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Review

# The Polymerization Motor

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**Polymerization and depolymerization of actin filaments and microtubules are thought to generate force for movement in various kinds of cell motility, ranging from lamellipodial protrusion to chromosome segregation. This article reviews the thermodynamic and physical theories of how a nonequilibrium polymerization reaction can be used to transduce chemical energy into mechanical energy, and summarizes the evidence suggesting that actin polymerization produces motile force in several biological systems.**

**Key words: Actin, Brownian ratchet, force generation, microtubule, protein polymerization**

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For convenience and flexibility, cells store much of their disposable chemical energy in common currencies that can be used for multiple types of cellular metabolism. Chemical energy can be stored in the form of nucleoside triphosphates, transmembrane chemical and electrical gradients, or molecules that carry high-energy reducing electrons. Biological motors, celebrated in this inaugural issue of *Traffic*, are protein machines that convert chemical energy to mechanical energy and force. A transmembrane proton gradient powers the bacterial flagellar rotary motor, and nucleotide hydrolysis allows molecular motors such as myosin and kinesin to walk in a stepwise fashion along their linear cytoskeletal tracks, as well as allowing polymerases and helicases to run along DNA. A different type of cellular use of chemical energy to generate mechanical force uses a specialized currency rather than a general one, nonequilibrium protein polymerization. Polymerization of small protein subunits to form large-scale structures is an important general feature of biological organization in all forms of cellular life. The most striking instances of this form of organization are found in the eukaryotic cytoskeleton, particularly in microtubules and actin filaments. Although the term 'motor' usually refers to discrete protein machines like myosin, kinesin, or the flagellar rotor, protein polymerization can also convert chemical energy into mechanical force and can therefore be considered another type of motor.

Experimental attention to the biophysical mechanism of force generation by the protein polymerization motor has lagged far behind equivalent studies of force generation by classical motor proteins such as myosin and kinesin. Over the past decade, many clever techniques have been devised to make ever more precise measurements of the amount of force generated by individual myosin and kinesin molecules (reviewed in (1–3)). In contrast, there is only a single published report of a direct biophysical measurement of force produced by an individual growing microtubule (4), and no equivalent measurements for individual actin filaments.

Although there is a relative paucity of quantitative experimental work on force generation by protein polymerization, there is a wealth of theoretical literature on this topic. The purpose of this review is to summarize the thermodynamic and physical basis of force generation by protein polymerization, framed in a biological context. Biological evidence for force generation by microtubule polymerization and depolymerization has been summarized in an excellent review (5), so here I will focus primarily on biological examples of force generation by actin polymerization. The theoretical requirements outlined here, however, are relevant to both microtubules and actin.

## Actin' Like a Motor

Actin is one of the most abundant proteins of eukaryotic cells and has been strongly conserved throughout eukaryotic evolution. Actin is often associated with biological force generation, either by virtue of association with a myosin (as in muscle contraction) or due to its own polymerization motor. A few cases where actin polymerization is thought to produce force are schematized in Figure 1.

Cells that crawl across solid substrates must produce two types of force for locomotion; protrusion force to extend the leading edge of the cell margin forward, and traction force to translocate the cell body (6,7). Both protrusion and traction are actin-dependent. Since the best-studied form of actin-dependent movement is skeletal muscle contraction, where chemomechanical energy transduction is performed by myosin hydrolyzing ATP, the discovery of nonmuscle myosin in crawling cells engendered the proposal that force for crawling is also produced by myosin (8). This model had to be revised after 1987 when it was found that *Dictyostelium* amoebae completely lacking myosin II heavy chain are capable of crawling, with protrusion perfectly normal and traction only partially affected (9,10). Of course, cells contain an abundance of other myosin isoforms, including some that are localized to leading edge, but knockout experiments have thus far failed to provide unequivocal evidence that any myosin isoform is required for protrusion. Drugs that prevent

actin filament elongation such as cytochalasin D, however, stop all common forms of cellular protrusion including extension of pseudopodia, lamellipodia, and filopodia. It is now widely believed that actin polymerization rather than myosin ATPase activity provides much of the force for protrusion (11,12). Quantitative measurements of cell deformability at the leading edge are consistent with this hypothesis (13).

