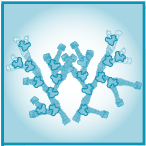


ACTIN DYNAMICS, ARCHITECTURE, AND MECHANICS IN CELL MOTILITY

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Blanchoin L, Boujemaa-Paterski R, Sykes C, Plastino J. Actin Dynamics, Architecture, and Mechanics in Cell Motility. *Physiol Rev* 94: 235–263, 2014; doi:10.1152/physrev.00018.2013.—Tight coupling between biochemical and mechanical properties of the actin cytoskeleton drives a large range of cellular processes including polarity establishment, morphogenesis, and motility. This is possible because actin filaments are semi-flexible polymers that, in conjunction with the molecular motor myosin, can act as biological active springs or “dashpots” (in laymen’s terms, shock absorbers or fluidizers) able to exert or resist against force in a cellular environment. To modulate their mechanical properties, actin filaments can organize into a variety of architectures generating a diversity of cellular organizations including branched or crosslinked networks in the lamellipodium, parallel bundles in filopodia, and antiparallel structures in contractile fibers. In this review we describe the feedback loop between biochemical and mechanical properties of actin organization at the molecular level in vitro, then we integrate this knowledge into our current understanding of cellular actin organization and its physiological roles.

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I. PREFACE ON ACTIN ORGANIZATION AND CELL MECHANICS

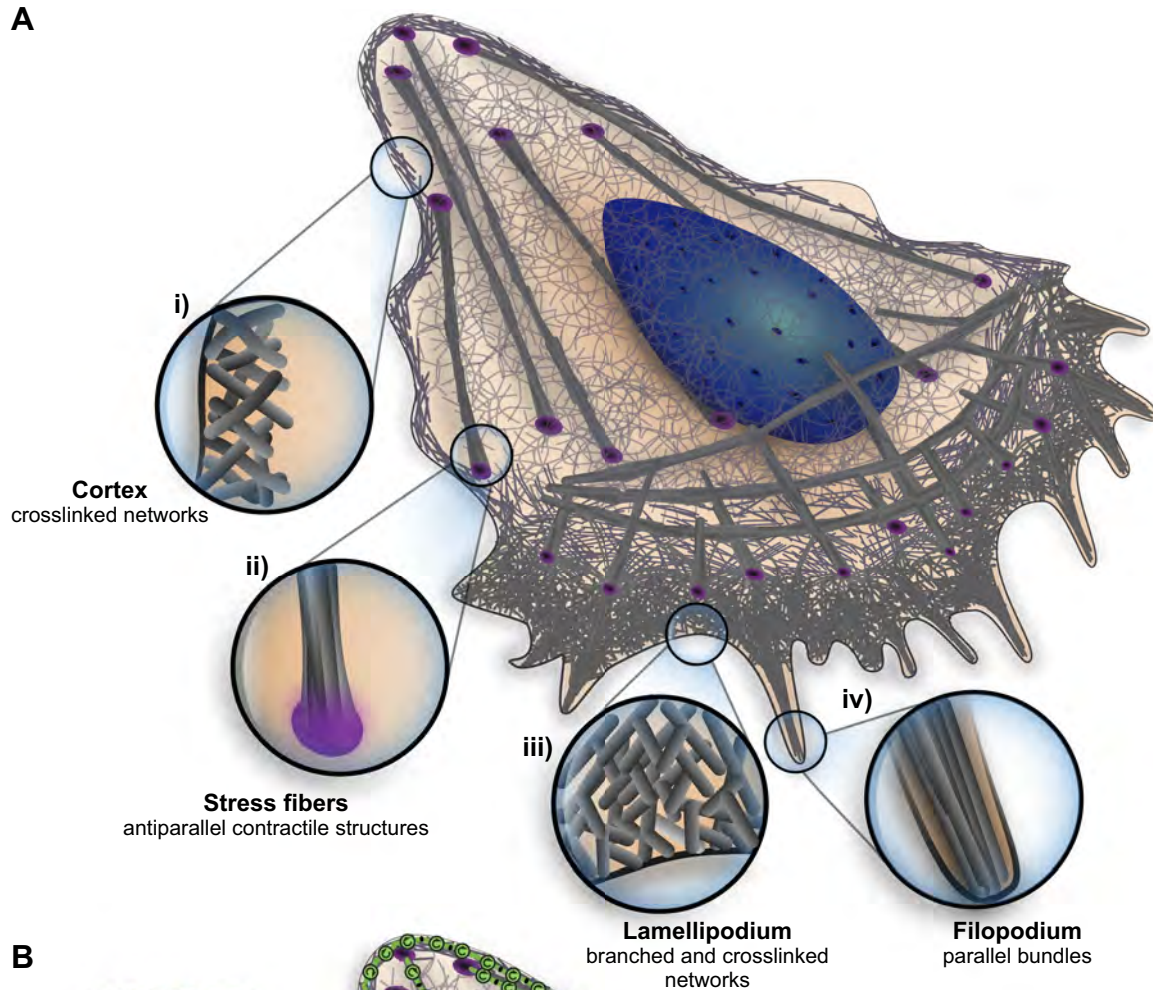
Animal cells (i.e., those without a cell wall) have the ability to change their shape to adapt to their environment, move through narrow spaces, divide, or allow exo- and endocytosis. The machinery of these shape changes relies on the assembly of proteins, in particular actin, a globular protein that polymerizes into filaments of different types of organization: branched and crosslinked networks, parallel bundles, and anti-parallel contractile structures (**FIGURE 1A**). These different architectures can be envisioned as a series of interconnected active springs and dashpots (green and red symbols in **FIGURE 1B**, respectively) that act as mechanical elements to drive cell shape changes and motility. The purpose of this review is to correlate recent progress in our understanding of the interplay between biochemical elements and mechanical properties. Instead of describing biochemical and mechanical properties separately, our main goal here is to address piece by piece the integrated feedback loop between biochemistry and mechanics.

At the front of the cell, branched and crosslinked networks in a quasi two-dimensional sheet make up the lamellipodium,

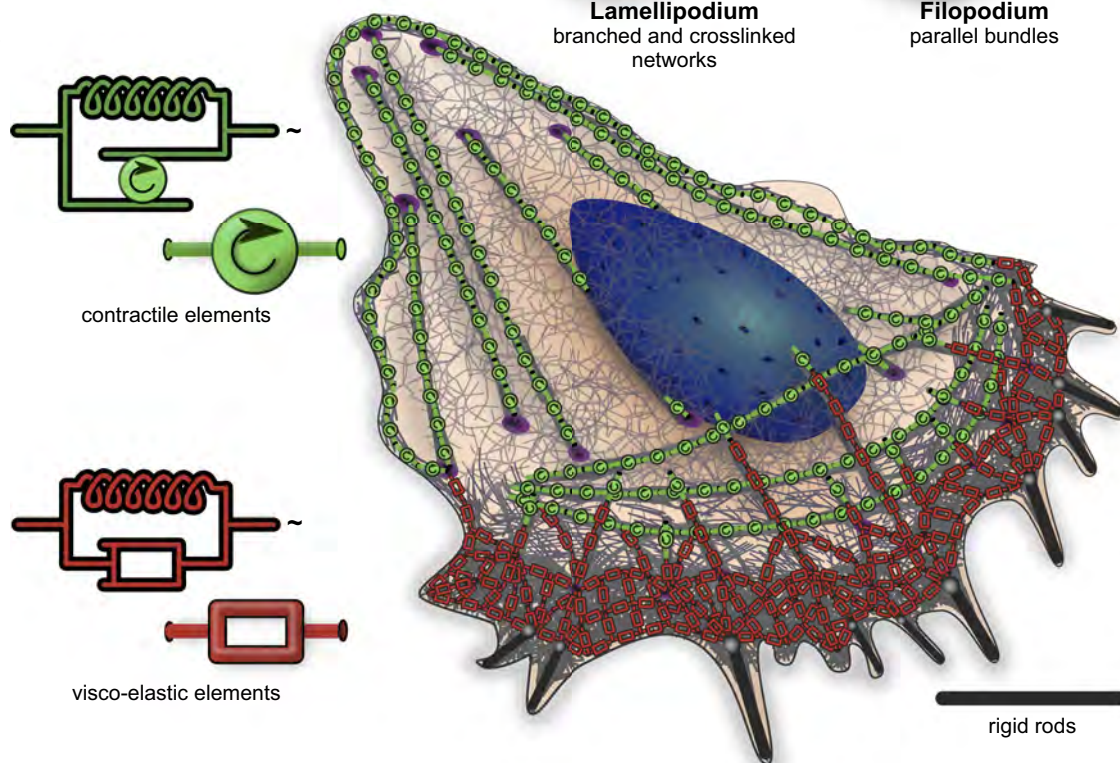
and are the major engine of cell movement since they push the cell membrane by polymerizing against it (**FIGURE 1A, iii**). Aligned bundles underlie filopodia that are the fingerlike structures at the front of the cell, important for directional response of the cell (**FIGURE 1A, iv**). A thin layer of actin, called the cell cortex, coats the plasma membrane at the back and sides of the cell, important for cell shape maintenance and changes (**FIGURE 1A, i**). The rest of the cell contains a three-dimensional network of crosslinked filaments interspersed with contractile bundles, including stress fibers that connect the cell cytoskeleton to the extracellular matrix via focal adhesion sites (**FIGURE 1A, ii**). Contraction in the cell is produced by the molecular motor protein myosin. Myosin assembles into antisymmetrical mini-filaments that, once incorporated within an actin network, provoke actin filament gliding and thus global contraction in the cell body and tension at focal adhesion sites.

To build such an array of different architectures, cells employ a battery of accessory proteins that sculpt the network and control the actin cytoskeleton mechanical response. In turn, mechanical cues feed back to control the biochemical activity of actin filaments and actin-binding proteins. In the following, we will describe this integrated view of actin cytoskeleton dynamics starting from the simplest element, an actin filament, and increasing the complexity to the cellular context. Since the main goal of this review is to bring to light how the dynamics and the mechanics of actin structures control their physiology in the cell, we will focus our attention on the four different

A



B



types of actin architecture depicted in **FIGURE 1A** that have well-documented mechano-biology. Other types of cytoskeleton organization such as podosomes or fibrillar adhesions are important for cell migration but will not be reviewed extensively here since the link between dynamics and mechanical properties is still unclear. However, the basic molecular players discussed here, including actin-nucleation machinery (Arp2/3 complex and formins), actin disassembly machinery (ADF/Cofilin), and actin contractile machinery (myosins), are conserved in all actin organizations. As such, the general mechanisms that we describe here linking actin structure formation, dynamics, and mechanical properties could be used as a springboard to understand other complex actin structures in the future.

II. ACTIN DYNAMICS AND MECHANICS

A. Single Actin Filaments

In this section we describe the basic building block of actin cytoskeleton architecture: the actin filament. Actin filament dynamics have been studied for decades, and the field is lively with controversies (120, 216). For exhaustive reviews of actin biochemistry and cytoskeletal mechanics, see References 244, 246, 299, 337. Here we will summarize the main points only. The actin monomer, a 42-kDa protein, is the basic unit for building a double-stranded helical actin filament (125, 141). The kinetics of actin filament assembly are thermodynamically limited by the nucleation step, consisting of the formation of actin dimers and trimers (**FIGURE 2A** and Ref. 285). As soon as trimers are formed, they elongate rapidly as a function of the concentration of available actin monomers (333). Polymerization of actin monomers at filament ends is followed by hydrolysis of the ATP bound to the actin subunits inside the filaments at a rate of 0.3 s^{-1} and phosphate dissociation at a rate of 0.002 s^{-1} (**FIGURE 2A** and Refs. 27, 28, 197). In addition, actin monomers bind divalent cations like calcium and magnesium, and the nature of the bound cation affects polymerization dynamics (27). Since cellular concentrations of Mg^{2+} are above millimolar, actin monomers under physiological conditions are loaded with MgATP (244).

Actin filaments are polar polymers with a right-handed helical twist and two ends that are dynamically different called barbed and pointed ends. The barbed end is the more dynamic end of the actin filament and elongates 10 times faster than the pointed end, at a rate of $11.6 \mu\text{M}^{-1}\cdot\text{s}^{-1}$ (243). Since cellular monomer concentrations can be as high as $300 \mu\text{M}$, actin filament barbed ends can assemble as fast as 3,000 subunits/s,

meaning that an actin filament can reach length scales relevant to the cell ($10 \mu\text{m}$) in $<2 \text{ s}$ (244).

