REVIEW: CELL BIOLOGY

Mechanism of Actin-Based Motility

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Spatially controlled polymerization of actin is at the origin of cell motility and is responsible for the formation of cellular protrusions like lamellipodia. The pathogens *Listeria monocytogenes* and *Shigella flexneri*, which undergo actin-based propulsion, are acknowledged models of the leading edge of lamellipodia. Actin-based motility of the bacteria or of functionalized microspheres can be reconstituted in vitro from only five pure proteins. Movement results from the regulated site-directed treadmilling of actin filaments, consistent with observations of actin dynamics in living motile cells and with the biochemical properties of the components of the synthetic motility medium.

Eukaryotic cells have the ability to organize directed movements to migrate, feed, divide, or drive internal transport of materials (*1*). The direction of movement is mediated in different ways in cells and is associated with dissipation of energy. Molecular motors are the archetype of protein machines that transport organelles along microtubules and actin filaments in a unidirectional fashion, converting the free energy derived from ATP hydrolysis into directed movement.

Another type of directional, ATP-consuming movement is the amoeboid crawling motion [(*1*, *2*); see also supplemental slides 1 to 4 (*3*)], mediated by the polarized assembly of a polymer. The most widespread example is actin-based motility, driven by the assembly of actin filaments. Actin-based motility describes a variety of cellular processes through which living cells change shape in response to environmental signals, or extend protrusions like lamellipodia and filopodia, or wrap around a particle in a phagocytic cup. Progress in understanding the mechanism by which actin polymerization generates movement resulted from advances in different fields. Genetic and immunocytochemical studies identified several important actin-binding proteins in motility; biochemical analysis characterized the function of these proteins; "live" observations of actin dynamics in motile regions of cells provided hints about the reactions involved; and last, but not least, the bacterial pathogens *Listeria monocytogenes* and *Shigella flexneri*, which exhibit actin-based movement in the host cytoplasm, have been instrumental in identifying essential factors in motility and in developing biophysical assays for movement analysis (*4*, *5*).

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From Lamellipodium Extension to *Listeria* Propulsion

Actin is one of the most abundant protein in all eukaryotes. [For this section, see supplemental slides 5 and 6 (*3*).] In physiological medium, this 43-kD globular protein polymerizes itself into polar, helical filaments in which subunits are connected by a 167° rotation and 2.7-nm axial rise. Irreversible hydrolysis of the bound ATP associated with polymerization is at the origin of treadmilling (*6*) and destabilizes the filament (*7*). Treadmilling plays a crucial role in the function of actin in motility (Fig. 1A).

In the 1980s, it was recognized that the polarized array of actin filaments turns over rapidly in the lamellipodium. Filament barbed ends facing the plasma membrane at the leading edge of the lamellipodium were growing, while pointed ends of the filaments were depolymerizing at the rear, in a treadmilling process (*8*). A similar orientation of filaments exists in the "comet tail" of *Listeria* in infected cells, barbed ends facing the surface of the bacterium (*9*). This arrangement suggested an insertional polymerization mechanism for force production. During lamellipodium extension and *Listeria* propulsion, the rate of barbed-end growth equaled the rate of movement (*10*, *11*), bringing the first clear evidence for actin assembly as the driving force and for the potential of *Listeria* and *Shigella* as models of the leading edge of the lamellipodium. The in vitro monitoring of *Listeria* movement in cell extracts (*12*) then converted a complex cell biology problem into a biochemically tractable problem, and opened the way to the full reconstitution of actin-based movement from a minimum set of purified components.