The fact that actin polymerization alone is capable of providing sufficient force to push out a lipid bilayer has been directly demonstrated in experiments where monomeric actin is enclosed in lipid vesicles and polymerization is induced by increasing the salt concentration. The lipid vesicles start off roughly spherical, but are drastically deformed by the polymerizing filaments, forming flattened discs with filopodial-like protrusions (14–16). These results lend credence to the proposition that similar forces are at work in cellular protrusion.

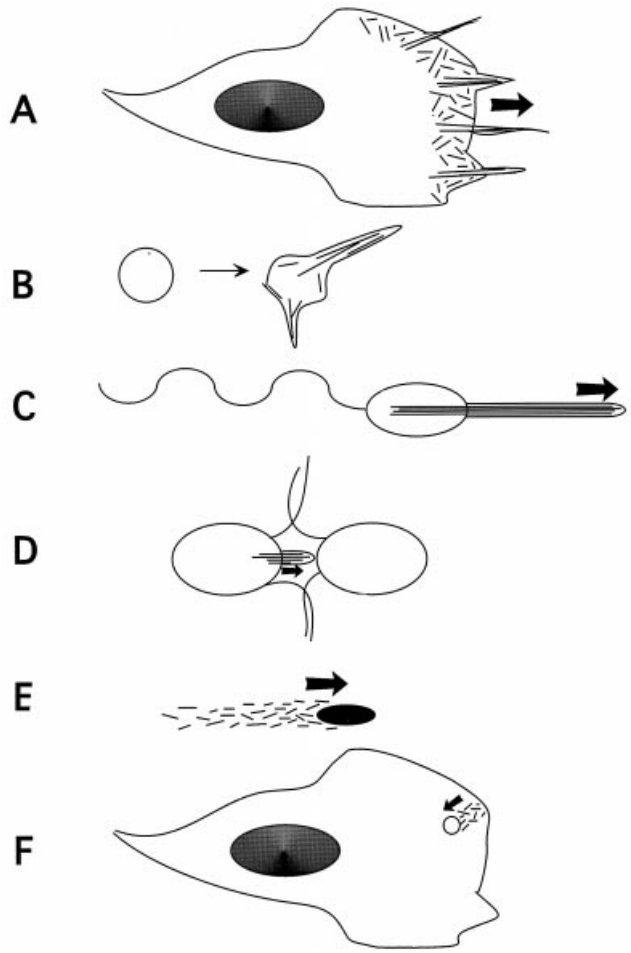


**Figure 1: Schematic of systems where actin polymerization is associated with the generation of pushing force.** Force is generated in the direction shown by the thick arrows. Details and references are provided in the text. Drawings are not to scale. A) Lamellipodial and filopodial protrusion at the leading edge of a locomoting fibroblast. B) Distension of an actin-containing liposome after polymerization is induced. C) Extension of the acrosomal process from *Thyone* (sea cucumber) sperm. D) Extension of fertilization tubule from *Chlamydomonas* cell of the plus mating type toward the minus mating type partner. E) Actin-based movement of *Listeria monocytogenes*. F) Inward movement of a nascent endosome.

Fertilization appears to be a biological function where actin polymerization is commonly harnessed for force generation. When the sperm of the sea cucumber *Thyone* encounters the egg jelly coat, an explosive actin polymerization reaction is initiated that pushes out an acrosomal process, enabling the sperm plasma membrane to reach and fuse with the plasma membrane of the egg (17). A similar reaction occurs during *Chlamydomonas* mating. Two cells of opposite mating type (plus and minus) recognize each other by specific adhesion receptors on the flagella. After this recognition has occurred, one of the two partners (the plus mating type) extends a fertilization tubule, a membrane-bound bundle of actin filaments that closely resembles a tiny *Thyone* acrosome (18). In these cases, water influx is coupled with actin polymerization and may contribute to force generation by a hydrostatic mechanism (19,20).

