Mathematical Models and Simulations of Cellular Processes Based on Actin Filaments*

Actin filaments help to maintain the physical integrity of cells and participate in many processes that produce cellular movements. Studies of the processes that depend on actin filaments have progressed to the point where mathematical models and computer simulations are an essential part of the experimental toolkit. These quantitative models integrate knowledge about the structures of the key proteins and the rate and equilibrium constants for the reactions for comparison with a growing body of quantitative measurements of dynamic processes in live cells. Models and simulations are essential because it is impossible to appreciate by intuition alone the properties that emerge from a network of coupled reactions, particularly when the system contains many components, and force is one of the parameters.

We use a few examples to illustrate how mathematical models advance the understanding of the actin system from side chain motions of proteins to the behavior of whole cells. Readers will find references to experimental work in the papers cited. Diverse methods (supplemental data) are required given the range of complexity (single proteins to cells), dimensions (10⁻⁹–10⁻⁴ m), and time (10⁻¹²–10² s).

Actin Molecule and Polymerization

Internal Motions of Actin Monomers—Actin consists of four subdomains surrounding a cleft that binds ATP or ADP (Fig. 1). In molecular dynamics (MD) simulations, the DNase I loop in subdomain 2 is the most flexible part of the protein with a weak tendency to form a π- or an α-helix (1, 2), so crystal contacts may stabilize the helix when it is present. The nucleotide-bind-0ing cleft of actin remains closed in MD simulations with bound ATP or ADP or without bound nucleotide (1–3), whereas the cleft of the actin-related protein Arp3 tends to open. This difference depends on a C-terminal extension of Arp3, which fits into the groove between subdomains 1 and 3 and stabilizes the open conformation (2). Profilin binding in this groove also promotes cleft opening and nucleotide exchange (4).

Actin Filament Nucleation and Elongation—Pure actin monomers (Fig. 1) spontaneously polymerize into helical filaments under physiological conditions. Kinetic simulations of the complete time course of polymerization of actin monomers showed that formation of dimers and trimers is extremely unfavorable (5). Brownian dynamics simulations showed that the long pitch (end-to-end) dimer is favored over the short pitch dimer and that the third subunit binds laterally to form a trimer nucleus (6). Brownian dynamics simulations showed that electrostatic forces favor elongation at the barbed end over the pointed end as observed (7).

Effect of Bound Nucleotide on Polymerization—The nucleotide bound to actin influences every aspect of polymerization. In cells, actin monomers are saturated with ATP. When incorporated into a filament, actin hydrolyzes bound ATP 40,000-fold faster than monomeric actin (8). Despite enough crystal structures and MD simulations to formulate a hypothesis (2, 9, 10) for conformational changes associated with the ATPase cycle, we do not understand how polymerization stimulates hydrolysis or how the presence of γ-phosphate influences the affinity of actin for profilin, thymosin-β4, and cofilin.

At the fast-growing barbed end of filaments, ADP-actin binds slower and dissociates faster than ATP-actin. ADP-P-actin associates only slightly faster than ADP-actin but dissociates much slower (11). All of these reactions are slower at the pointed ends of filaments. An enduring mystery has been how ATP hydrolysis by polymerized actin makes the critical concentration for elongation ~10 times more favorable at barbed ends than at pointed ends. Analytical models and Monte Carlo simulations consistent with experimental data show that the difference arises from faster dissociation of phosphate from ADP-P subunits near both ends than from the interior subunits and lower affinity of phosphate for terminal subunits at pointed ends than at barbed ends (11). At steady state with ATP in the medium, these reactions coupled with random ATP hydrolysis and Pₐ release in filaments produce gradients of subunits containing bound ATP, ADP-Pₐ, and ADP from the ends toward the interior of filaments (12), small length fluctuations at barbed ends (13, 14), and net addition of subunits at barbed ends balanced by net loss of subunits at pointed ends (~0.1 s⁻¹).

Force Production by Polymerizing Actin—Experiments and theory agree that polymerizing actin filaments produce a few piconewtons (pN) of force. Single filaments as short as 700 nm long buckle when they elongate between two attachment sites (15). Given the stiffness of actin filaments, the force is >1 pN (16).