Actin networks continuously generated at the leading edge or at the surface of *Listeria* display features characteristic of a steady-state process: the filament array has a constant length, shows a stationary gradient of density from the leading edge to the rear of the lamellipodium (or from the surface of the bacterium to the end of the actin comet tail), and the array treadmills rapidly. Typically, a 3-μm-long filament turns over in 1 min, that is, 100 times as fast as pure actin in vitro. How can these observations be translated in terms of biochemical reactions? The steady-state concentration of monomeric actin reflects the dynamic state of actin filaments (Fig. 1A). For pure actin, it is very close to the barbed-end critical concentration, so that slow, steady barbed-end growth balances slow pointed-end depolymerization. Steady barbed-end growth (as well as pointed-end depolymerization) is two orders of magnitude faster in lamellipodium and in the actin tails of *Listeria*. This fact implies that, in vivo, the concentration of active monomeric actin is maintained at a much higher value by factors that control the dynamics of assembly at the two ends of the actin filament.

Regulatory Factors Controlling Actin Treadmilling

Two proteins control actin dynamics independently to achieve this function: actin depolymerizing factor (ADF, also called cofilin) and capping proteins. ADF proteins are essential in morphogenetic and motile processes [For this section, see supplemental slides 7 to 15 (*3*).] Most of them are regulated by phosphorylation in a stimulus-responsive fashion (*13*). A Rac-regulated LIM kinase inactivates ADF; the activating phosphatase is unknown (*14*). The biochemical properties of ADF account for its biological function. ADF accelerates pointed-end depolymerization, which is the rate-limiting step in the treadmilling ATPase cycle (*15*). As a result, a higher steady-state concentration of monomeric ATP-actin is established in F-actin solutions, which supports faster barbed-end growth, balancing faster pointed-end depolymerization (Fig. 1B). In enhancing treadmilling, ADF increases the rate of actin-based motility. ADF has also been proposed to affect motility by severing the filaments (*16*). However, cutting the filaments creates as many growing barbed ends as depolymerizing pointed ends, thus increasing the bulk turnover but not the intrinsic treadmilling of filaments. Therefore, severing the filaments in itself cannot affect motility (*17*). In addition, in lamellipodia, growing barbed

ends are restricted to a narrow (0.1 to 0.2 μm wide) zone at the leading edge (*18*), and yet ADF is present throughout the lamellipodium (*19*).

Profilin is involved in motile processes mediated by actin polymerization. Newly discovered proteins of the actobindin family, like *Drosophila* Ciboulot (*20*), are functional homologs of profilin. Profilin specifically binds ATP-monomeric actin (ATP–G-actin) in a complex that has the unique property of associating exclusively with barbed ends (*21*, *22*). Profilin shifts the distribution of monomeric actin at steady state: The large pool of ADF–ADP–G-actin, which undergoes association-dissociation reactions at the pointed ends, is converted into the ATP-bound profilin-actin complex, which polymerizes at barbed ends only. The processivity of treadmilling therefore is enhanced by profilin. In vitro, treadmilling is accelerated 125-fold by the synergistic effects of ADF and profilin, reaching values close to those found in vivo (*23*).

Not all G-actin–binding proteins affect the rate of motility the way profilin does. Proteins like β-thymosins make a complex with G-actin that does not participate in assembly at either end (*24*). Hence, they do not modify filament dynamics or the rate of movement (*20*). These proteins are sometimes thought to buffer the free ATP–G-actin concentration. Instead, the amount of sequestered actin is determined by the value of the concentration of ATP–G-actin imposed by filament dynamics, according to the law of mass action (*25*). The changes in free ATP–G-actin elicited by regulators like ADF lead to changes in sequestered actin that are more than one order of magnitude greater. Motile cells contain a large pool of sequestered actin.

Capping proteins are required for efficient motility of many cells (*26*– *28*). By blocking a large fraction of the barbed ends, capping proteins funnel the flux of pointed-end depolymerization to feed the growth of a few noncapped filaments, which individually grow faster than if the other filaments were not capped. In conclusion, ADF and capping proteins cooperate in promoting fast actin-based motility (Fig. 1, C and D).

In the treadmilling cycle, capping proteins are recycled after depolymerization of capped filaments and eventually cap the growing barbed ends. Maintenance of a steady number of transiently growing barbed ends requires the constant generation of barbed ends. The cellular factor that generates new filaments in a site-directed, signaling-controlled fashion is the Arp2/3 complex.