Measurements in vitro indicate that single actin filaments are semi-flexible at the scale of a cell (110). This means that actin filaments shorter than cell size, $\sim 10 \mu\text{m}$, behave like rigid rods, whereas longer filaments are able to bend and thermally fluctuate because of thermal energy, $k_B T$, on the order of $4 \times 10^{-21} \text{ J}$ at room temperature, where k_B is the Boltzmann constant and T the temperature. This characteristic length of $10 \mu\text{m}$ is called the “persistence length” designated by l_p , which is exactly defined as the length over which correlations in the direction of the tangent to the filament contour are lost (**FIGURE 2B**). In other words, a filament shorter than l_p is straight and makes a constant angle with a reference, whereas a longer filament changes orientation along its length as soon as it is longer than l_p . Due to their persistence length of $10 \mu\text{m}$, pure actin filaments are virtually straight at the cell scale. This is even more the case for microtubules with a persistence length of 1 mm that are straight tracks within the cell, suiting their main function as rails for transport (**FIGURE 2B**). In comparison, DNA molecules with a persistence length of 45 nm are built to be compacted inside the nucleus (**FIGURE 2B** and Ref. 337). The persistence length l_p depends on the temperature and the rigidity of the filament. Indeed, higher temperatures result in increased flexibility and a smaller persistence length since thermal energy ($k_B T$) will be greater. Conversely, increased rigidity leads to decreased flexibility, and thus a longer persistence length. In general, the persistence length scales with thermal energy as $l_p = EI/k_B T$, where EI is called the flexural rigidity of the filament (110, 128).

However, mechanical constraints imposed by confinement/geometry or by motor protein activity can bend actin filaments at the micron scale, well below their persistence length (22, 161, 220, 327). Moreover, in the cell lamellipodium, microtubules are sometimes observed to be bent, again well below their l_p (35). From in vitro buckling experiments, we have an idea of the force that needs to be applied to bend an actin filament. Imagine a beam of length L under compression, or a plastic ruler that we press on both sides. First, the beam is compressed as an elastic material, and then it suddenly bends or “buckles” under a certain compression force (**FIGURE 2C**). This compression force can be estimated considering that it should increase with increasing elastic modulus (or increasing persistence length) and decrease when the beam length increases (it is easier to buckle a long beam). Then for homogeneity reasons, the buckling force reads $F_B = (\pi^2 k_B T/L^2)l_p$ (128). This buckling force was measured for actin filaments constrained on one end by nucleating agents like formin and

FIGURE 1. Overlay of actin architecture and mechanics in the moving cell. *A*: schematic representation of the cell with the different architectures indicated: *i*) the cell cortex; *ii*) an example of a contractile fiber, the stress fiber; *iii*) the lamellipodium; and *iv*) filopodia. The zoom regions highlight architectural specificities of different regions of the cell. *B*: overlay of the actin architecture and its mechanical profile. The red rectangles are the shock absorbers (dashpots) that represent the actin network, while the green circles are active springs due to myosin motor activity.

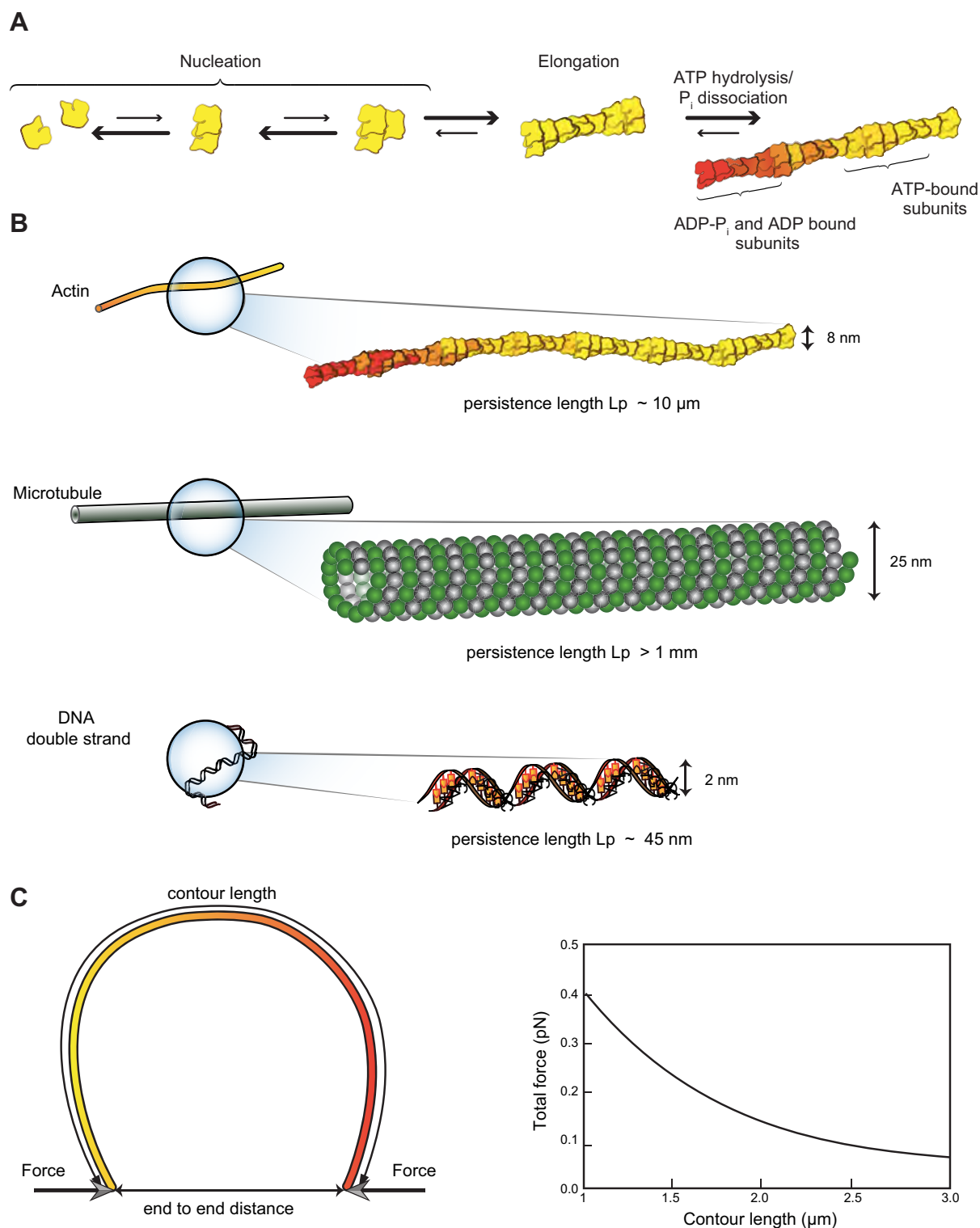


FIGURE 2. Single filament assembly and mechanics. *A*: the kinetics of actin assembly. Actin polymerization from the pool of actin monomers happens in two phases. The thermodynamically limiting step for actin assembly, nucleation, is the formation of dimers and trimers. This is followed by rapid elongation at the more dynamic end, the barbed end, at $11.6 \mu\text{M}^{-1}\cdot\text{s}^{-1}$, ATP hydrolysis in the filament at 0.3 s^{-1} , and phosphate dissociation at 0.0022 s^{-1} . *B*: persistence lengths of different cytoskeletal elements. Actin filaments are semi-flexible polymers with a diameter of 8 nm and a persistence length of $10 \mu\text{m}$. Microtubules are rigid with a diameter of 25 nm and a persistence length greater than 1 mm. Double-stranded DNA are flexible molecules with a 2 nm diameter and a persistence length of $\sim 45 \text{ nm}$. *C*: at the scale of the cell, actin filaments are almost straight structures, but they can nevertheless buckle under a load. The force exerted to bend the filament varies as a function of its contour length [22].

on the other end by an inactivated myosin to be on the order of 0.4 pN for a micrometer long filament (FIGURE 2C and Refs. 22, 161). For shorter filaments, this buckling force is greater since it scales inversely proportional to the square of the filament length. In fact, in a physiologically relevant scenario where filaments shorter than 0.5 μm elongate through polymerization of actin monomers at a concentration of 50 μM , modeling suggests that this buckling force can be as large as 10 pN (22, 132). So single filaments growing against a load exert considerable forces before they buckle. Interestingly, both the nucleotide and the divalent cations bound to an actin filament modulate its bending properties; for example, actin filaments become four times stiffer when the concentration of free cations is increased (132, 146). In addition, as we will see in more detail in section IIG, actin binding proteins such as ADF/cofilin not only modify the mechanical properties of the actin filament, but also the nucleotide state of actin monomers in the filament (28, 76, 194, 251).

The polymerization of actin filaments and their association with actin regulatory proteins produce a variety of architectures. The following paragraphs detail the dynamic in vitro formation of the major types of actin architectures encountered in cellular structures and their mechanical properties: branched actin networks, crosslinked meshworks, bundles of parallel actin filaments, and bundles of antiparallel actin filaments. These organized actin structures are built in space and time in competition with the spontaneous assembly of unorganized actin filaments. Therefore, before going into detail on how specific structures assemble, we will first describe how cellular proteins inhibit spontaneous, uncontrolled actin polymerization to focus assembly at sites where actin growth is needed.

B. Keeping Actin Assembly Under Control: Role of Profilin

Actin polymerization nucleation is thermodynamically unfavorable; however, once oligomers are formed, spontaneous actin assembly can occur if the concentration of actin monomer is above what is called “the critical concentration” at the barbed ends (i.e., 0.1 μM) (244). This is the case in cells where the concentration of actin monomers in the cytoplasm can range from a few to hundreds of micromolar depending on cell type, with platelets and neutrophils weighing in at hundreds of micromolar while *Xenopus* egg cells and HeLa cells grown in suspension have less than 10 μM (244 and references therein and Ref. 242).

Profilin, an abundant actin monomer binding protein, plays an important role in actin homeostasis (7, 145, 234, 244, 325). Profilin functions by inhibiting the spontaneous formation of actin dimers or trimers, the building blocks for the nucleation of new actin filaments (244). Actin monomers complexed with profilin can only be used for de novo actin assembly catalyzed by cellular nucleation factors. These factors include the Arp2/3

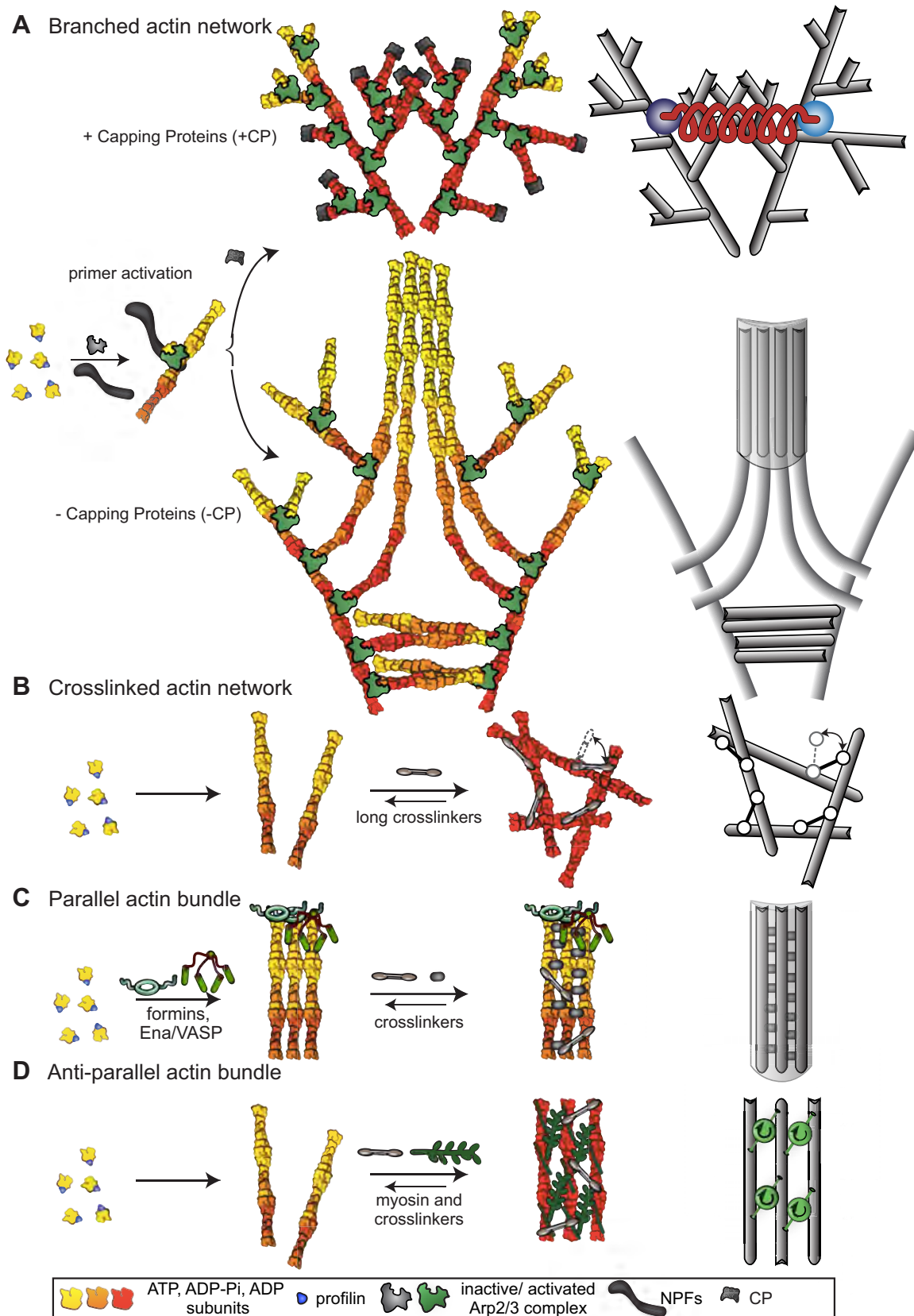
complex, activated by WASP/WAVE family proteins (see sect. IIC) and formins (see sect. IIE). Indeed, profilin is key for the rapid elongation of filaments in the presence of formin: actin filament free barbed ends elongate at a rate of 10 $\mu\text{M}^{-1}\cdot\text{s}^{-1}$, whereas the formin/profilin tandem increases this rate of elongation up to 90 $\mu\text{M}^{-1}\cdot\text{s}^{-1}$ (119, 160, 209, 264, 322). Another key role of profilin is to exclusively drive actin assembly at the fast-growing barbed end of actin filaments, while preventing polymerization at the pointed end, thus giving a polarity in the growth of any type of actin architecture (250).