A line of evidence for direct force generation by actin polymerization which does not involve the cell plasma membrane (and therefore cannot involve a hydrostatic force) comes from studies of intracellular bacterial and viral pathogens that use the host cell actin cytoskeleton for motility, the most familiar examples being *Listeria monocytogenes* and *Shigella flexneri*. These bacteria use specific virulence factors to catalyze local polymerization of actin filaments near their surface in an asymmetric fashion, generating an actin-rich 'comet tail' (reviewed in (21,22)). Actin filament dynamics in the comet tail resemble a simplified form of actin dynamics in lamellipodia, where the bacteria seem to be imitating a fragment of the leading edge (23). The rate of movement of bacteria through the cytoplasm is identical to the rate of new actin filament polymerization at the front of the comet tail (24,25). No myosin isoform has yet been found associated with the comet tail and the broad-spectrum myosin ATPase inhibitor butanedione monoxime has no effect on bacterial movement (26). Like protrusion, it is now generally accepted that actin polymerization rather than myosin ATPase activity provides the force for bacterial movement. Rigorous proof for this proposition should soon be at hand. This form of actin polymerization-dependent motility can be accurately reconstituted *in vitro* using cytoplasmic extracts (27,28) and efforts are underway in several laboratories to reconstitute movement using only purified proteins. Preliminary laser trap studies have indicated that the amount of force that is produced by actin polymerization in this case is remarkably large, at least 90 piconewtons (pN) by a single comet tail (L. Cameron, K. Visscher, S. Block and J. Theriot, unpublished results).

A few recent reports have shown that actin-rich comet tails also form in normal, uninfected cells (29,30). In cells performing endocytosis, comet tails are seen pushing nascent endosomes inward from the cell edge. The endosomes appear to

be kicking off from the membrane with a burst of actin polymerization, perhaps in order to deliver themselves to waiting microtubule tracks (30).

What all these forms of actin-based motility have in common is that force seems to be generated when an assembly of actin filaments undergo polymerization in close physical proximity to a barrier or load. The load may be the plasma membrane, a pathogenic bacterium, or an endosome, and the actin filament assembly may be a bundle (in filopodia, the *Thyone* sperm, and *Chlamydomonas*) or a crosslinked web (in lamellipodia, pseudopodia, and comet tails). Myosin-dependent filament sliding need not be invoked for any of these cases; filament assembly alone seems sufficient to push.

## Pushy Polymerization

What exactly is the form of the chemical energy that is transduced into mechanical pushing force by protein polymerization? It is stored in the difference in chemical potential (Gibb's free energy) between the free monomer form and the polymer form of a protein subunit (31). In general, protein polymerization can be thought of as a simple bimolecular binding reaction, where a free subunit binds to the end of a filament that contains n subunits to generate a filament of length $n + 1$ (Figure 2A). At chemical equilibrium, the rate of addition of new subunits to the filament ends is exactly balanced by the rate of subunit dissociation: $k_{on}[C] = k_{off}$, where C is the concentration of free subunits. The concentration of free subunits left in solution at equilibrium is frequently referred to as the 'critical concentration,' abbreviated $C_c$, where $C_c = k_{off}/k_{on}$. Filament elongation proceeds spontaneously when the free energy of the subunit in solution is greater than the free energy of the subunit in the polymer; this is the case when the concentration of subunits in solution exceeds the critical concentration. Likewise, filament shrinkage proceeds spontaneously when the concentration of subunits in solution is less than the critical concentration (Figure 2B).

Whenever there is excess free energy ($\Delta G$) liberated from a chemical reaction, it can in principle be harnessed and converted into another form of energy. To see how $\Delta G$ from spontaneous polymerization can be used to provide a pushing mechanical force, consider the case of a simple filament, fixed at one end, that encounters a moveable load at its other tip (such as an actin filament that abuts a plasma membrane). For the filament to add a single subunit, the load must be pushed forward by a distance, $\delta$, that corresponds to the size of the protein subunit. Applying a force through a distance on the load is mechanical work. Thus, after a filament adds a subunit while it is pushing on a load, the final free energy state of the system is higher than it would be for an equivalent filament that encounters no load. However, as long as the overall $\Delta G$ is still negative, this reaction will still proceed spontaneously; elongation will occur and the load will be pushed forward (Figure 2C). Conversely, spontaneous depolymerization of a filament can in principle generate a