WASP Family Proteins Connect Signaling to Polarized Assembly of Actin

Evidence for a role of the Arp2/3 complex in the spatial control of actin assembly first came from studies of actin-based motility of *Listeria*. The Arp2/3 complex, a conserved ubiquitous complex of seven polypeptides comprising actin-related proteins Arp2 and Arp3, stimulates actin polymerization at the surface of the bacterium when it is activated by the *Listeria* protein ActA (*29*, *30*). [For this section, see supplemental slides 16 to 18 (*3*).]

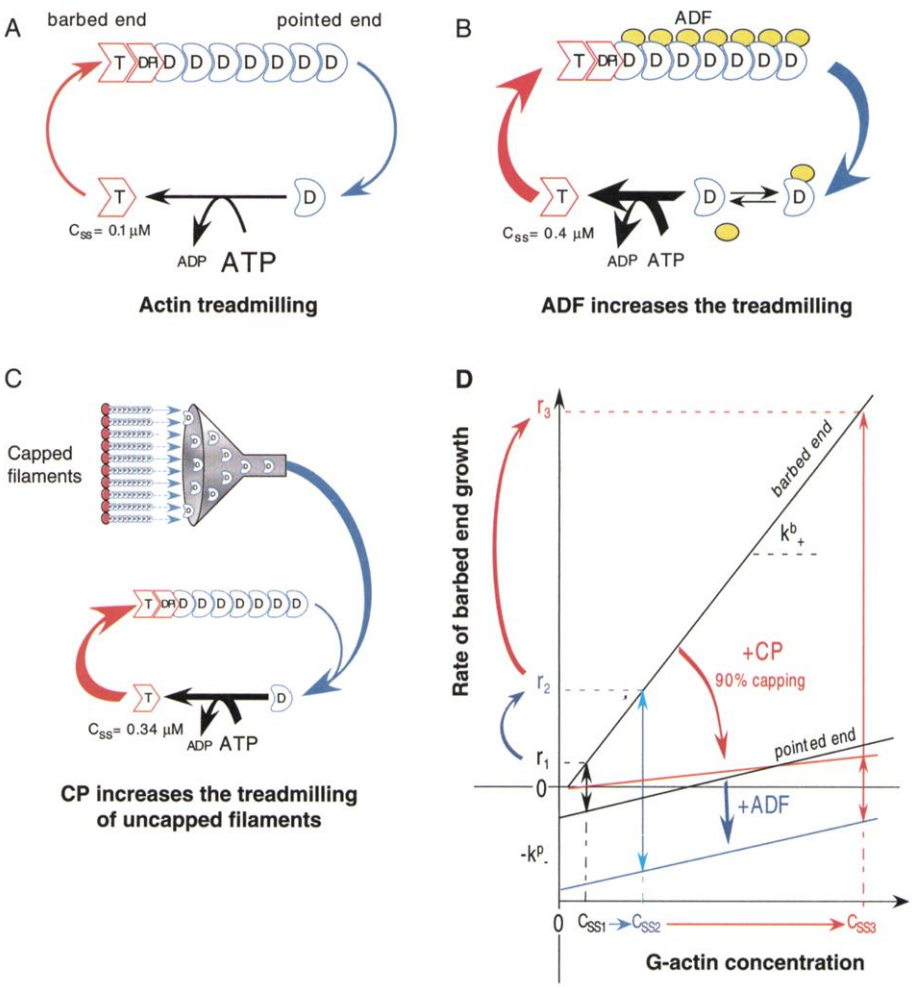

Fig. 1. Treadmilling of actin filaments and its regulation in motility. **(A)** Intrinsic treadmilling of actin filaments reflects the energetic imbalance between the barbed and the pointed ends. Pointed-end disassembly, the rate-limiting step of the actin ATPase cycle, controls the rate of barbed-end growth and the steady-state concentration of monomeric ATP-actin, C_{SS}. The rate of treadmilling is $r = k_+^B(C_{SS} - C_C^B) = k_+^P(C_C^P - C_{SS})$, where k_+^B and k_+^P are the forward rate constants for association of G-actin to barbed and pointed ends, and C_C^B and C_C^P the corresponding critical concentrations. For pure actin, $C_{SS} = 0.1$ μM, $C_C^P = 0.6$ μM, $k_+^B = 10$ $\mu M^{-1} s^{-1}$, $k_+^P = 0.6$ $\mu M^{-1} s^{-1}$, $r = 0.3$ s^{-1}. **(B)** ADF accelerates treadmilling by increasing the reverse rate constant k_-^P. C_{SS} therefore settles at a higher value so that the rate of barbed-end growth, $r = k_+^B(C_{SS} - C_C^B)$, balances faster pointed-end depolymerization. **(C)** Capping proteins (CPs) bias the treadmilling process. Blockage of a large fraction of barbed ends increases C_{SS}. Noncapped barbed ends therefore grow faster. When 90% of barbed ends are capped, $C_{SS} = 0.34$ μM, $r = 2.7$ s^{-1}. **(D)** Graphic representation of the effects of ADF and capping proteins on treadmilling. Lines represent the dependence of the rates of filament elongation at barbed and pointed ends on the concentration of ATP–G-actin. Black lines: actin alone (no effectors). The value of C_{SS} is C_{SS1}, which satisfies equal rates of barbed-end growth (r_1) and pointed-end disassembly (black double-headed