C. Branched Actin Networks

One specific form of actin architecture involved in actin-based force generation for cell movement and shape changes is the branched network initiated by a complex made of seven proteins: the Arp2/3 complex (FIGURE 3A and Refs. 184, 218, 219, 260). Arp2/3 complex-branched actin organization is found for example at the leading edge of motile cells (FIGURE 1, Ref. 304), at the site of clathrin mediated endocytosis (335), and is necessary for meiotic spindle positioning (177, 348) and for the motility of some bacteria and viruses in host cell cytoplasm (47, 91).

In vitro in cell extracts or pure protein mixes, the growth of a branched network at the surface of bacteria, micrometer-sized polystyrene beads or oil droplets generate movement, thus mimicking lamellipodium extension (20, 33, 46, 181). This mechanism of branch nucleation also generates a stress in a growing network on a spherical surface (223). This stress is observed in the deformation of endosomes and biomimetic objects resembling endosomes that are able to move using the actin machinery (33, 103, 309, 318). All together these results indicate that branched networks can generate force and do work when assembled in proximity to a surface.

The polymerization of a branched actin network in the presence of the Arp2/3 complex is explosive, and in vivo, a multiple-switch mechanism is necessary to finely tune the kinetics of branch formation (122, 190). The whole process is seeded by a preexisting filament or “primer” whose side interacts with the Arp2/3 complex (FIGURE 3A and Refs. 3, 186, 292). In addition to a primer, nucleating-promoting factors (NPFs) from the Wiskott-Aldrich Syndrome protein (WASP)/WAVE family of proteins are necessary to activate the Arp2/3 complex (185, 186, 261). A feature of the Arp2/3-activating domain of all NPFs is the presence of one or several WH2 domains NH_2 terminal to the Arp2/3 complex-binding motif. WH2 domains are short domains (<50 amino acids) that bind to monomeric actin and have a range of attributed roles including actin filament nucleation (130, 266). In NPFs, WH2 domains are invariably preceded on the NH_2 -terminal side by a polyproline domain that can bind profilin-actin. This arrangement suggests a loading model where the polyproline region binds profilin-actin and then hands it off to the WH2



domain to participate in actin filament nucleation by the Arp2/3 complex (62).

Somewhat counterintuitively, to build a branched network dense enough for efficient force production, branch elongation needs to be limited by interaction with capping proteins that block growth at filament barbed ends (FIGURE 3A and Refs. 3, 4, 75, 150, 323, 324). Indeed, in the absence of capping protein, branches elongate with no limitation, resulting in most cases in an array of parallel bundles growing away from the NPF sites, and thus this condition is not optimal for force production (FIGURE 3A and Refs. 3, 4, 115, 150, 233, 256). Instead, capping protein or proteins with similar activities work in synergy with the Arp2/3 complex to build a dense actin network of capped filaments that are sterically displaced by new branches formed at NPF sites (3, 4, 25, 96, 150, 351). In the presence of capping proteins, the mechanism of activation of the Arp2/3 complex results in an actin network that should not be viewed as a homogeneous structure at the filament scale, but as a combination of subnetworks, each seeded by different primers. These subnetworks merge together depending on the biochemical conditions to build a homogeneous network that can generate a force due to entanglement of new branches generated at the NPF sites. Subnetworks can thus be visualized as independent networks that are entangled if filaments are long enough to reach the neighboring network. This creates a repulsion effect that can be depicted by a spring, although it might also involve a viscous component, and no myosin is at work here (FIGURE 3A, mechanical representation and Refs. 3, 150).

The degree of branching is of course important for defining the mechanical state of a branched actin structure. The mechanics of a network is defined by its mesh size ξ that is variously taken as the spacing between actin filaments inside the network or the distance between fixed crosslink points. In the case of branched networks, these points are both the Arp2/3 complex branch points and the entanglement points. This parameter is controlled by the actin monomer concentration, the Arp2/3 complex activity, and the amount of capping protein (150, 252). Mesh size and stiffness (elastic modulus, E) are related through $E = kTl_p/\xi^4$ with the same notations used above, which highlights how strongly (power 4) the mesh size affects the elastic modulus (223). In a nutshell, increasing the network

density dramatically increases its stiffness through decreasing its mesh size.

The mechanical properties of branched actin networks are measured by “rheology” experiments, where the network is submitted to a force per unit surface or a pressure, and the consequent deformations are observed (59, 191, 252, 340). Such experiments were pioneered in the mid 1980s (275, 350). A deformation is quantified by the “strain,” a dimensionless parameter that measures the displacement divided by the distance over which the deformation is applied. If the strain is proportional to the applied force, the material is “elastic,” meaning that it returns to its original shape when the force is removed. If the material does not come back to its original shape, this means that there is dissipation or flowing, and thus a viscous component is present in the system. In the elastic response, stress (or force per unit surface) is proportional to strain, and the proportionality coefficient is the elastic modulus. In the viscous response, stress is proportional to the rate of strain, and the proportionality coefficient is the viscosity component. Therefore, an elastic modulus has the units of Pascal, whereas a viscous component has the units of Pascal second. Mechanical properties of actin networks are also studied by microrheology experiments, where beads are embedded in the network. This technique allows for the measurement of the network deformation when beads are displaced by optical or magnetic tweezers, called active microrheology. Alternatively, since the beads are small, they undergo Brownian motion because of thermal energy, and the amplitude of this motion can be related to the rheological properties of the actin network, called passive microrheology. For a clear description of active or passive microrheology, see Reference 340. Whatever the measurement technique, actin networks are found to be “viscoelastic” materials in that they are generally elastic at small time scales (time shorter than a minute) and viscous at larger times (longer than minutes) due to rearrangements in the network. This is why a cell, or by extension, our epidermis that is made of many cells, comes back to its original shape when pinched briefly, but remains baggy when deformed during a long time, for example, on our elbows and knees.

How then does the microscopic filament organization of the branched actin network and its mechanical properties determine force production in the cell? Despite an array of different

FIGURE 3. Distinct actin filament organizations and their mechanical description. *A:* a branched actin network results from the autocatalytic branching activity of the Arp2/3 complex. Activated by nucleation promoting factors, NPFs, the Arp2/3 complex generates a branched network from the side of a preexisting actin filament, called a “primer.” In the presence of capping proteins (+CP), branches are shorter. This yields dense and rigid subnetworks that evolve into an entangled meshwork. The entanglement of filament subnetworks leads to mechanical interactions that are represented by a spring (in red) connecting barycenters (spheres) of adjacent subnetworks. In the absence of capping proteins (–CP), actin filaments grow longer and can either align into an antiparallel organization or bend and coalesce into a parallel bundle. These parallel filaments form stable filopodia-like bundles that act as a solid body. *B:* long crosslinkers organize actin filaments into networks. These bonds act as rigid links and control the global elasticity of the actin network depending on their binding kinetics and concentration. *C:* short crosslinkers tightly pack unbranched filaments, such as those generated by formins or Ena/VASP proteins, into stiff, straight bundles. *D:* molecular motors, myosins, are dynamic links that gather antiparallel filaments into a contractile unit. They act as active springs. On the right, gray diagrams represent mechanical analogs of each molecular structure.

studies concerning the mechanical properties and force generation of branched networks, no clear consensus has emerged relating the detailed actin structure to how good the actin network is at moving an object (3, 4, 20, 87, 195, 257, 338). New methods using NPF-micropatterning to orient actin network growth and control its geometry in combination with physical methods to measure force production, such as magnetic colloids in magnetic fields, atomic force microscopy, or optical tweezers, should make it possible in the future to correlate the biochemical composition of the actin network and its microscopic organization to the force generated by the network (34, 59, 96, 217, 252, 256).

D. Crosslinked Actin Networks

For the sake of simplicity, we define a crosslinked actin network as any structure of actin filaments that is connected by proteins that bridge actin filaments together, excluding the Arp2/3 complex that has been described above. Crosslinked networks are involved in controlling cell shape and mechanical integrity (64, 87, 98, 156, 254, 331). Unlike the Arp2/3 complex, which is involved both in the initiation of actin assembly and in the organization of the network, crosslinking proteins play no or little role during actin assembly, but connect already polymerized actin filaments together to generate a complex macroscopic organization (**FIGURE 3, B-D**, and Refs. 85, 129, 148, 331). An identifying property of each crosslinking protein is the distance by which it bridges two actin filaments (**FIGURE 3, B AND C**); crosslink distances range from 10 nm for fimbrin to 160 nm for filamin (153, 282, 297). Crosslinkers that impose small crosslinking distances, such as fimbrin or fascin, tightly pack actin filaments into bundles with actin filaments oriented in a parallel, antiparallel, or mixed polarity fashion depending on the crosslinker (178, 289). Larger crosslinkers, such as filamin or α -actinin, are present in either bundles or networks depending on their concentrations (69, 148, 199, 277, 329, 330). In addition, recent results show that the rate of assembly of actin networks can influence how crosslinkers do their job through crowding effects (85). Indeed, increasing the rate of actin assembly abolishes the formation of actin bundles generated by α -actinin because fast actin assembly generates long filaments that have limited mobility, preventing them from being aligned into bundles by this crosslinker (85). This observation could have a major impact on our understanding of network architecture formation in a crowded cell cytoplasm.

Like for branched networks, the mechanical properties of crosslinked networks can be studied by rheology experiments. It is observed that if the crosslinked network is a homogeneous structure (**FIGURE 3B**, mechanical representation), applying a force on a long time scale gives time for redistribution of the crosslinkers, and global shape changes that remain once the force is released like for a viscous material (343). If the force is applied on a short time scale, crosslinkers do not have time to reorganize and resist against the load; the network then be-

haves like an elastic material, returning to its original shape once the force vanishes (343). The presence of crosslinkers in an actin network increases the elastic modulus and decreases the viscosity. However, crosslinked networks in most cases are a mixture of entangled and bundled filaments that prevent the network from having a linear viscoelastic response under force, since the different architectures respond differently. A nonlinear viscoelastic response where stress is no longer proportional to strain or strain rate can also be obtained in homogeneous semi-flexible networks considering that some filaments are elongated under stress, whereas others are compressed (137). So a detailed understanding of the actin architecture is necessary to understand the relationship between actin organization and mechanical response (87, 178, 299).

E. Parallel Actin Bundles

Parallel actin filament bundles are a type of structure found in a large number of cellular contexts including filopodia, microvilli, and hair cells (14, 254, 324, 345, 346). They are made of filaments oriented with their barbed ends in the same direction, most of the time facing the cell membrane (64). Actin filaments in a bundle are maintained in close contact via crosslinking proteins including α -actinin, fimbrin, and fascin that bind/unbind from the filament on the order of seconds, but this can vary if a load is applied to the crosslink (**FIGURE 3C** and Refs. 15, 86, 206).