Pioneering studies (17, 18) proposed that elongating filaments move objects by a Brownian ratchet mechanism. The original model considered how elongation of rigid filaments could rectify the thermal motion of a diffusing object. A later elastic Brownian ratchet model considered not only the motion...
of the distribution of positions of the object over time gave a logarithmic dependence of force (in the pN range) on the rate of elongation (in the range of 0–110 subunits/s). The velocity depends on actin monomer concentration, elongation rate constant, length of the filaments, and angle of incidence between the filament and the barrier, with an optimum angle near 45°. Remarkably filament growth and branching at the leading edge of motile cells select filaments oriented at angles near 45° relative to the membrane (19). Note that long filaments buckle, filaments parallel to the barrier exert no force, and bending of filaments normal to the barrier opens only a small gap for elongation. Langevin dynamics simulations (20) and Monte Carlo simulations (21) confirmed the general features of the elastic Brownian ratchet mechanism.

Physical Properties of Actin Filaments—Massive all-atom MD simulations and normal mode analysis of coarse-grained models (3) reproduced the observed stiffness of ATP- and ADP-actin filaments. The α-helical DNase loop assumed for ADP-actin has weaker short pitch interactions and no long pitch interactions, accounting for the greater flexibility of ADP-actin filaments. However, we do not understand how γ-phosphate dissociation from filaments alters their structure and influences subunit reactions at the ends.

Proteins That Regulate Actin Polymerization

Cells use dozens of proteins to regulate the time and place of actin polymerization. Other proteins shape and reinforce structures composed of actin filaments. Here, we use two proteins that direct actin assembly and one that promotes disassembly to illustrate how modeling contributes to research on actin-binding proteins.

Arp2/3 Complex—The Arp2/3 complex nucleates actin filaments as 78° branches on the sides of pre-existing actin filaments. Five protein subunits hold the two actin-related proteins, Arp2 and Arp3, close together but separated enough to prevent them from initiating an actin filament. Kinetic simulations (22) based on a partial set of rate constants showed that the favored pathway begins with a nucleation-promoting factor such as WASp binding actin and then the Arp2/3 complex. This ternary complex has no nucleation activity until it binds very slowly to the side of a filament. Then, a daughter filament grows at its free barbed end from the side of the “mother” filament (Fig. 2).

Formins—Formins are homodimers with multiple domains, including formin homology (FH)-2 domains that associate with barbed ends of actin filaments (reviewed in Ref. 23). Formins stimulate formation of un-
branch actin filaments in cables, filopodia, and cytokinetic contractile rings. Simulations of experimental data suggest that FH2 domains nucleate filaments by stabilizing actin dimers (24) and show that free actin monomers account for all nucleation in the presence of profilin (25).

Doughnut-shaped FH2 dimers (Fig. 3) encircle an actin filament (26) and remain associated with a growing barbed end through thousands of cycles of subunit addition at rates up to 100 s⁻¹ (25, 27, 28). FH2 domains slow elongation of barbed ends by 10–99%. Much work remains to determine how FH2 domains track reliably on growing barbed ends. One idea (26) with solid theoretical support (29) is that the leading FH2 domain steps off the end before the next actin subunit binds (Fig. 3A, lower). Alternatively, the step may occur after addition of each new actin subunit (Fig. 3A, upper) (25). One might expect an FH2 dimer to rotate along the path of the growing actin helix, but this is not observed if the formin and distal parts of the filament are both anchored to a surface (15). One idea is that an FH2 domain tracks with the growing helix for about six subunits and then steps around the filament axis in the opposite direction to relieve any accumulated torsion (30).

Flexible FH1 domains adjacent to FH2 domains contain multiple polyproline sequences that bind complexes of profilin-actin and transfer actin onto the barbed end of the filament (Fig. 3B) at rates of >1000 s⁻¹ (31). Transfer is more favorable from proximal than distal polyproline sequences (25).

Theoretical work (32) showed that elongation in association with a protein like an FH2 domain can produce more force if subunit addition is coupled to hydrolysis of ATP bound to actin. However, formins can use ADP-actin monomers to elongate filaments (28), and elongation rates mediated by FH1-FH2 constructs with ATP-actin monomers can exceed the ATP hydrolysis rates by 300-fold, so any coupling must be indirect.