arrow). Blue line: effect of ADF on pointed-end kinetics. The value of C_{SS} is C_{SS2}, and the rate of barbed-end growth is r_2. Red line: the effect of 90% capping on the barbed-end kinetics (90% decrease in slope to one-tenth of barbed-end growth, red arrow curving downward). In the presence of both ADF and capping proteins, the value of C_{SS} is C_{SS3} and the rate of barbed-end growth is r_3.

Cellular activators of the Arp2/3 complex are members of the Wiskott-Aldrich syndrome protein (WASP) family (*31*–*34*). These scaffolding multimodular proteins connect actin to a variety of signaling pathways involving receptor tyrosine kinases, heterotrimeric GTP-binding proteins (G proteins), or the small GTPases Cdc42 and Rac through the Arp2/3 complex (Fig. 2). The conserved isolated COOH-terminal domain of WASPS constitutively activates the Arp2/3 complex. The other domains are targets of diverse signaling molecules. Structural and biochemical studies using shorter versions of the full-length WASP or N-WASP (the ubiquitous neural isoform of WASP) indicate that an intramolecular bond maintains these proteins in an autoinhibited conformation (*35*–*37*). The cooperative binding of the Cdc42-GTP, phosphatidylinositol-4,5-bisphosphate (PIP_2), or SH3 domain-containing proteins (like Grb2), or profilin in the presence of lipids (*38*–*41*), targets N-WASP to the sites of signaling and changes the structure of the protein, allowing interaction with the Arp2/3 complex. By this mechanism, actin polymerization is stimulated locally, in a manner that may be modulated up or down by the input of several signals, possibly cooperating in a lipid "raft," at the leading edge. It is remarkable that IcsA, the surface protein of *Shigella* responsible for bacterial motility, binds N-WASP (*42*) and activates the N-WASP–Arp2/3 machinery (*43*). *Shigella* thus mimics the leading edge better than *Listeria*. The NH_2-terminal domain of the *Listeria* protein ActA is a functional homolog of the COOH-terminal domain of WASP proteins (*44*). The exact mechanism by which the focal adhesion protein VASP enhances *Listeria* movement in binding the central domain of ActA is not understood (*6*, *7*).