It is an open question as to how the actin filaments that make up the bundle are initiated. Two nonexclusive mechanisms are generally admitted, one that requires the Arp2/3 complex and another that involves barbed end elongation enhancement proteins like formins or Ena/VASP proteins that will be discussed in the next paragraph (3, 4, 39, 115, 200, 256, 264, 276, 305, 323, 324, 345). The first situation occurs when capping protein is absent from Arp2/3 complex-generated networks (**FIGURE 3A**, -CP). As uncapped filaments elongate freely at their barbed ends, they tend to bundle via electrostatic interactions, and then a transition from branched to parallel actin filaments is observed. Geometrical constraints and the angle by which filaments are contacting each other can alter this transition and generate either parallel or antiparallel actin structures (256). As the filaments start to organize in parallel structures, they can be captured by crosslinkers such as fascin that further stabilize the bundled conformation and stiffen the structure (**FIGURE 3, A**, mechanical representation, **AND C**). Interestingly this transition between branched and parallel actin organization is observed in the actin comet tails of *Listeria monocytogenes*, a bacterium that uses cytoplasmic actin machinery to move (42). In this study, the Arp2/3 complex is removed from already moving *Listeria*, and movement continues via parallel actin structures, crosslinked by fascin.

An alternative for initiating parallel actin bundles is through formin proteins (63, 112). Formins are a large family of pro-

teins characterized by the presence of the formin homology 1 and 2 (FH1 and FH2) domains that work in concert during actin assembly (63, 121, 159, 238). The FH1 domain is an unstructured domain that acts as a lasso to rope in profilin-actin molecules while the FH2 domain interacts with the barbed ends of actin filaments (60, 104). For most formins, the FH2 domain stays attached to the growing barbed ends as the filaments elongate, making formin a processive elongation machine (37, 70, 113, 119, 139, 161, 209, 352). In some cases, formins work together with the Arp2/3 complex or with the tumor suppressor adenomatous polyposis coli (APC) for actin assembly (26, 30, 37).

Some formins are not processive and move during actin assembly from the end to the side of actin filaments (201). This latter property allows formin to be not only an elongator during actin assembly but also a crosslinking protein that organizes actin filaments into large parallel or antiparallel structures (114, 201, 203, 214). However, formins do not necessarily form bundles *in vivo*. A case in point is FMNL1, which is able to bundle actin filaments *in vitro* but lacks the ability to generate filopodia in cells (111). For *in vivo* bundle formation, it is likely that formins work in concert with bundling proteins such as fascin (for filopodia) or fimbrin (for cables in yeast).

Another component of actin bundles *in vivo* is Ena/VASP proteins, also associated with protrusive actin structures in general (for review, see Ref. 316 and **FIGURE 3C**). Anti-capping and barbed end elongation enhancement activity, shown using purified Ena/VASP proteins *in vitro*, may explain the role of Ena/VASP in filopodia formation (16, 38, 39, 109, 236). However, Ena/VASP could also contribute to mechanical rigidity of bundles, since it is multimeric with filamentous actin binding sites, a common motif in crosslinking proteins. Indeed, Ena/VASP is found to magnify the effect of fascin on network rigidity, perhaps explaining the presence of these two proteins in filopodia (301). However, on their own, Ena/VASP proteins only very modestly increase the rigidity of actin networks, possibly due to the flexibility the Ena/VASP tetramer (101, 302).

The mechanical properties of bundles depend on the presence of crosslinkers and on whether the crosslink attaches actin filaments tightly together or lets them slide over each other. Mechanical properties of actin bundles can be estimated through their buckling force, which is, as for a single filament described above, the compression force necessary to bend the bundle. Since for a single filament this buckling force is $F_B = (\pi^2 K_B T / L_2) l_p$, for N filaments that are in a non-crosslinked bundle this becomes $F_B = N(\pi^2 K_B T / L_2) l_p$. For N filaments that cannot slide in the bundle since they are crosslinked, the buckling force scales with the power 4 of the radius of the bundle, therefore with N^2 . Thus the persistence length of an actin bundle reads $L_p = N l_p$ for filaments freely able to slide over each other and $L_p = N^2 l_p$ when all filaments are statically attached to one another. Another type of force that can affect

bundle mechanics is the force exerted by the actin crosslinkers, or the applied force needed to break the actin-crosslinker bond (86, 298). This rupture force depends on the type of actin binding proteins but ranges from 30 pN for α -actinin to 50 pN for filamin (86).

Similar to a branched actin network, elongating actin bundles can exert enough force to move an object (200, 264). In a study using optical traps to measure the force generated by elongating bundles, a force of 1 pN is enough to stop the growth of a bundle made of a few filaments (88). Similar force stops the growth of a single filament, suggesting that in the case of an actin bundle elongating freely against a load, only one filament is in contact with the load at a given time. The reason why actin bundles generated by formin are able to maintain bead motility is that all the barbed ends are elongated at the surface by a processive formin instead of arriving freely in contact with the load, and also the filaments form a bundle, preventing the active force of actin polymerization from being lost to buckling. As a result, more than one filament is pushing the bead, and therefore, forces add to push the bead in a coordinated fashion. This is the case for formin but could also be true for any processive elongation machinery, such as Ena/VASP proteins. A challenge for further investigation will be to correlate the number of actin filaments in a processively elongating bundle and the force exerted by this bundle as a function of its length. Indeed, short stiff bundles stay straight as they apply a force against a load, whereas long actin bundles have a tendency to buckle and deform themselves instead of maintaining an increasing force against a load (200, 264). Interestingly, formin-mediated force generation via actin bundles has been shown to drive the motility of the bacterium *Rickettsia* (108).

F. Antiparallel Actin Organization

Antiparallel actin structures with myosin-induced contraction are necessary for cytokinesis and for stress fiber function during the establishment of cell/cell and cell-matrix adhesions (45, 126, 170, 281, 289, 311, 315). As for parallel actin bundles, antiparallel organizations are stabilized by crosslinking proteins that favor this specific configuration (**FIGURE 3D** and Refs. 155, 170, 312). Fimbrin and α -actinin are good candidates to stabilize actin bundles in an antiparallel conformation (170). Recent reconstitution of contractile networks *in vitro* reveals two steps during myosin/antiparallel actin bundle interaction in the presence of crosslinking proteins: contraction and myosin-induced disassembly (116, 220, 327). The latter will be described in the subsequent section. In the absence of crosslinking proteins, antiparallel structures contract to a large extent under myosin action before disassembly, whereas in the presence of crosslinking proteins such as α -actinin, the extent of contraction is limited before disassembly is initiated (255). In addition to biochemical composition, the length of filaments in the antiparallel network has a direct impact on its contractile properties as the number of myosin heads per unit

length will vary and the tension along the contractile structure is proportional to the number of myosin heads (FIGURE 3D, mechanical representation, and Ref. 313). The mechanism of bundle contraction has been addressed theoretically by analytical continuous models or by a microscopic description of bundle architecture (24, 175, 176). Minimum reconstituted systems for antiparallel contractile bundles, consisting of just a few components, seem to mimic most of the mechanical properties of sophisticated contractile units in vivo (255, 313). The velocity of contraction depends on filament architecture: contraction is faster for antiparallel filaments compared with branched networks. The orientation of the actin filaments in the contracting network is thus a major determinant for controlling the rate of contraction (155, 255). Moreover, the dynamics of actin polymerization and depolymerization have to be taken into account in addition to myosin activity for explaining phenomena such as actin ring contraction during cytokinesis (354). Obviously more work is necessary to correlate biochemical composition and geometrical parameters of antiparallel bundle organization and their mechanical properties under tension generated by myosin motors, but recent reconstituted systems are promising tools to unveil this connection (217).

G. Disassembly of Actin Networks

In the previous sections, we described how the different actin organizations are built. In the following section, we address how actin structures are disassembled. Two key protein factors are involved during actin disassembly, ADF/cofilin and myosin, both acting on actin filament mechanics.

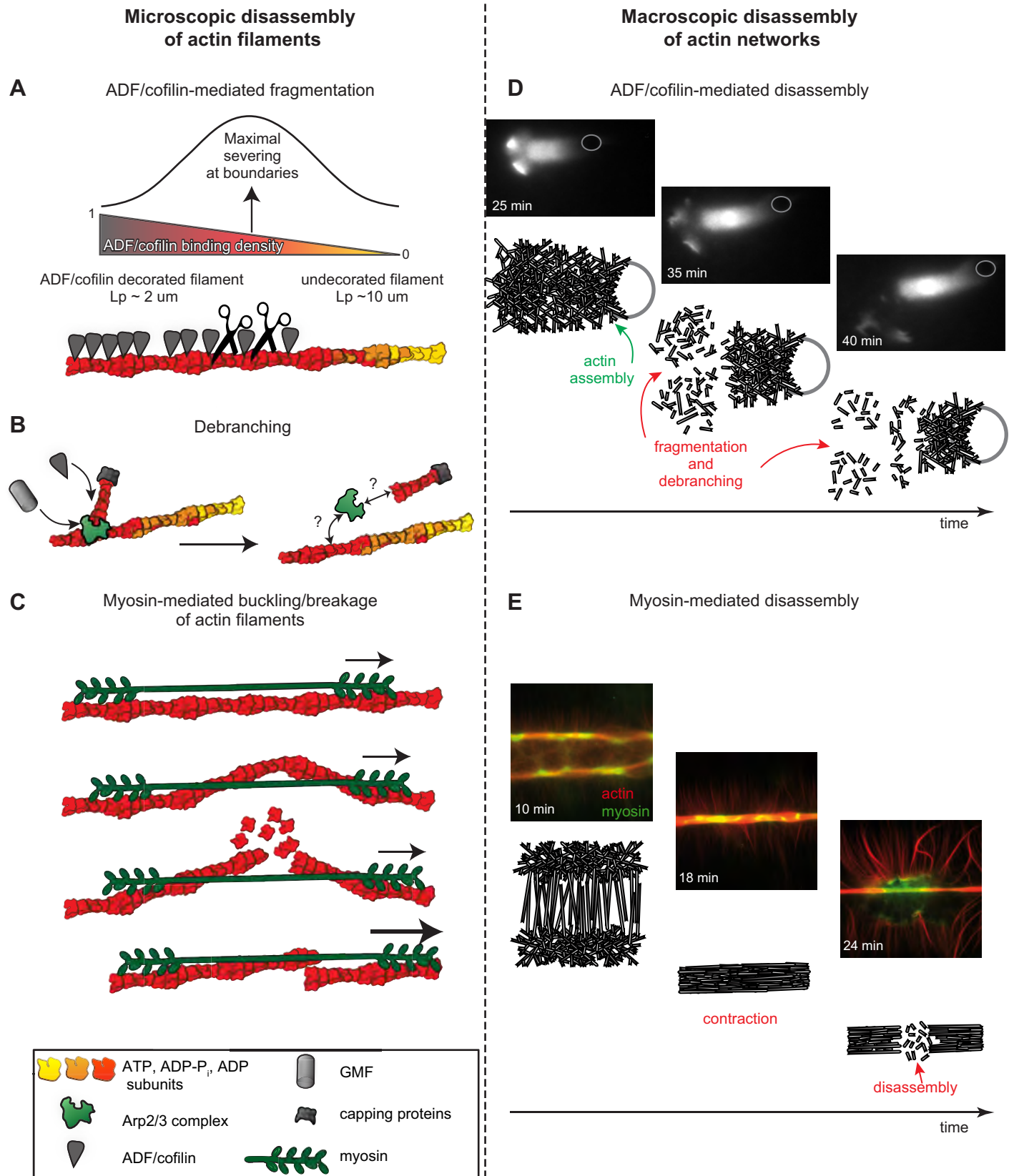
1. ADF/cofilin-induced disassembly

ADF/cofilin was discovered in the 1980s in the brain as an actin disassembly factor (12). Inspired by this pioneering work and almost simultaneously, the same protein going by the names of actophorin, destrin, cofilin, and depactin was discovered in different organisms including amoeba, starfish, and mammals (21). By general consensus, these proteins are now grouped under the name ADF/cofilin (21). It is somewhat unfortunate that the “D” in ADF stands for “depolymerization” since, as we will see in the following, ADF/cofilin is in fact a

disassembly factor that uses fragmentation or severing to break down actin organizations (131, 257), as opposed to a depolymerization machine that affects the rate of depolymerization at the end of actin filaments (51). Part of the confusion stems from the fact that in a bulk assay ADF/cofilin is reported to increase by 25-fold the rate of actin dissociation from pointed ends; however, evanescent wave microscopy (also known as TIRF microscopy) of single filaments in the presence of ADF/cofilin does not confirm this finding (9, 40, 58, 300).