Cofilin—Actin-depolymerizing factor (ADF)/cofilin proteins stimulate actin filament turnover. ADF/cofilins bind ADP-actin subunits with higher affinity than ATP- or ADP-Pₐ-actin subunits and sever filaments. Stochastic simulations (33) and mathematical analysis (34) showed that ATP hydrolysis and phosphate dissociation by actin subunits lead to a gradient of ADF/cofilin severing activity from the oldest to the youngest part of a filament. This aging process can explain the rapid turnover and large stochastic fluctuations in the length of growing filaments observed experimentally (33).

**Models of Actin-based Cellular Motility**

Polymerization of branched actin filaments pushes the plasma membrane forward at the leading edge of motile cells. Variations of the dendritic nucleation hypothesis (Fig. 2) are the basis for models of these processes. Nucleation-promoting factors associated with the inside of the plasma membrane are proposed to activate the Arp2/3 complex to form many generations of growing branches, which produce force by an elastic Brownian ratchet (17) (18). Capping proteins terminate branch growth, and all of the proteins recycle back to the cytoplasmic pool.

Analytical and numerical solutions of a system of partial differential equations describing the dendritic nucleation hypothesis operating at steady state produced several insights (35). All of the filaments were assumed to share the load equally, and actin subunits diffused after disassembly. The model was approximately one-dimensional in space. When the concentration of growing filaments is high, polymerization consumes actin monomers and creates a modest sink of monomers at the leading edge, such that diffusion of actin monomers bound to thymosin-β4 and profilin to the leading edge is rate-limiting for movement. The rate of movement depends on the density of growing filaments, reaching an optimum of ~0.2 μm/s with 20–60 filaments/μm, depending on the resistance. Resistance slows polymerization at suboptimal end densities, and monomer depletion slows polymerization at high end densities.

Stochastic models consider each individual filament in a heterogeneous population. This approach allows consideration of how geometry determines the work (force × distance) performed by each filament. Carlsson (36) made Monte Carlo simulations of the growth of networks of rigid, branched actin filaments against a rigid obstacle, using the reactions in the dendritic nucleation model and taking into account the positions of every subunit in each filament. He assumed a uniform concentration of reactants, branch formation only near the obstacle (at rates to give spacing similar to that in cells), and resistance to polymerization similar to a Brownian ratchet. Simulations produced different geometries depending on other assumptions such as branching from the sides of filaments or only at barbed ends. Velocity was remarkably independent of resistance (as observed in experiments with *Listeria*) because resistance bends the leading filaments, allowing more filaments to contact the obstacle.
MINIREVIEW: Modeling of Actin Structures

Schaus et al. (19) made a stochastic two-dimensional simulation of every filament in a dendritic nucleation model under the plasma membrane, an extension of Maly and Borisy (37) without their simplifications. They assumed actin monomer diffusion, spontaneous formation of branches at some distance from the previous branch, a zone with “protection from capping” within 5 nm of the plasma membrane (essential but unproven), 70° branches (not critical) and elastic behavior of both the filaments (assumed stiffness critical) and the membrane (assumed stiffness not critical). Starting with randomly oriented filaments, the mechanism generated a self-organized network of branched filaments strongly oriented at ±35° to the plasma membrane as observed by electron microscopy. This resulted from capping being faster than branching for filaments of other orientations. The model moved at 8 μm/min and was able to change direction in ~1 min after 15 generations of branches. The maximum velocity was achieved if the filaments shared the work equally, but this is impossible with stiff filaments and hard objects. If elongating filaments are flexible, they can bend to various degrees to share the load, push rapidly, and approach perfect thermodynamic efficiency. Flexibility of the membrane contributes effectively to load sharing. Tethers between the load and the filaments reduce performance. The performance of such a system depends on the size of the subunits in the polymer, and the size of the actin molecule is nearly ideal for a Brownian ratchet mechanism driven by filaments with the physical properties of actin.

Models have also addressed other remarkable features of the leading edge, nucleation of most filaments very near the plasma membrane, and growth of filaments in a plane only 200 nm thick oriented at about ±35° relative to the inside of the membrane. To restrict nucleation to the front of the cell, Atilgan et al. (38) proposed that nucleation-promoting factors concentrate where the plasma membrane has the smallest radius of curvature, but the relevant transmembrane anchors have yet to be identified. Maly and Borisy (37) proposed that contact of growing barbed ends with the plasma membrane inhibits capping. This favors elongation of filaments growing toward the front and termination of filaments growing in other directions. Their model correctly reproduced the distribution of orientations of filaments relative to the leading edge.