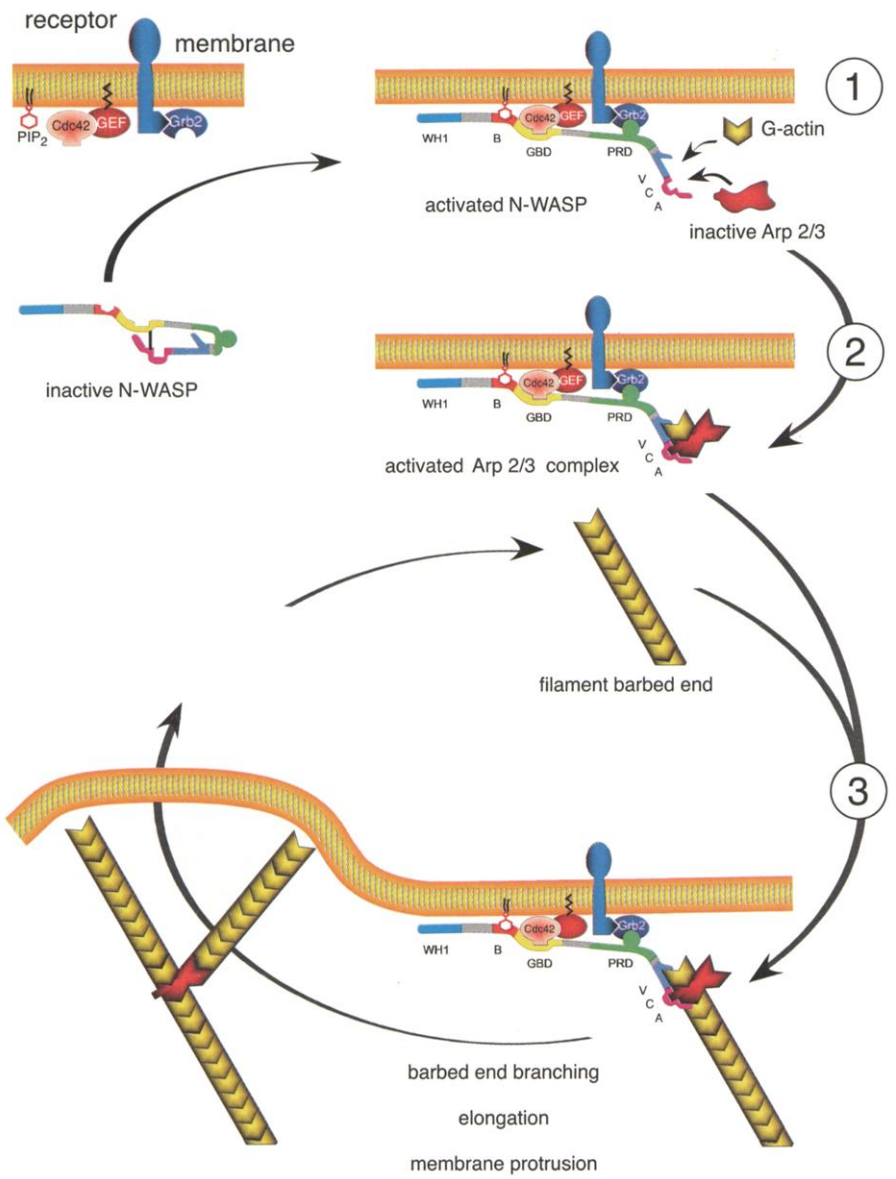

Fig. 2. Signaling to actin at the leading edge of the lamellipodium. N-WASP is activated and targeted to the membrane by signaling molecules (circled 1). G-actin and the Arp2/3 complex bind the exposed COOH-terminal domain of N-WASP (VCA), forming a branching complex (circled 2). Association of the branching complex with a filament leads to the formation of a branch (circled 3). The two branches grow at equal rates. N-WASP catalyzes several consecutive cycles of branching.