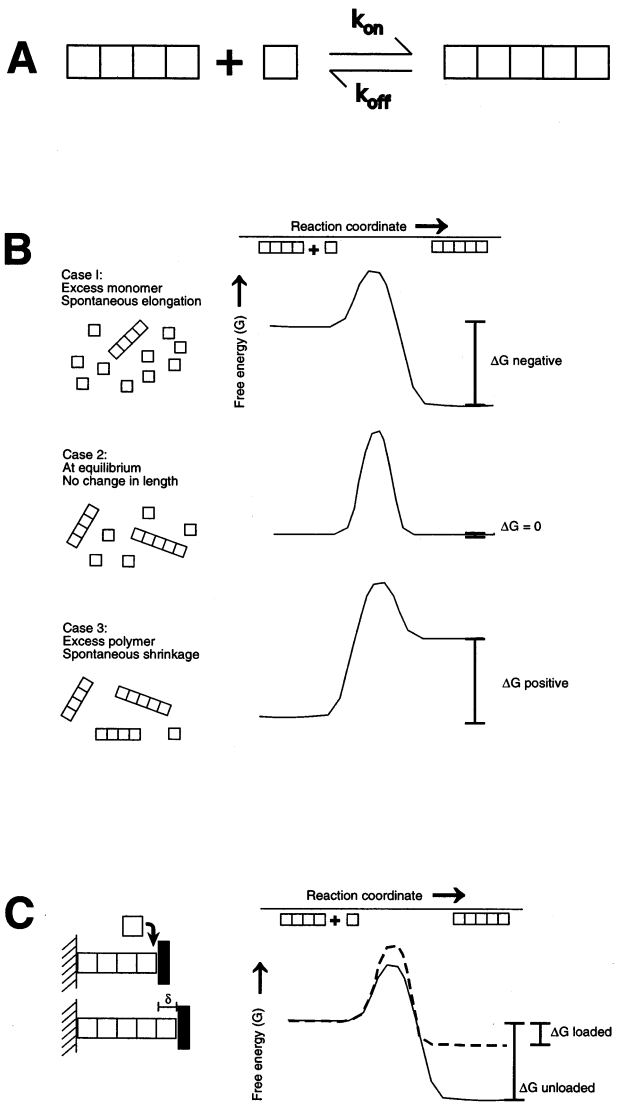


**Figure 2: Thermodynamics of protein polymerization.** A) The addition reaction is characterized by a forward kinetic rate constant, $k_{on}$ (units of $sec^{-1}M^{-1}$), and a backward kinetic rate constant, $k_{off}$ (units of $sec^{-1}$). B) Free energy diagrams for the polymerization reaction for three different starting conditions. C) Coupling between $\Delta G$ released by spontaneous polymerization and mechanical work. A filament is anchored at the left end; its right tip abuts a moveable load (black rectangle). Elongation of the filament produces useful work by moving the load through a distance, $\delta$, equal to the size of the protein subunit. The presence of the load normally raises the activation energy barrier for the elongation reaction as well as decreasing the final amount of free energy released. The maximum amount of work that can be done against the load is determined by the magnitude of $\Delta G$, which in turn depends on the amount of free subunit in excess over the critical concentration.

pulling force if the load remains attached to the filament while the concentration of free monomers falls below $C_c$; it is likely that microtubule depolymerization helps to pull chromosomes apart in the spindle in this way (5). In 1982, Hill and

Kirschner elaborated a comprehensive thermodynamic treatment of protein polymerization and depolymerization for several different physical states (capped and uncapped filaments; with tips free, next to moveable loads, or next to immovable barriers, with and without coupling between polymerization and nucleotide hydrolysis) (31). I will summarize only the most relevant cases here.