Whereas the observation of actin filaments by classical epifluorescence microscopy requires the use of fluorescent phalloidin to preferentially label filaments so they can be seen against the noise of free fluorophores in solution, evanescent wave microscopy is an alternative to observe single actin filaments formed from fluorescently labeled monomers since it cuts down on the background by illuminating a restricted region of the sample. Therefore, evanescent wave microscopy is key for revealing the real mechanism of ADF/cofilin since it avoids filament visualization via fluorescent phalloidin-stabilized filaments, a real problem in the study of ADF/cofilin because phalloidin inhibits the interaction between ADF/cofilin and the actin filament (8, 93, 187). With the use of evanescent wave microscopy, the first important observation is that ADF/cofilin fragmentation efficiency depends on the degree of saturation of ADF/cofilin along actin filaments (FIGURE 4A and Refs. 9, 76, 83, 193, 300). Indeed, poorly decorated actin filaments fragment more readily than fully decorated filaments that seem instead to be stabilized by ADF/cofilin decoration. This was puzzling until ADF/cofilin was shown to decrease the persistence length of actin filaments fivefold to a l_p of 2 μm (FIGURE 4A). In other words, actin filaments decorated by ADF/cofilin are more flexible (193). This suggests a tight coupling between biochemistry and mechanics during actin fragmentation by ADF/cofilin, a unique property that could be the object of an entire review on its own. From these observations a model has emerged to explain fragmentation by ADF/cofilin based on local stress accumulation at mechanical discontinuities, i.e., at boundaries of bare and ADF/cofilin-decorated filament segments (FIGURE 4A and Refs. 76, 194). This is confirmed by direct observation by two-color evanescent wave microscopy of the interaction of ADF/cofilin with actin fila-

FIGURE 4. Remodeling and disassembly of dynamic actin structures. ADF/cofilin or myosin-mediated disassembly of individual or complex actin filament structures occurs through important modifications of their global mechanical properties. At the microscopic level, *A*: fragmentation of individual filament happens at the boundary of bare and ADF-decorated portions, whose persistence length is significantly decreased. *B*: disassembly by ADF/cofilin can also occur by debranching of the Arp2/3 complex network. Additional proteins such as GMF can also induce branch dissociation. *C*: directed motion of myosins can induce filament buckling and eventually breakage when one end of the myofilament moves faster than the other one. [Adapted from Vogel et al. (327).] At the macroscopic level, *D*: ADF-mediated severing and debranching activities lead to the stochastic macroscopic fragmentation characterized by the loss of large network portions of actin tails polymerized at the surface of functionalized beads in the presence of ADF/cofilin (257). This mechanism favors rapid actin turnover of actin structures away from the surface of the bead, due to the localization of ADF/cofilin in older parts of the tail (ADF/cofilin is fluorescently labeled). *E*: myosin-induced contraction and disassembly of a reconstituted contractile network on functionalized micropatterned bars (255). Myosin-induced contraction of a coherent meshwork ends in mechanical breakage of filaments beyond a certain deformation limit. Waves of myosin (green) appear leading to actin disassembly, as visualized on functionalized micropatterned bars. For *D* and *E*, the cartoons illustrate the different steps over time during actin disassembly mediated by ADF/cofilin or myosin.



ments, which demonstrates that fragmentation occurs at the boundary (40, 300).

In addition, the biochemical control of ADF/cofilin binding to actin filaments is complex: ADF/cofilin binds with a stronger affinity to ADP subunits than ADP-P_i or ATP subunits inside the filaments (28, 183). Interestingly, binding of ADF/cofilin to actin filaments also increases the rate of phosphate dissociation from actin subunits, making the aging of actin filament from ATP to ADP subunits faster (28). The ATP-loaded growing barbed end of actin filaments is always excluded from decoration by ADF/cofilin, and therefore, fluorescently labeled ADF/cofilin appears to be an excellent probe to monitor the nucleotide state of growing actin filaments (300). In keeping with this, only the presence of capping proteins that block the elongation of actin filaments allows for full decoration of the actin filament by ADF/cofilin (300).

As a result, a stochastic fragmentation of growing actin filaments is observed with ADF-cofilin, with filament lengths showing phases of growth and shortening, a mechanism that is different from the vectorial disassembly process originally envisioned (200, 262, 295). The link between the mechanics of actin filaments, ADF/cofilin, and severing was further investigated using optical tweezers to stretch actin filaments, revealing that stretched actin filaments are resistant to ADF/cofilin binding/severing (95, 117). This observation is in line with the idea that actin filaments may act as tension sensors to modulate their own dynamics.

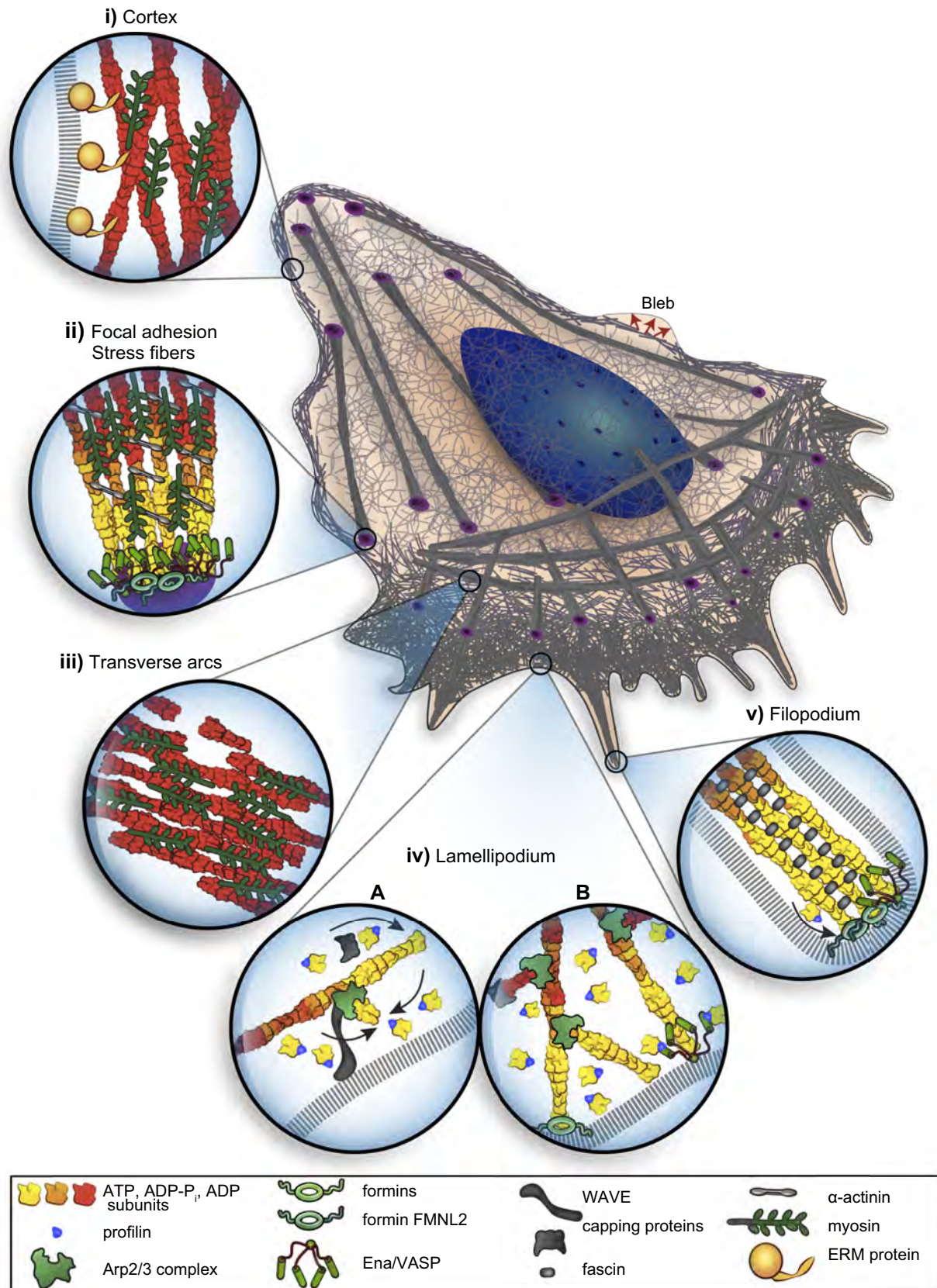
It is very difficult to predict how ADF/cofilin will act on actin filament bundles stabilized by crosslinking proteins (83, 294). On one hand, ADF/cofilin has been shown to synergize with fascin to disassemble parallel actin filaments (40). On the other hand, crosslinking proteins have been shown to inhibit fragmentation induced by ADF/cofilin, and direct visualization of bundle fragmentation by ADF/cofilin demonstrates that bundles are more resistant to fragmentation than single filaments (129, 200, 278). The question as to how actin filament bundles are disassembled by ADF/cofilin is still open, especially in the context of actin dynamics in cells.

Concerning branched networks, ADF/cofilin severs them, but also dissociates branches generated by the Arp2/3 complex (FIGURE 4B). The mechanism of branch dissociation is not fully understood, but a combination of dissociation of the Arp2/3 complex from the mother filament and/or from the pointed end of the daughter filaments has been proposed (25, 52, 189). Another member of the ADF/cofilin superfamily, glia maturation factor (GMF), targets the junction between actin subunits and the Arp2/3 complex of the daughter filament, dismantling branch points and inhibiting new actin assembly (31, 97, 182, 347). This microscopic effect of ADF/cofilin family proteins on the stability of Arp2/3 complex branch stability can be observed at the larger scale of dense branched networks reconstituted in vitro (FIGURE 4D). As for single growing actin filaments, ADF/cofilin targets the aged ADP part of the growing actin network, thus focusing disassembly at sites that are removed from locations of active assembly (131, 163, 164, 257). It is also observed that stochastic severing or debranching by ADF/cofilin facilitates network turnover through macroscopic network fracture where large portions of the network suddenly disintegrate, rather than by gradual filament depolymerization (FIGURE 4D and Refs. 131, 257). In the future, it will be interesting to understand how the effect of ADF/cofilin on the mechanical properties of single actin filaments is integrated at the level of a branched network as a whole.

2. Myosin-induced disassembly

Myosin-induced contraction has recently emerged as another way to disassemble actin networks (FIGURE 4, C AND E, and Refs. 116, 155, 220, 255, 292, 327, 339). This was initially reported for actin filament bundles stabilized by fascin during an in vitro filament gliding assay (133). In this study, actin bundles are able to slide along the myosin-coated surface at low myosin density, whereas they are disassembled at high myosin density. In solution, myosin-induced actin bundle disassembly occurs in two steps: first bundle dissociation, then actin filament disassembly (116). An “orientation selection” mechanism is proposed where selective contraction and disassembly by myosins occurs depending on the actin architecture (255). Myosins are able to reorganize branched networks into an antiparallel organization and induce their contraction and dis-

FIGURE 5. Specialized actin organizations in vivo. Motile cells have distinct actin organizations in different locations in the cell, specialized for precise functions. *i)* The actin cortex is anchored to the plasma membrane through ERM proteins and is contractile via myosin activity. *ii)* One category of contractile bundles, the stress fibers, are found spanning the cell body, usually oriented parallel to the direction of movement. They are attached to focal adhesions and involve a specific set of regulatory intracellular factors, among them formins, Ena/VASP proteins, α -actinin, and myosin. *iii)* Transverse arcs are specific antiparallel actin filament formations found at the back of the lamellipodium. They are contractile through myosin activity. *iv)* The motor organelle, the lamellipodium, hosts rapid, massive, and localized polymerization of branched actin networks. *A:* initiation of this dendritic network occurs via the concerted activity of locally activated Arp2/3 complex binding to the side of an actin filament “primer” and by interaction with members of the WAVE family of proteins. Elongation of the network occurs by addition of the profilin/actin complex (black arrows) to the barbed ends of actin filament in close contact with the plasma membrane. *B:* Ena/VASP proteins, the formin FMNL2 and capping proteins control the elongation of the network by modulating the dynamics at filament barbed ends (right zoom inset). Ena/VASP and FMNL2 favor barbed end elongation whereas capping protein blocks it. *v)* The sensors organelles, filopodia, are filled with parallel actin bundles elongated by the actin polymerases, Ena/VASP and formins, and tightly packed by the short bundler fascin. Another type of leading edge protrusion are blebs, initially formed as cytoskeleton-free membrane bulges driven by the internal pressure of the cell (brown arrows).



assembly, while parallel actin bundles are unaffected (**FIGURE 4D**). This mechanism has major implications for cellular actin dynamics and contractile properties as will be discussed in the following section.

A possible molecular mechanism for myosin-induced contraction and disassembly based on mechanical fragmentation of a single actin filament was recently proposed by two elegant studies (**FIGURE 4C** and Refs. 220, 327). Fragmentation occurs via actin filament buckling generated by myosin contraction. It is interesting to draw a parallel between this mechanism of fragmentation and severing by ADF/cofilin. In both cases, the fragmentation results from a mechanical effect on actin filaments. Indeed, bending of actin filaments due to myosin contraction or thermal fluctuations induces high curvature and mechanical stress that favor fragmentation (194, 220, 327). It is interesting to note that the inverse has also been shown to be true: actin filament curvature seems to favor branch formation (259). The mechanics of actin filaments are obviously an important intrinsic property that controls both actin assembly and disassembly.