The Arp2/3 Complex Multiplies Filaments by Branching

How does Arp2/3 locally stimulate the formation of new filaments? Full understanding of Arp2/3 function will require much more structural and biochemical data than presently available. Immunolocalization of Arp2/3 in the lamellipodium, microscopic observations, and polymerization studies show that when activated by WASP proteins or by ActA, Arp2/3 multiplies the filaments by branching them. [For this section, see supplemental slides 19 to 27 (*3*).] Actin filaments form a dendritic Y-branched array in the lamellipodium; daughter branches grow at 70° angles from the mother branch (the long branch of the "Y"). Arp2/3 is located at the branching points, and the barbed ends of the two branches face the leading edge of the lamellipodium (*19*). In solution, the WASP- or ActA-activated Arp2/3 complex initiates the formation of morphologically similar branched filaments (Fig. 3, B and C) (*44*–*47*). The kinetics of actin polymerization into branched filaments is autocatalytic, different from the nucleation-growth process for polymerization of pure actin, indicating that Arp2/3 generates new filaments by interacting with the product of the polymerization reaction, F-actin (*47*, *48*). No definite agreement has yet been reached concerning the exact mechanism by which the Arp2/3 complex causes the filaments to branch in solution. Side branching (*48*, *49*) and barbed-end branching (*44*, *47*) have been proposed. The two views have different implications regarding the length correlation of mother and daughter branches, and the ability of filaments with free or capped barbed ends to activate branching. In the barbed end–branching model, mother and daughter branches are expected to be equal in length, which was observed when branched filaments were formed in the absence of phalloidin (*47*). In the side-branching model, daughter branches should always be shorter than mother branches, and the expected length correlation coefficient is 0.6. In the presence of phalloidin, a total loss of correlation was observed (*49*), suggesting that some other mechanism than the proposed side-branching model must have been operating. Gelsolin-capped fila-

ments did not activate branching (*44*, *47*), consistent with the barbed end–branching model. The opposite result was obtained using a weaker capping protein, which, however, did not block all barbed ends (*49*).

In solution, branching of filaments by the Arp2/3 complex is followed by spontaneous debranching (*44*, *47*, *50*). Debranching is about 20% as fast as P_i release after ATP hydrolysis on F-actin. The nature of the event that causes debranching is not elucidated yet. The localization of the Arp2/3 complex after debranching is not known either. The regulation of ATP binding and/or hydrolysis on the Arp2/3 complex in the branching and/or debranching reactions and in the recycling of the Arp2/3 complex is of great interest regarding the morphogenesis of the dendritic arrays and the physics of movement.

What are the features of filament branching at a surface? In *Shigella*-infected cells, N-WASP remains bound to the bacterial surface during movement, whereas Arp2/3 colocalizes with actin in the comet tail (*43*). Similarly, N-WASP remains bound to the surface of rocketing endosomes (*51*). In addition, no branched filaments are seen with their barbed ends growing toward the rear of the lamellipodium. These facts indicate that branching is catalyzed locally by an immobilized enzyme (ActA or N-WASP), which transfers the Arp2/3 complex and actin to filament ends, to form a branch. When activated at the surface of a bead placed in a cell extract, the Arp2/3 complex initiates the formation of multiply branched filaments (Fig. 3D) (*52*), suggesting that individual filament ends are transiently attached to the surface while branching. Mechanical studies of *Listeria* movement (*53*, *54*) also indicate that filaments are transiently attached to the bacterium during movement.

How Do Quiescent Cells Switch to a Motile Stationary State?

In quiescent, nonpolarized cells, all barbed ends are capped. Concerted regulation of different players may switch on motility, inducing a new polarized actin meshwork and rapid actin dynamics. We suggest that changes in the steady state of actin assembly elicit changes in the motile state of cells as follows. First, activation of ADF, by increasing ATP–G-actin concentration, leads to an increased amount of actin sequestered by β-thymosins and profilin, i.e., causes massive depolymerization of F-actin. Second, the high level of ATP–G-actin both favors nucleation and prompts rapid growth of barbed ends, generated upon activation of the Arp2/3 complex. The creation of barbed ends shifts the steady state of actin assembly toward the de novo formation of a polarized actin meshwork in the lamellipodium.