The presence of the load may affect either $k_{on}$ or $k_{off}$, or more likely both. Intuitively, it makes sense that subunit addition will be inhibited by the presence of an object at the filament tip by simple steric hindrance, decreasing $k_{on}$. Also, an object pushing on the tip may weaken the bond between the terminal subunit and its neighbor, increasing $k_{off}$. The degree to which $k_{on}$ as opposed to $k_{off}$ is affected by the load can not be predicted in general, since this depends on the geometry of the interaction between the filament and the load and also on the nature of the filament involved. The ratio between $k_{off}$ and $k_{on}$ is equal to the critical concentration, so the presence of a load force has the net effect of increasing the critical concentration, regardless of the detailed mechanism. The exact amount by which a particular load force changes the critical concentration can be calculated using thermodynamic arguments (31): $C_c(\text{loaded}) = C_c(\text{unloaded})\exp(\delta F/kT)$ where $\delta$ is the length of the subunit, F is the load force, k is Boltzmann's constant and T is the absolute temperature.

Rearranging this equation, we can calculate the maximum amount of force that a polymerizing filament can generate at a given concentration of free monomers, $F_{max} = (kT/\delta)\ln(k_{on}[C]/k_{off})$. For actin at a concentration of 50 μM, the stall force for a single filament is about 9 pN (32), equivalent to several molecules of myosin.

Note that this type of force generation does not in any way require the ATPase activity of actin or the GTPase activity of tubulin. Mechanical force is derived solely from the chemical potential of protein polymerization. This is dramatically illustrated in the case of sickle cell hemoglobin. In humans carrying the sickle cell mutation (a point mutation that changes a single glutamate residue to valine on the surface of the hemoglobin β chain), the deoxy form of the hemoglobin is slightly stickier than normal deoxyhemoglobin and has a tendency to polymerize. The filaments formed by aggregated deoxyhemoglobin are regular helices with 14 protofilaments, somewhat reminiscent of the structure of microtubules (33). When the blood oxygen level drops, the hemoglobin polymerization reaction is initiated, deforming the membrane of the red cell to form spiky projections, exactly the same way that initiation of actin polymerization in a lipid vesicle deforms the vesicle as described above (14–16).

The limitation of this sort of polymerization-induced force generation is that the cell can only use it once. The polymerization reaction is initiated and then runs to equilibrium. At equilibrium, no more chemical energy can be extracted from polymerization and no more force can be generated. For the *Thyone* sperm acrosome reaction, it need only extend its process once and it is fine for the polymerization motor to run down. In most forms of cell motility, however, there must be an external energy input that effectively reverses the polymerization reaction and takes it back far from chemical equilibrium. In the case of sickling red cells, the external energy input can come from breathing to reoxygenate the blood. Better controlled forms of the polymerization motor, embodied by actin filaments and microtubules, are operated in continuous cycles at the expense of nucleotide hydrolysis.

## Nucleotide Hydrolysis Pays for Recycling

Motor proteins, of course, also cycle through multiple steps of force generation by repeatedly hydrolyzing ATP, where the free energy released from phosphate hydrolysis is used to induce a large-scale conformational change in the protein. For actin and tubulin, the coupling between polymerization and nucleotide hydrolysis enables polymerization to occur constantly and steadily without ever reaching chemical equilibrium. To see how this is managed, consider a filament such as a microtubule or an actin filament where the subunits assemble in a head-to-tail fashion, forming a filament with distinct structural polarity. This makes the two ends of each polymer different in ways that have a profound effect on their growth rates. Addition of a subunit to either end of a filament of n subunits will result in a filament of $n+1$ subunits. There can be no overall energetic difference between two different pathways that generate the same products from the same reactants, so the free energy difference and therefore the association constant (and the critical concentration) must be the same for addition of subunits to both ends of the polymer. This means only that the ratio $k_{off}/k_{on}$ must be identical at the two ends; there is no similar constraint on the absolute values of these kinetic rate constants, and they are usually different at each end (Figure 3A).

In structurally polar filaments, such as actin filaments and microtubules, the kinetic rate constants $k_{on}$ and $k_{off}$ are typically much greater at one end than at the other. Thus if an excess of purified subunits are allowed to assemble onto marked fragments, one end can be seen to elongate much faster than the other (34,35). Similarly, if filaments are rapidly diluted so that the free subunit concentration is below the critical concentration, one end will depolymerize much faster than the other (Figure 3A). The more dynamic of the two ends, where both growth and shrinkage are fast, is the barbed end on actin filaments and the plus end on microtubules.