3. Disassembly versus depolymerization

We argue here that most of actin structure destruction occurs by a mechanism of actin disassembly instead of depolymerization from filament ends (208). Both ADF/cofilin- or myosin-induced fragmentation disrupt the mechanical integrity of the actin organization. For branched networks, debranching and fragmentation by ADF/cofilin induce large fractures in the network and a macroscopic disintegration of the network (**FIGURE 4D**). However, the integrity of the network at the site of force generation (i.e., against the load) is preserved by the fact that ADF/cofilin does not bind to ATP-actin subunits at sites of active assembly. In addition, proteins such as GMF that specifically target dissociation of branch junctions help in this mechanical destruction of the network. Similarly, myosin-induced actin disassembly at least in vitro has little to do with depolymerization, but instead is probably controlled by mechanical fragmentation that can propagate at a mesoscopic scale (**FIGURE 4E**). In the following section, we discuss in more detail the implications of these recent observations in a cellular context and why the debate is still open concerning the mechanism controlling actin disassembly in vivo.

III. CELLULAR SCALE: DYNAMICS AND MECHANICS OF INTEGRATED ACTIN STRUCTURES

We saw in preceding sections how different protein factors regulate individual actin filament dynamics and mechanics and overall actin network properties. In **FIGURE 5**, we revisit the moving cell schematized in **FIGURE 1** to understand how these different structures are integrated on the scale of the cell to produce efficient force and movement.

In the classic vision of the moving cell, largely inspired by studies of fibroblasts moving on rigid surfaces, motility is the result of the protrusion of the front of the cell (the leading edge), which then adheres to the substratum, followed by de-adhesion at the back of the cell concomitant with contraction to squeeze the cell body forward (1, 2, 172, 205). The actin cytoskeleton is a major player in all parts of this process (196, 245). In **FIGURE 5** and the subsequent sections, we zoom in on different parts of the moving cell and discuss what is known about their actin dynamics and mechanics. Lamellipodia and filopodia (**FIGURE 5**, *iv* and *v*, respectively) are the main organelles driving motility while contractile structures such as transverse arcs (*iii*), focal adhesion-anchored stress fibers (*ii*), and the cell cortex (*i*) ensure mechanical integrity and coherent movement of the cell as a whole. In addition, in recent years, another kind of protrusion, the cellular bleb (**FIGURE 5**), has garnered increasing attention. Blebs can appear as leading edge protrusions, driving cell motility independently of or concomitantly with lamellipodia and filopodia.

It should be pointed out that with the advent of culture conditions that more closely mimic the in vivo environment (3-dimensional geometries with adjustable mechanical properties), as well as developments in in vivo imaging of cell motility, it is becoming increasingly apparent that not all migrating cells in vivo actually look like the typified cell shown in **FIGURE 5**, and properties such as shape and speed as well as the balance between bleb-based and lamellipodia-based motility can be modified (79, 102, 198, 240). However, the molecular ingredients behind cell motility are universal: actin filament formation is nucleated by either formin or the Arp2/3 complex, and some sort of protrusion is formed via either directed actin assembly or via myosin contractility of the actin cortex, followed by cytoskeleton disassembly. **TABLE 1** summarizes this idea, showing how the molecules introduced in section II are integrated into the four main cellular structures shown in **FIGURE 5** and examples of the various incarnations of these structures in different cell types or in in vivo conditions.

A. The Lamellipodium

1. Lamellipodia construction

The lamellipodium of the moving cell is a quasi-two-dimensional actin network formed via the assembly filaments beneath the leading edge membrane, beautifully visualized by a variety of optical techniques (**FIGURE 5**, *iv*, *A* and *B*, and Refs. 290, 304, 310, 326, 344). Numerous polymerization nucleation factors play a role in lamellipodia formation, but the main mode of filament assembly is via the Arp2/3 complex activated by a specific NPF, the WAVE complex, although formins may also play a role (48). The WAVE complex is itself

Table 1. Actin-binding protein composition of the major actin architectures and cellular examples

Actin Structures	Signature Biochemical Components	Star Examples of Cell Types Displaying This Architecture	Reviews Associated With Each Cell Type
Lamellipodia *	Branched actin filaments Nucleation: Arp2/3 complex via WAVE activation, FMNL2 formin via Cdc42 activation Cross-linkers: alpha-actinin, filamin, fimbrin Ena/VASP proteins Capping protein ADF/cofilin	Fish keratocytes in 2D Migrating cancer cells in 2D and 3D White blood cells in 2D, 3D, and in vivo	169, 245, 273
Filopodia	Parallel actin filaments Nucleation: mDia2 and FMNL2/3 formins, Arp2/3 complex Crosslinker: fascin Ena/VASP proteins	Neuronal growth cone cells in 2D Melanoma cells in 2D Endothelial tip cells in vivo	80, 82, 84, 345
Contractile fibers (stress fibers and transverse arcs)	Antiparallel actin filaments Nonmuscle myosin II Nucleation: formins, Arp2/3 complex Crosslinker: alpha-actinin, myosin II Ena/VASP proteins	Motile fibroblasts in 2D Cells in 3D subjected to sheer stress (example: endothelial arterial cells)	45, 221
Cell cortex→blebs	Branched and unbranched actin filaments Nonmuscle myosin II Nucleator: unclear Formin-based? Ezrin/radixin/moesin (ERM) proteins	Amoeba like <i>Dictyostelium discoideum</i> and <i>Entamoeba histolytica</i> (only examples of bleb-based movement in 2D) Motile cells in vivo (examples: germ cells in zebrafish, fundulus deep cells)	169, 232

See text for a full list of components and references.

*Other variants include pseudopodia, invadopodia, and lobopodia (36).

intrinsically inactive and needs Rac, a membrane-bound GTPase, and lipids to become functional for activating the Arp2/3 complex (61, 173). The end result of these layers of activation is the very tight control of Arp2/3 complex-based polymerization at the leading edge of the cell.

However, up until recently, there was a long-standing controversy in the field as to whether the meshlike appearance of the lamellipodium was due to filament branching via the Arp2/3 complex or to the criss-cross of straight filaments. Recent results involving reanalysis of electron microscopy data originally used to support the criss-cross theory have definitively shown that filaments are branched in the lamellopodia (291, 319, 346). However, branches are not always positioned with the fork facing toward the leading edge membrane as is commonly portrayed in the textbooks (306). This is even more obvious in in vitro studies where the orientation of branches with respect to the polymerizing surface is random with many barbed ends growing away from the surface (3). This is a direct consequence of the fact that surface/membrane-bound Arp2/3 complex starts a new filament off the side of a filament primer as described in a previous section on branched networks (FIGURE 5, *iv*, A; Ref. 307). This is likewise observed in vivo in an intracellular wound healing system, where primer filaments run parallel to the wound edge and branches are oriented obliquely to the protruding membrane (326). In a related

study, an analysis of the correlation of cell velocity with actin filament orientation shows that, in fact, faster cells display filaments oriented in an oblique manner, whereas filaments in slower cells are oriented mainly toward the direction of movement (334). Future studies should shed light on how obliquely oriented actin growth produces movement and why in vivo many filaments are oriented with their barbed ends abutting the membrane. One hypothesis for the latter is that cellular factors such as formins, Ena/VASP proteins, or WASP family members may hold the barbed ends in this orientation (30, 39, 66, 109).

Although N-WASP, downstream the RhoGTPase Cdc42, also activates the Arp2/3 complex to form actin branches, up until recently only WAVE has been implicated in lamellipodia formation. N-WASP has been exclusively associated with filopodia formation and specialized protrusions of invasive cancer cells called invadopodia (258, 274). However, new studies have shown that N-WASP, not WAVE, is the major actin polymerization regulator in cells moving in three dimensions (308), and in another study, that cells in three-dimensional environments are motile despite the fact that active Rac is completely mislocalized from the protruding edge of the cell (240). These results modify the traditional view that the lamellipodium is formed via Rac signaling to WAVE and suggest

that actin polymerization machinery may depend on cell environment via mechanisms that remain to be elucidated.

Recent studies have even shown that leading edge protrusion can occur in the absence of the Arp2/3 complex. When the Arp2/3 complex is knocked down more thoroughly than had previously been achieved or even knocked out entirely, cells are still motile, moving via fingerlike protrusions resembling filopodia, although various defects in translocation are observed (266, 303, 342). In addition, a role for Cdc42 in lamellipodial protrusion has been discovered as an activator of a formin FMNL2 (FIGURE 5, *iv*, B), so again driving home the idea that the Arp2/3 complex is not the only way to polymerize actin and push out a plasma membrane (30). Another major player in lamellipodia dynamics is the Ena/VASP proteins, which are found at the leading edge of the moving cell and are associated with increased protrusion (FIGURE 5, *iv*, B; Refs. 18, 166, 265).

2. Role of the plasma membrane in lamellipodia dynamics

In the preceding section, we have essentially ignored the plasma membrane despite the fact that the membrane is in close proximity to the growing barbed ends of the actin network. Physical models of membrane-cytoskeleton interaction treat the membrane as the load opposing polymerization, with the magnitude of the load being proportional to how tense (or tight) the membrane is, also known as the “membrane tension.” In keeping with this, a study on cell spreading shows that protrusion is enhanced when the membrane tension is reduced (253). On the microscopic scale of a single filament growing up against a membrane, there is no doubt that reduced membrane tension allows more growth. However, on the macroscopic level of the entire cell, it is becoming increasingly apparent that the mechanics of the membrane play an active role in sculpting and organizing the actin cytoskeleton deeper in the cell body (77, 151).

This idea that membrane tension can be a necessary and positive regulator of cytoskeleton architecture was recently demonstrated in cells in two separate studies. First, in crawling *Caenorhabditis elegans* sperms cells, increasing membrane tension suppresses lateral lamellipodial protrusions, thus streamlining cytoskeleton assembly in the direction of movement and enhancing motility (17). Tension is believed to drive the coalescence of filaments, overcoming the energetic penalty of bending to bring filaments together in a bundle in the absence of crosslinking proteins or other clustering factors, as has been shown in vitro for actin filaments growing up against a membrane (179, 210). Likewise in locomoting neutrophils, membrane tension is a major polarity factor: high membrane tension leads to a high directionality of actin polymerization (127). As in the sperm cell, high membrane tension in neutrophils appears to suppress protrusions that are not going in the direction of movement, thus probably working with signaling pathways to confine lamellipodia assembly to one side of the

cell only. Conversely, membrane tension has also been implicated in crushing the actin network at the back of the cell and thus enhancing disassembly to permit retraction of the trailing edge (225). Open issues include why the main protrusion itself is not suppressed by the membrane tension and the interplay between membrane tension and biochemical and signaling pathways (211). As concerns the latter point, membrane tension has been shown to affect membrane trafficking and myosin contractile activity, with high tension leading to a burst of exocytosis and also triggering myosin contraction (99, 100). Overall, whereas we once thought of the membrane as a passive sack impeding leading edge actin polymerization, we now see it as an active player in actin organization and dynamics for protrusion.

3. The mechanics of the lamellipodium

The Arp2/3-branched network in the lamellipodium is very homogeneous compared with other parts of the cell, a property that is explained by the homogenizing effect of branches, since their growth fills in the voids in the network, also stiffening it (317). Myosin motors present at the rear of the lamellipodium have been shown to increase disassembly, and thus accelerate the turnover of actin filaments in the lamellipodium (225, 339). Therefore, at short time scales on the order of seconds, the lamellipodium is elastic, whereas at longer time scales, because of this turnover of actin filaments, the lamellipodium is viscous.

Many controlled studies of lamellipodial dynamics have been done on fish keratocytes, the fast-moving “Ferrari” of the cell motility world, and a useful system for studying how much force an active lamellipodium can exert. Fish keratocytes move at a velocity of micrometers per minute. In one study, forces generated during lamellipodium activity were measured by recording the deflection of glass fibers as cantilevers in contact with the cell. The maximal force necessary to stall the whole cell body of a moving keratocyte is on the order of 35 nN, whereas the stall force necessary to stop lamellipodium extension is on the order of 3 nN for a contact surface of $\sim 1 \mu\text{m}^2$, on the same order of magnitude as the force generated by Arp2/3 complex activation on micrometric beads (118, 191, 249). In the case of the keratocyte, the force-velocity curve has a bent-down shape: at low force, the cell velocity is independent of the force until 50% of the stall force is reached, and then velocity decreases. The force velocity curve can be described with the equation $v_0 = v_0[1-(F/F_s)^w]$, where v_0 is the velocity at zero force and F_s is the stall force. The exponent w is positive for keratocytes, between 6 and 8, and was determined independently by aspect ratio observations and force measurements (118, 152). Contrary to cell measurements, in vitro measurements and theoretical models of actin growth show a rapidly decreasing force-velocity curve, thus proving that actin dynamics alone are insufficient to explain cell measurements, and that either motor activity should be taken into account, or the length change of actin filaments under force (353). Moreover, the ability of the keratocyte cytoskeleton to

return to its original shape between successive stalling experiments is striking since no effect of force or velocity change is found after the lamellipodium has been stalled, contrarily to atomic force microscopy *in vitro* measurements of growing actin networks (118, 235). Therefore, the dynamic reorganization of the lamellipodium through actin polymerization, disassembly, and motor activity results in a very robust structure that can resist a series of obstacles. However, adhesions, especially nascent ones, may be less robust than the lamellipodium in resisting loads. This is shown in a micromanipulation experiment involving shear flow, where a weak flow of a few picoNewtons per micron acting on the leading edge of the keratocyte stops protrusion due to interference with adhesion while polymerization is unaltered (32).