The maintenance of a steady branched filament array may be viewed tentatively as follows. Constant filament branching occurs at the leading edge by activation of the Arp2/3 complex. Barbed-end growth is supported by a high concentration of monomeric ATP-actin, maintained by ADF, profilin, and capping proteins. A steady number of growing barbed ends results from the balance between the creation of barbed ends by Arp2/3 and the blockage of these ends by capping proteins; hence, the free barbed ends measured at a given time seem to escape capping (*55*). A steady gradient of filament density results from the balance between the multiplication of filaments by branching and the debranching reaction followed by cooperative pointed-end depolymerization of filaments fiber by fiber, elicited by ADF. A question remains open: how are barbed ends first nucleated in a medium containing capping proteins? The high steady-state concentration of ATP–G-actin supports significant spontaneous formation of barbed-end nuclei, which cyclically appear, get capped by capping proteins, and disassemble from their pointed ends. Nuclei and filaments escape this abortive cycle when they are captured by activated Arp2/3 at the leading edge of the lamellipodium or at the bacterial surface and are multiplied by branching.

A Minimum Motility Medium for Sustained Actin-Based Movement

The design of a minimal motility medium comes as a logical conclusion from the biochemical mechanism of control of actin dynamics. [For this section, see supplemental slides 28 to 34 (*3*).] The medium should contain ATP as an energy source, the Arp2/3 complex and actin filaments in the presence of ADF, profilin, and capping proteins, making a chemostat of monomeric actin (ATP–G-actin + profilin–ATP–G-actin). The Arp2/3 complex multiplies barbed ends locally, by interacting with an activator bound to a particle like *Listeria*, or N-WASP–coated *Escherichia coli* expressing IcsA (which is a good substitute for *Shigella*), or a functionalized (ActA- or N-WASP–coated) bead. Rapid actin-based movement of all these particles is actually observed in this medium (*56*) (Fig. 3, D to F). In the absence of ADF, capping proteins alone support extremely slow movement, actin tails are observable after 18 hours. Profilin improves motility but is not essential, as previously demonstrated (*57*, *58*). Efficient ActA-induced motility of *Listeria* or beads also requires VASP. It is noteworthy that no myosin motor is involved. The concentrations of all components that yield optimum motility are similar to the in vivo concentrations, except for capping proteins, which are one order of magnitude more abundant in vivo, suggesting that their function might be regulated. The physical parameters of movement (particle velocity of several microns per minute, length of the actin comet tails of a few microns, trajectories) are similar to those observed in vivo or in cell extracts. The onset of movement is preceded, as in cell extracts, by the formation of a nonpolarized "cloud" of F-actin around the particle, followed by breakage of symmetry (*59*) and establishment of a stationary regime of movement.

The simplicity of the composition of a minimum medium required for a self-organizing motile system is consistent with the functioning of treadmilling supporting actin-based motility (*60*). Because movement is linked to the ATPase cycle of actin, effectors acting on a critical step of this

