from technical difficulties of visualizing the fine architecture of the actin cytoskeleton at these “inconvenient” locations. However, ongoing technological developments in light, electron and atomic force microscopy pave the way toward solving these problems in near future.

Acknowledgments

This work was supported by the National Institutes of Health grant # R01 GM 095977.

The author declares no conflict of interest.

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Figure 1.

Load-dependent changes in geometry of branched actin networks.

(a) Two major branched network geometries characterized by either $\pm 35^\circ$ ("slingshot") or $-70^\circ / 0^\circ / +70^\circ$ ("trident") angle distribution.

(b) Geometry of branched networks depends on resistive load. Conventional slingshot geometry exists under intermediate loads (middle), such as during steady state cell migration. Under increased resistance (left), branched networks switch to the trident geometry with higher filament density and more barbed ends. Under reduced load (right), branched networks switch to the trident geometry with lower density and predominant $\sim 0^\circ$ angle.

Figure 2.

Roles of actin filament nucleators and elongators in lamellipodia.

Both Arp2/3 complex and formins can nucleate lamellipodial filaments. Filament elongation can be promoted by the same or different formins, and by Ena/VASP proteins. NMII bipolar filaments reorganize branched networks into various bundles with potential preference for formin-polymerized filaments.

Figure 3.

Formation of stress fibers from random networks by NMII activity.

(a) NMII filaments form clusters within random actin networks in the cell lamella. Cluster formation occurs through spontaneous nucleation of NMII filaments followed by their local amplification. In clusters, NMII filaments interact with each other at the ends.

(b) NMII filaments reorganize isotropic actin networks into stress fibers by moving along and pulling on actin filaments. Simultaneously, NMII filaments become aligned to form stacks, which can be separated by gaps of variable lengths.

(c) Actin filaments in stress fibers have mixed polarity. Their barbed and pointed ends are preferentially enriched at NMII stacks and inter-NMII gaps, respectively.