Now consider what happens when the subunits are also capable of nucleotide hydrolysis. For actin and tubulin, hydrolysis on the soluble subunits proceeds very slowly, however it is accelerated when the subunits are incorporated into filaments (36–38). Sometime after incorporation of an actin or tubulin subunit in a filament, hydrolysis occurs and the free phosphate group is released from each subunit. However, the nucleoside diphosphate remains trapped in the

filament structure. (On tubulin, the nucleotide binding site lies right at the interface between two neighboring subunits (39), whereas in actin the nucleotide is deep in a cleft near the center of the subunit (40).) Thus, two different types of filament structures can exist, one with the 'T' form of the nucleotide bound (ATP for actin, GTP for tubulin), and one with the 'D' form bound (Figure 3B).

When the nucleotide is hydrolyzed, much of the free energy released by cleavage of the high-energy phosphate–phosphate bond is stored in the polymer lattice. That is, the free energy of the D-containing polymer is higher than the free energy of the T-containing polymer. Therefore, the critical concentration $C_c = k_{off}/k_{on}$ (the dissociation equilibrium constant) for the D-form of the polymer is higher than for the T-form of the same polymer. Consequently, when the actual free subunit concentration lies between these two critical concentrations, D-form polymers will shrink while T-form polymers will grow (Figure 3B).