A simple analytical model (39) accounts for several features of motile keratocytes, including constant surface area, limited variation of shapes, constant velocity, and ability to recover these characteristics after an insult, which rounds up the cell. The model assumes protrusion force produced by actin polymerization against a uniform surface tension in a fluid but inextensible membrane. A key feature is a gradient of barbed ends (measured with a fluorescent natural product) from the middle of the leading edge to the margins of the cell, where the force produced by actin polymerization matches the tension resisting movement. The biochemical origin of this gradient is not known.

Models of Actin-based Bacterial Motility

Certain intracellular bacteria usurp the cellular actin system to assemble a comet tail of filaments for propulsion. For example, ActA on the surface of Listeria is a nucleation-promoting factor for the Arp2/3 complex. ActA attached to plastic beads also produces actin comet tails in cellular extracts or mixtures of purified proteins. Tethers to the actin filament comet tail limit diffusion of the bacterium. Both deterministic and stochastic models show that transient tethers are compatible with an elastic Brownian ratchet (40).

Stochastic object-oriented simulations of dendritic nucleation by ActA on a bacterium (41) followed reactions of thousands of molecules in short time steps. Collisions produced forces, which were dissipated by movements apart, but the model did not include force-velocity relationships. Pauses between intervals of constant velocity emerged in complicated ways from the ensemble of reactions rather than from a fundamental step such as subunit addition to barbed ends.

Macroscopic theories consider the tangled actin filaments at the rear of Listeria as a continuous viscoelastic gel. Stress accumulates in the gel as polymerization takes place at the surface of a bacterium. Release of this stress can produce sustained or intermittent movements as observed. Mathematical analysis of an expanding gel model gave a nonlinear force-velocity relationship, and simulations reproduced the hopping movements of bacteria (42). Lipid vesicles (43) and oil droplets (44) coated with ActA produce comet tails, which compress the sides and pull at the rear of these spherical particles. A model with compression by a viscoelastic gel accounts for the observed shapes of these particles (44).

Filopodia

Filopodia (also called microspikes or microvilli) are slim projections of the plasma membrane supported by a bundle of actin filaments, similar to a finger in a glove. In some cases, the filaments turn over by addition of subunits to the barbed ends of the filaments at the tip balanced by loss at the base of the bundle. Single filaments cannot support the forces (tens of pN) required to protrude the membrane, but packing n filaments into a bundle increases their stiffness by a factor of n to n², depending on the extent of cross-linking and breaks in the filaments (45). Elongation of many barbed ends depletes the local pool of monomeric actin, which is limited by diffusion along the length of the filopodium and restricted by the close apposition of the membrane (46). A calculation made before formins were implicated showed that 30 filaments are optimal to produce a process a few μm long (46), similar to numbers observed in cells. Cross-linking restricts the thermal motion of the barbed ends, so the ability of the filaments to grow against the membrane is attributed to fluctuations of the membrane (47).

Cytokinesis

It has been appreciated for 3 decades that a contractile ring of actin filaments and myosin II is responsible for cleavage of cells at the end of mitosis, but progress on mechanisms awaited extensive inventories of the numerous participating proteins from genetics in yeast and RNA interference experiments in flies and worms. Both yeast and animal cells depend on formins associated with the plasma membrane for assembly of the actin filaments. Myosin II might simply capture these filaments and pull them into a ring, but Monte Carlo simulations of contractile ring assembly in fission yeast ruled out a simple search and
capture between the filaments (50). Further experiments using fission yeast suggested that connections between growing actin filaments and clusters of myosin II break about every 20 s. Simulations of models including search, capture, traction, and release account for cellular observations (48). Analytical solutions to partial differential equations show that force between clusters of myosin around the midsection of a cylindrical cell can generate a contractile ring and cleavage furrow (49). The force generated by such a bundle of actin filaments and myosin depends on the lengths of the filaments and the extent of cross-linking between the filaments (50).

REFERENCES