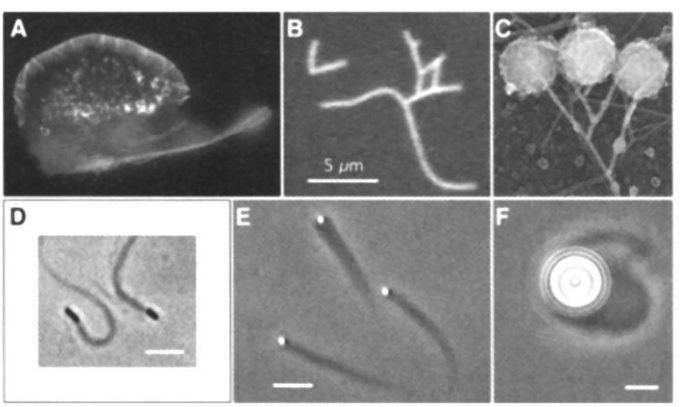

Fig. 3. Site-directed filament branching and actin-based motility. (**A**) A migrating GFP-actin transfected melanocyte displays fluorescent actin array in the extending lamellipodium and in filopodia (courtesy of K. Rottner and J. V. Small). (**B**) Branched filaments formed in solution upon polymerization of actin in the presence of the Arp2/3 complex and ActA. Filaments are stained with rhodamine-phalloidin immediately before observation by fluorescence microscopy. (**C**) ActA-coated beads placed in a *Xenopus* egg extract initiate a branched actin meshwork [from T. M. Svitkina and G. G. Borisy (*52*)]. Note the length of mother and daughter branches. (**D** through **F**) Actin-based movement of particles in the reconstituted motility medium (phase-contrast microscopy). (D) *Escherichia coli* (IcsA); (E and F) N-WASP–coated beads of 2 and 10 μm in diameter. [Picture F courtesy of C. Syke.]

cycle control other steps at the same time. Several putative factors for nucleation, rapid depolymerization, rapid barbed-end growth, or inhibition of nucleation in the cytoplasm are not required. Analysis of the movement at different concentrations of the individual components of the motility medium elucidates the different facets of their function. At suboptimal concentrations of capping proteins, movement is slow, and the actin tails exhibit a fishbone pattern, demonstrating that branched filaments continue to grow away from the surface of the bacterium until a capping protein stops their growth (*47*). The length and lifetime of the filaments in the actin meshwork, therefore, are controlled not only by ADF, but also by capping proteins, which restrict filament growth to the sites where force must be produced. These observations reconcile two apparently opposed models for actin-based motility, treadmilling, and nucleation-release (*61*).

Open Issues in the Mechanism of Actin-Based Motility

The reconstitution of actin-based movement sets up the important issues that have to be solved concerning the concerted regulation of motility by different signaling pathways, and the relations between the reactions of filament branching and growth at a surface and the resulting production of force.

The minimum motility medium allows testing of the function of putative regulators of processes like phagocytosis, discovery of new activators of the Arp2/3 complex, screening of inhibitors of motility, or development of a strategy for drug therapy design. The forthcoming challenges are to understand the interplay between microtubule dynamics and the actin response, mediated by small GTPases Rac and Rho (*62*, *63*), and the coupling between protrusion and adhesion in motility.

How general are the principles that govern actin-based motility? Remarkably, cell-cell adhesion appears mediated by directed actin polymerization (*64*), showing that actin partners involved in cell protrusion may be used in other directed processes. The importance of the regulation of the steady state of assembly may also apply to microtubules, the other dynamic fibers of the cytoskeleton. Most intriguing, the major sperm protein (MSP)–based crawling motion of the nematode sperm (*65*) displays the same treadmilling behavior as actin, whereas MSP does not bind or hydrolyze ATP and assembles into a nonpolar polymer, virtually unable to treadmill or to support motor translocation. ATP nonetheless is required for nematode motility. Whether a membrane-associated ATP-consuming machinery energetically favors assembly at one end of the MSP fibers, close to the plasma membrane, thus inducing treadmilling in this symmetric polymer, is an open question.

Biological engines are often thought to rectify brownian motion. Theoretical brownian ratchet models of actin-based motility first relied on the polymerization-biased diffusion of *Listeria*, then on the elastic properties of the actin filament (*66*, *67*). Another model at the mesoscopic scale relies on the elastic properties of the actin meshwork and the shear stress due to the growth of the gel at the bacterium surface (*68*, *69*). However, a microscopic model for force production is still pending. Progress requires deeper knowledge of the structure-function relation of the components of the membrane-bound motile machinery. High-resolution trajectory analysis of a bead (*70*) in a chemically controlled motility assay, microrheology of the transiently branched filament network will provide information on the molecular mechanism of movement. Single molecule measurements may be feasible in the future.

References and Notes

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71. We acknowledge financial support from La Ligue Nationale Contre Le Cancer and a grant from Human Frontiers in Science.