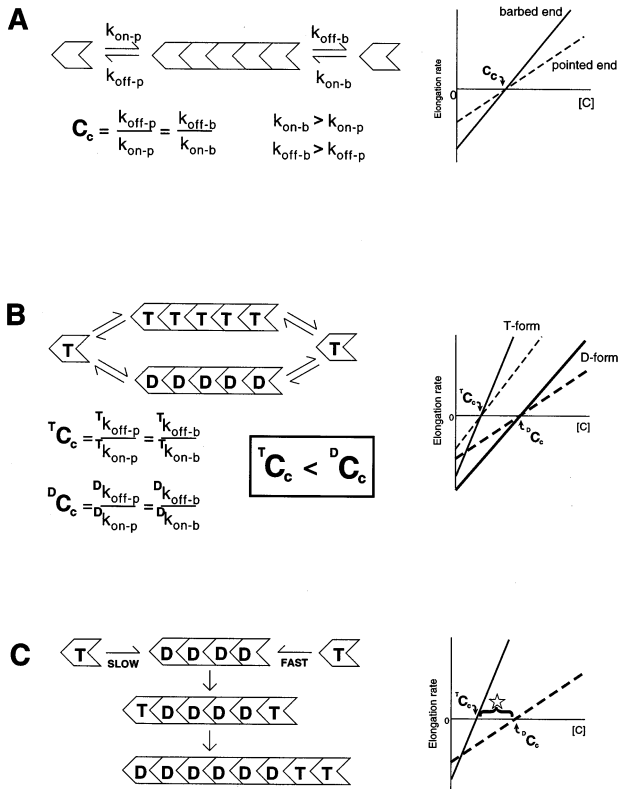


**Figure 3: Consequences of filament polarity and nucleotide hydrolysis.** A) For a structurally polar filament, the kinetic rate constants at one end (to the right) are greater than those at the other end (to the left). For actin, these structurally distinct ends correspond to the barbed and pointed ends respectively. The principle of detailed balance dictates that the ratio between $k_{on}$ and $k_{off}$, also known as the critical concentration, must be the same at both ends. Elongation rate as a function of protein concentration is shown at the right; $v = \delta(k_{on}[C] - k_{off})$ where $\delta$ is the size of the protein subunit and C is the concentration of free subunits in solution. At equilibrium, when $C = C_c$ (the critical concentration) there is no net elongation or shrinkage at either end. Away from equilibrium, the barbed end (solid line) grows faster or shrinks faster than the pointed end (dashed line). B) For actin filaments and microtubules, the subunits in the filament can exist in either the NTP-bound form (T) or the NDP-bound form (D). For each type of filament lattice, the critical concentration at the barbed and pointed ends must be identical as in A above. However, there is no requirement that the critical concentrations be identical for the two different filament types. Some of the energy released from nucleotide hydrolysis is stored in the polymer lattice, and therefore the critical concentration for the D-form filament is always higher than the critical concentration for the T-form filament. Elongation rate as a function of free subunit concentration is shown at the right. The four lines represent the curves for addition to the barbed end (solid lines) or pointed end (dashed lines) of T-form filaments (thin lines) or D-form filaments (thick lines). C) Kinetic consequences of coupling hydrolysis and polymerization. At the slowly growing pointed end, the rate of subunit addition is slow compared to the rate of nucleotide hydrolysis, so hydrolysis 'catches up' with the end and the filament tip is normally in the D form. At the rapidly growing barbed end, the rate of addition outstrips the rate of hydrolysis, and the end remains in the T form. At the right, elongation rate as a function of free subunit concentration is shown for the mixed polymer, with a T-form barbed end (thin solid line) and a D-form pointed end (thick dashed line). In the subunit concentration range marked with a star, there will be net elongation at the barbed end and net shrinkage at the pointed end, a situation known as 'treadmilling' (42).

Living cells, most of the free subunits are in the T form (since the free concentrations of both ATP and GTP are much higher than those of ADP and GDP) (41). The more time that subunits have remained in the polymer lattice, the more likely they are to have hydrolyzed their bound nucleotide. The identity of the nucleotide at the end of a filament therefore depends on the rate of hydrolysis relative to the rate of subunit addition. If the rate of subunit addition is high, that is if the filament is growing rapidly, then it is most likely that a new subunit will add on before the nucleotide in the terminal subunit is hydrolyzed, and the tip of the polymer will remain in the T-form. However, if the rate of subunit addition is low, hydrolysis is likely to occur first and the tip of the filament will be in the D-form.

The rate of subunit addition at the end of a filament is the product of the subunit concentration in solution and $k_{on}$. Remember that $k_{on}$ is much faster for one end of the filament (the barbed or plus end) than for the other end (the pointed or minus end), simply because of the structural distinction of the two ends. So at intermediate subunit concentrations, it is possible for the average rate of subunit addition to be faster than hydrolysis at the barbed end of an actin filament, but slower than hydrolysis at the pointed end. Thus, one end of the filament, the barbed end, can remain in the T conformation, while the other end, the pointed end, may be in the D conformation (Figure 3C). The D-form is at a higher energy state, with a higher critical concentration, than the T-form. If the concentration of free subunits in solution is higher than the critical concentration of the T-form (that is, the barbed end), but lower than the critical concentration of the D-form (that is, the pointed end), the filament will add

subunits on one end while it simultaneously loses subunits on the other end. This remarkable property is called treadmilling, which was first predicted as a consequence of nucleotide hydrolysis by Wegner in 1976 (42). Treadmilling has been frequently experimentally demonstrated for both actin filaments and microtubules *in vitro* and for microtubules it has even been seen in living cells (43,44).

In this way, the polymer can use the free energy of ATP or GTP hydrolysis to translate the kinetic differences between subunit addition rates at the plus and minus ends into energetic differences that can potentially be exploited by the polymerization motor. At a particular intermediate subunit concentration, filament growth at the barbed end will be exactly balanced by filament shrinkage at the pointed end, so that subunits will cycle rapidly between the soluble and filamentous states while the total length of the filament remains unchanged. Thus, chemical energy is spent by the cell to disassemble filaments and regenerate the monomer pool, so that polymerization is always energetically favored and the reaction never reaches equilibrium. A treadmilling filament placed between two barriers therefore has the ability to perform continuous mechanical work, for example it can carry along a weight that is attached to the middle of it like a clothesline (45) (Figure 4A). Alternatively, if a treadmilling filament is fixed to a surface at one point, the filament as a whole will seem to move forward even though the subunits within it are stationary (Figure 4B). If the treadmilling filament abuts a moveable load such as a plasma membrane or *L. monocytogenes*, the load can be pushed forward indefinitely while the concentration of the monomer remains constant, as long as ATP is in plentiful supply.