B. Filopodia

1. Filopodia formation

Filopodia are the fingerlike projections at the front of the cell, composed of unbranched, bundled actin filaments oriented with their growing ends toward the membrane (FIGURE 5, *v*). This orientation is due to the presence, in the filopodia tip complex, of formin and Ena/VASP proteins, both of which are capable of retaining the growing barbed ends at the cell membrane and enhancing filament growth, as previously described. While the mechanism of filopodia elongation is clear, how filopodia are initiated remains more of a mystery. In a nutshell and as discussed above with the Arp2/3 complex *in vitro* systems, the debate centers on whether the Arp2/3 complex plays a role in the initiation of filopodia formation or whether filopodia are nucleated exclusively by formin: the convergent extension model versus the tip nucleation model, nicely reviewed in Reference 345. Most evidence indicates that the former is the correct model and that filopodia are born out of the network via the coming together of branched filaments formed by the Arp2/3 complex, subsequently elongated by formin and VASP into straight filaments that are bundled by fascin (290). A time course analysis of spontaneous filopodia formation *in vitro* on supported lipid bilayers in cell extract shows this order of events: first the Arp2/3 complex machinery appears on the membrane, then actin and formin, and finally VASP and fascin (174). However, the role of the Arp2/3 complex in filopodia initiation may need to be revisited in light of the new experiments described above where the Arp2/3 complex was knocked out entirely, and filopodia-like structures were still formed (303, 342).

2. Filopodia mechanics

Besides cell migration, filopodia have a role in sensing the cell environment, initiating cell contacts, and transmitting cell-cell signals. The response of filopodia to the mechanical stiffness of the local environment occurs through a motor-clutch mechanism (53). Microorganisms exploit filopodia for their own advantage during infection for their capture by cells (263).

After capture, filopodia retract toward the cell body, leading to engagement of bacterial-cell contact and bacterial engulfment. This process was used to directly measure the force generated by filopodia by replacing the microorganism with a bead coated with the microorganism attachment proteins, coupled with an optical tweezer set-up. The force exerted by a retracting filopodia is on the order of 10 pN and can work over a distance of 10 μ m (212, 328). Filopodia retraction occurs at velocities of ~ 100 nm/s requiring the activation in some cases of the ERK1/2 pathway that controls retrograde flow via depolymerization in filopodia (263). Myosin II, V, and VI are not required for filopodia retraction, nor are microtubules (162). Therefore, filopodia mechanics and dynamics appear to entirely rely on actin assembly and actin dynamics, and maybe also cortex rearrangements at the root of the filopodium, but not on myosin or microtubule activity.

C. Contractile Fibers: Transverse Arcs and Ventral Stress Fibers

Excluded from the lamellipodia/filopodia region, but present throughout the rest of the cell are the contractile fibers. These structures are bundles of unbranched actin filaments of mixed polarity containing myosin II (221, 239). There are two main classes of contractile fibers: ventral stress fibers which run approximately parallel to the direction of movement, linking focal adhesion sites (FIGURE 5, *ii*), and transverse arcs which run parallel to the advancing leading edge, just behind the dendritic network of the lamellipodium and not anchored in focal adhesions (FIGURE 5, *iii*). Dorsal stress fibers are in the same class of structures, but since they are not contractile, we do not discuss them here.

Despite their unbranched structure, transverse arc formation depends on the Arp2/3 complex and on myosin activity (126). High-resolution imaging studies show that transverse arcs arise from the collapse of the dendritic network, powered by myosin (44). Transverse arcs mark out the region of the cell where actin flows slow down and where nascent cell-substrate contacts mature into stable focal adhesion structures (FIGURE 5, *iii*); however, transverse arcs are not believed to contact focal adhesions (5, 65, 247). Besides the role of myosin II in transverse arc formation, the formin FHOD1 with bundling and capping activity seems important for the transition between branched and antiparallel actin filament during arcs initiation (279). In addition, transverse arcs and stress fibers have also been shown to be seeded by dynamic filopodia that either collapse laterally into the lamellipodia to contribute to transverse arcs or remain perpendicular to the cell edge and contribute their distal end to stress fiber formation (222).

The role of transverse arcs in cell motility is not entirely clear. It seems that transverse arcs can be combined with noncontractile dorsal fibers to make ventral stress fibers (126). It is also proposed that transverse arcs, via their contractility, crush the actin network and accelerate its disassembly (107, 320,

339). Another possible role for arcs is that they may provide a starting block to push off from for subsequent rounds of leading edge protrusion, although not all protruding cells possess transverse arcs so there must be redundant mechanisms for supporting a lamellipodia (44).

Myosin is also present in ventral stress fibers that have a key role in mechanosensing via cell-substrate adhesions and in adhesion formation (FIGURE 5, *ii*). How ventral stress fibers are nucleated is not completely known, but seems to involve formins and Ena/VASP proteins and possibly Arp2/3 complex networks as well (143, 315). Laser nanosurgery of stress fibers reveals that the retraction of the contractile fiber triggers the recruitment of proteins such as zyxin to the cut end to make a new adhesion (67). Impeding stress fiber assembly impedes focal contact maturation and lamellipodium motility (224, 286, 287).

D. The Cell Cortex

1. Cortex assembly

In a preceding section, we saw how the cell membrane plays an active role in shaping the lamellipodia. This interplay is even more pronounced in the cell cortex, a thin actin shell that is contractile due to the presence of myosin in the network, underlying the inner face of the plasma membrane (FIGURE 5, *i*). This acto-myosin structure is several hundreds of nanometers thick and is a mix of bundled straight filaments and branched filaments, giving an average mesh size of ~ 50 – 200 nm depending on the cell type (213).

The cortex was neglected by the cell motility community for many years, as work was focused on the formation of protrusive structures like lamellipodia and filopodia. It is just over the last decade that we are beginning to get a clearer picture of cell cortex mechanics and biochemistry and its importance in cell shape changes and movement, from both an experimental and a theoretical point of view (140, 230, 231, 270). Cortical actin filament growth at the plasma membrane appears to be the result of different activation mechanisms, one of them relying on formin proteins (92). In order for myosin contractile forces in the cortex to be communicated to the membrane, the actin cortex must be attached. Various proteins involved in this linkage have been identified, among which proteins from the ERM (ezrin, radixin, moesin) family are one of the most important players (41, 56, 192). An excellent demonstration of the importance of both the contractility of the acto-myosin cortex and its degree of attachment to the cell membrane comes from a study of meiosis in oocytes where alterations in either parameter lead to defects in cell division (171). Another study shows that both the thickness and dynamics of the acto-myosin shell attached to the membrane play an important role in cell shape changes (147).

Cortical contractility in cells results in an inward pressure that tends to shrink the cell, which in turn generates an osmotic

pressure difference that tends to increase cell volume. This pressure balance explains the round shape of cells before mitosis, as is shown using atomic force microscopy cantilevers to measure the force the cell develops when it rounds up (296). Cortical contractility and cell internal pressure are the driving force behind a special type of cellular structure, the bleb (FIGURE 5). Blebs are membrane bulges that are dynamically extruded at the cell surface (54, 57). Blebs are triggered when holes form in the actin cortex or when the cortex is locally detached from the cell membrane (56, 229). In these situations, the internal or hydrostatic pressure in the cell forces the piece of membrane that is not attached to the cell cortex to balloon out, thus forming a bleb. Recently, blebs have been shown to be a way for the cell to relax excess tension. This is the case for example at cellular poles during cytokinesis where blebs play the role of valves releasing cortical contractility, thus ensuring the stability of cleavage furrow positioning (283).

However, in other cases, blebs subsequently fill with polymerized actin and myosin, and adhere to the substratum to drive motility. Called amoeboid motility, this is a mode of locomotion well-studied in the amoeba *Dictyostelium* (55, 89, 349). Blebbing has also been shown to be an important mode of motility in vivo during development, in specialized moving cells like leukocytes and possibly in other three-dimensional motility contexts such as cancer cell invasion (29, 168, 169). In fact, proving the versatility of cells, certain cells have been shown to switch between lamellipodia and blebs and reciprocally depending on their cortical tension, the dynamics of actin polymerization, and the three-dimensional environment (19, 341). Other cell types, like zebrafish embryonic cells, appear to maintain both lamellipodial/filopodial and bleb-type protrusion mechanisms together, and the balance between the two is important for proper morphogenesis (78).

2. Acto-myosin cortex mechanics

The acto-myosin cell cortex is characterized mechanically by its tension, which can be directly measured by micropipette aspiration, as was proposed a decade ago, where the force needed to suck a small region of the cell surface into a pipette is measured (123). This tension is directly related to the activity of myosin motors since decreasing their activity leads to a lower value of the tension (19). Interestingly, in the same study, reducing the activation of actin polymerization leads to an increase in cortical tension since more myosin motors are able to bind per unit length of actin filament, therefore increasing the total tension generated by myosin.

The membrane tension and the cortical (or cell) tension are not the same thing, although each can affect the other, and they are not measured by the same experimental technique. Based on membrane mechanics, membrane tension can be measured by tube pulling since the force to pull a tube depends on the square root of the tension, at least on a simple artificial membrane. In this case, tubes are pulled by optical tweezer trapping

of a membrane-adhering micrometric bead, and applied forces are in the tens of piconewton range (267). The situation is more complicated in cells where the membrane has a complex composition and is also connected to the underlying cytoskeleton. Tubes are devoid of actin cytoskeleton, so the lipids pulled into the tube must first detach and then experience friction over the cytoskeleton to be able to flow into the tube (43, 72, 124). The measured tube forces are then a mixed measurement of the in-plane membrane tension and the attachment of this membrane to the cytoskeleton. Indeed, for some cell types, it seems that measured tube forces reflect almost only the force needed to detach the membrane from the cytoskeleton, while in other cases, tube forces seem to reflect the real in-plane membrane tension (17, 73, 253). Moreover, the complex composition of the membrane in a cell, with transmembrane proteins that prevent the two lipid leaflets from sliding over each other, creates an additional friction force that places membrane tubes out of equilibrium (50). The take-home message concerning membrane/cortical tension is that while micropipette aspiration can directly measure cortical tension, it is difficult to quantify how much of this tension derives from the membrane as opposed to the cytoskeleton, unless the membrane detaches from its underlying cytoskeleton, allowing for both modules to be analyzed separately (49).

E. Dynamic Remodeling of Cellular Actin

So far we have discussed a steady-state view of the different cellular actin organizations. However, during most cellular shape changes, actin is continuously under intense reorganization, allowing cells to adapt to their environment (6). Cell motility is an extreme case of this major remodeling of the actin cytoskeleton. One of the main mechanisms for remodeling actin networks is to disassemble them as an ensemble, a concept referred to in the cellular context as the “treadmilling array” model (245). In this model, filament assembly at the front of the cell is matched by disassembly at the back of the lamellipodium, thus providing a pool of recycled monomers for subsequent rounds of polymerization and also keeping the width of the lamellipodium constant. This idea fits well with experimental observations from blebbing, photoactivation, and speckle microscopy where the actin network formed at the front of the cell travels backwards as a unit before disappearing in the cell body (310, 167, 248, 332). This is true also in yeast where photobleached actin structures in large endocytic structures in the *sla2Δ* mutant move inward (227). But the mechanism that generates this “treadmilling array” has probably little to do with a vectorial process where actin subunits are incorporated at the barbed end and then come off the pointed end one by one (208). It is more likely that the branched actin network disassembles by a macroscopic ADF/cofilin debranching/severing activity coupled to efficient suppression of elongation of the generated fragments by capping proteins or similar factors such as Aip1, an actin-binding protein that inhibits elongation of ADF/cofilin-decorated fila-

ments (FIGURE 6 and Refs. 163, 208, 226, 257). Interestingly, a detailed analysis of the different capping machineries involved in regulating branched network dynamics reveals that capping proteins are mostly involved at sites of active assembly, whereas Aip1 activity is tightly coupled with ADF/cofilin at sites of active disassembly (202). This is also consistent with the fact that capping protein is not present in the back half of the lamellipodium (207, 134). Recent work highlights the important role of Aip1 in recycling these small fragments back into the pool of actin monomers (228). These recent findings also point toward a polymerizable pool of actin made of short oligomers (293, 228). How these oligomers participate in the overall dynamic assembly/disassembly of actin networks in cells is still unknown.

In addition to actin reorganization modulated by ADF/cofilin disassembly, major actin structure reorganization can occur via contractility modulated by myosins that fragment actin filaments as explained above. Indeed, myosin not only reorients branched actin networks into parallel or antiparallel organizations, but also favors disassembly of these networks (FIGURE 6). This begs the question as to why an alternative mechanism of actin disassembly in addition to ADF/cofilin fragmentation is necessary to disrupt cellular actin organization. First, certain structures such as tight bundles seem resistant to ADF/cofilin-modulated actin disassembly (129, 278). Second, the myosin-induced disassembly of actin network generates a travelling wave of actin disassembly that can propagate rapidly and favor massive and rapid disassembly when necessary (6, 255). Just as waves of assembly have been shown to be important for motility of certain cell types, so waves of disassembly may be used to perform complex stop-and-go or turning movements (336).

F. Actin Cytoskeleton and Disease

Understanding how cytoskeleton mechanics and biochemistry add up to cell behavior can contribute to a molecular understanding of cytoskeleton-based diseases. Alterations in the actin cytoskeleton and its associated proteins have been linked to numerous disease states, ranging from microbial infections to deafness to immune system deficiencies. Indeed, much has been understood about the molecular mechanisms of actin-based motility from the study of how bacteria and viruses, such as *Listeria monocytogenes*, *Shigella flexneri*, *Rickettsia coronii*, and *Vaccinia virus*, move in infected host cytoplasm (68, 91, 105, 181, 314). These pathogens hijack the actin cellular machinery to move in the host cytoplasm and to increase infectivity and virulence. Reconstitution in vitro of bacterial and virus motility demonstrates that the minimum machinery for pathogen motility consists of actin, the Arp2/3 complex, and a capping factor with profilin and ADF/cofilin to ensure actin monomer recycling (91, 181). This minimum machinery has recently been extended to include formins since *Rickettsia* Sca2, a bacterial-formin-like protein, drives the motility of this bacteria, taking the place of the Arp2/3 complex as

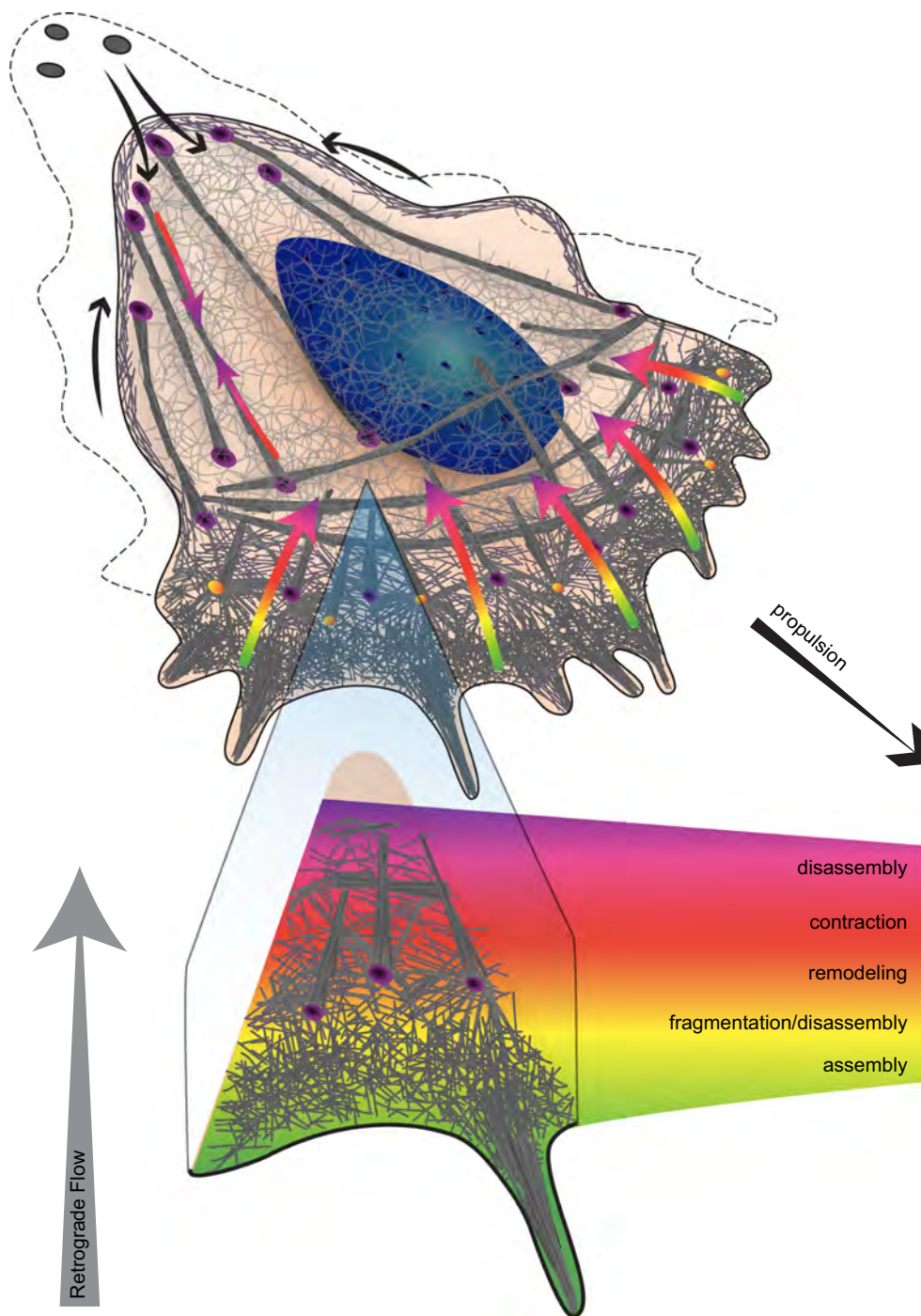


FIGURE 6. Dynamics of actin structures during motility. The specialized actin organizations described in **FIGURE 5** are involved in assembly and protrusive force production at the cell front and in contraction and disassembly at the center and at the trailing edge of motile cells. The leading-edge actin organizations are extremely dynamic. They are characterized by a massive and fast assembly underneath the plasma membrane responsible for membrane protrusion. This assembly occurs in the lamellipodium, a 1- μm -width region, and is finely balanced by continuous ADF/cofilin-mediated fragmentation and myosin-induced remodeling followed by contraction and disassembly of aged actin organizations. This tight spatiotemporal coordination completes the actin turnover cycle and gives rise to the actin retrograde flow toward the cell center. Simultaneously with leading edge protrusion, the trailing edge of the motile cell is retracted. Contractile stress fibers ensure this coordination, permitting the continuum of events to occur in tightly regulated succession starting from protrusion at the front to retraction at the rear. The color codes in the zoom region correspond to the different mechanisms controlling actin dynamics (assembly, fragmentation/disassembly, remodeling, contraction, and disassembly). This color code is used in the large arrows in the cell to illustrate where these different mechanisms occur during cell motility.

nucleator (108, 188). Interestingly, another formin (INF2) localized at the endoplasmic reticulum of mammalian cells is reported to modulate mitochondria fission and mutations in INF2 can result in Charcot-Marie-Tooth neuropathy (157).

A striking example of the link between actin mechanics and pathology, and one that has been receiving increasing attention over the last decade, is cancer metastasis and invasion (165, 204). Invasion is a step in the metastatic program when tumor cells break through extracellular matrix barriers and invade surrounding tissues and the circulatory system. This

process is driven by the lamellipodia, filopodia, and blebs described in this review, which allow the cell to translocate, but also specialized structures, invadopodia that allow the cell to make holes in the extracellular matrix. Invadopodia appear to be on the fence between lamellipodia and filopodia, with both branched and unbranched filaments and both Arp2/3 complex and mDia2 (formin) nucleation of actin formation (180, 280). In another study, the protein composition of these protrusions was profiled using subcellular fractionation techniques and proteomics, and in addition to actin, the Arp2/3 complex activator N-WASP, the actin bundler fascin and the Ena/VASP

family protein Mena, just to highlight a few, were associated with invadopodia formation (154 and references therein).

The stiffness of the extracellular matrix surrounding tumors as well as the mechanics of the tumor mass itself, mostly determined by actomyosin cytoskeleton dynamics, has also been shown to be different from normal tissue (106, 271). Furthermore, enhanced contractility of both invading cancer cells and the fibroblasts that accompany them has been linked to more aggressive cancers (94, 269, 272). The interest in looking at the mechanical properties of tumors is twofold: to identify possible targets for new anti-metastatic therapies, but also to use the tumor's aberrant mechanical profile to evaluate its metastatic potential and adapt therapies accordingly.

Another role of the cytoskeleton in disease is indirect via transmission of external forces to the nucleus, which in turn activates different pathways of gene expression and alters cell behavior (71, 90, 135, 136, 138, 321). One well-known example is the transcription factors YAP and TAZ, implicated in cell proliferation and growth, but also in human cancers. Recently, YAP and TAZ, which act in specific locations in tissues that are mechanically stressed, have been shown to be down-regulated by the actin machinery at locations not submitted to tension (10, 81). This is a very nice example of how the mechanical and biochemical (dynamical) control of the actin cytoskeleton is tightly coupled to transcriptional activity, termed a mechanical checkpoint (10).

As our understanding of the link between actin dynamics, architecture, and mechanics has improved and technological tools have facilitated our ability to mechanically perturb actin organization, other diseases such as atherosclerosis have been added to the list of actin mechanics-based pathologies (74, 149, 158, 237). The narrowing of arteries during atherosclerosis happens at the site of arterial branches and curvature where cells are put under tension by blood flow (74, 149). This mechanical tension on the actin cytoskeleton (215) is proposed to trigger signaling pathways leading to cell death (74, 241).

Much attention has been focused on how defects in actin assembly may be at the origin of a large number of pathologies; however, actin disassembly may be equally important. Altered ADF/cofilin regulation is associated with diseases as diverse as Alzheimer's disease and ischemic kidney disease (11, 13). In Alzheimer's disease, hyperactivation of ADF/cofilin during neuronal dysfunction leads to stabilization of actin filament/ADF/cofilin rods via a mechanism described in the section IIG on actin disassembly. These stable rods are sites for accumulation of a phosphorylated form of tau that is characteristic of tau-induced pathology during Alzheimer's disease. Interaction of tau with rods might favor tau modifications or assembly into filamentous structures, the major component of neurofibrillary tangles (NFTs). Rods could also block both neurite

growth and vesicle transport. Thus actin rods because they are abnormally undynamic may act as the trigger to mediate the loss of synapses and the formation of NFTs, both pathological hallmarks of Alzheimer's disease.

IV. CONCLUSION AND PERSPECTIVE

Our goal in this review has been to emphasize the dual biochemical/mechanical nature of the actin cytoskeleton and bring together the different communities of actin biochemistry, actin mechanics, and actin cell biology to provide a setting for future advances in the field. The field of actin cytoskeleton benefits already to a large extent from the interdisciplinary work between biochemistry, cell biology, and physics, and we must continue to bridge the gaps between biochemistry and mechanics in the coming decade.

There are still mysteries to be solved in the relationship between actin dynamics and force production (6, 87, 208). For example, why are different types of disassembly machineries necessary, and how are these different mechanisms coordinated in space and time to sustain the dynamics of the whole network and preserve cell integrity? Another challenge is transferring what we have learned about actin networks in vitro to the cellular environment, for example, how a specific actin organization correlates with a particular mechanical output. Such a quest has already benefited from new super-resolution imaging tools that allow for a precise cartography of the cell cytoskeleton (143, 144, 284, 344). However, we are still far from being able to follow in real time the dynamics of a single filament in a cellular environment and how associated proteins affect these dynamics, except in very specific cellular conditions (295). Great promise for the field of actin biomechanics lies in the emergence of quantitative cell biology approaches that allow for the quantification of the number of molecules involved in a physiological event such as cytokinesis, clathrin-mediated endocytosis, or lamellipodium protrusion (23, 142, 268, 288, 292). In addition, since we are on the way to surmounting technical hurdles associated with simultaneously visualizing and manipulating the cell cytoskeleton by physical tools such as optical tweezers or atomic force microscopy combined with fluorescence and optical methods, we may have a way in the future to correlate molecular activity with mechanical properties. In the field of cell motility, the relevance of studying molecular mechanisms in vitro or cell behavior in an environment far from a tissue is an open debate. However, we would argue that this variety of complementary approaches is the backbone of progress in the field, since once a phenomenon has been characterized in cells in culture, its mechanism can be tested in vitro with a limited number of variables, to intelligently design further experiments in the more complex context of a tissue.

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