This does appear to be the situation in the most familiar forms of force generation by actin polymerization. In both lamellipodia of rapidly moving cells and the *L. monocytogenes* comet tail, actin filaments remain stationary with respect to the substrate, growing at the front to push the load forward (24,46). This thermodynamic treatment predicts that the barbed end of the filament should point forward for force generation by this mechanism; this is also experimentally observed in both cases (47–49).

This important point deserves emphasis: force generation by protein polymerization does not require nucleotide hydrolysis. The sole contribution to force generation of the ATP hydrolysis by actin, or the GTP hydrolysis by tubulin, is to allow the polymerization motor to be recycled and reused over and over in a living cell. It is clear from the thermodynamic arguments that energy for force generation is available from the polymerization motor, and likewise clear from the examples of sickling red cells and actin deforming vesicles that force is in fact generated by polymerization alone. However, we cannot predict the speed or efficiency of this type of motor mechanism without a detailed physical model.

## Brownian Ratchets

Oster and colleagues have proposed physical models for how chemomechanical energy transduction is carried out at the microscopic level by protein polymerization (32,50). These models are variations on the theme of the 'Brownian ratchet'. The name comes from the perpetual motion machine proposed by Feynman (51). Feynman imagined a box full of gas that encloses an axle with vanes on it. Thermal motion of the gas molecules results in random bombardment of the vanes, causing the axle to turn in a jiggly way in both directions. Next, he hooked the axle up to a toothed wheel, a ratchet, that can turn freely in one direction but whose backward rotation is prevented by a pawl on a spring that catches on the asymmetric teeth (Figure 5). Now, when the axle tries to jiggle in one direction, the direction permitted by the ratchet, it turns freely, but when it tries to jiggle in the other direction, it is stopped. Step by step, the axle turns only one way, even though the thermal motion of the gas is random and undirected. In this way, thermal motion of the gas molecules in the box could be used to generate useful work such as lifting a weight attached

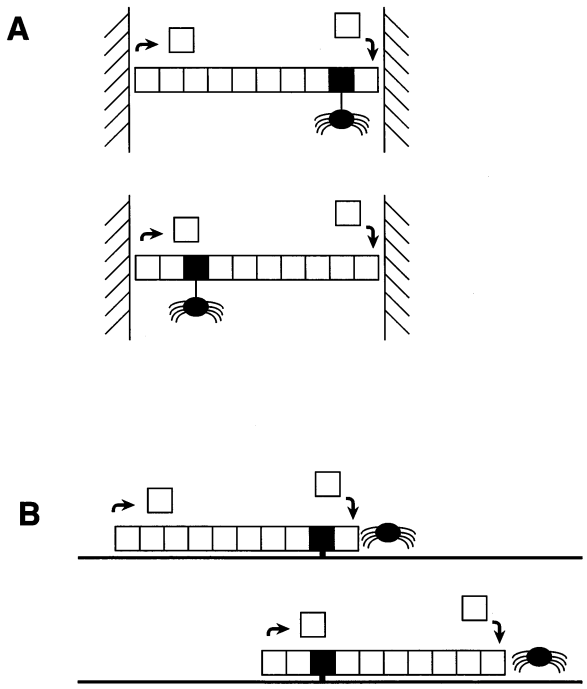


**Figure 4: Steady-state treadmilling can be used to generate force without a net change in filament length.** A) A treadmilling filament is trapped between two rigid barriers. Subunit addition at the right is exactly balanced by subunit loss at the left. Although the amount of polymer in the system remains constant, nucleotide is continually hydrolyzed and the system does not approach equilibrium. A marked subunit within the filament would be seen to move from right to left. A small load such as a spider attached to that subunit would experience a force, moving it from right to left. B) A treadmilling filament is stuck down to a solid substrate. Again, subunit addition at the right is exactly balanced by subunit loss at the left. Although the overall length of the filament remains unchanged, it translocates from left to right. A small load such as a spider resting at the tip would experience a force, nudging it from left to right.

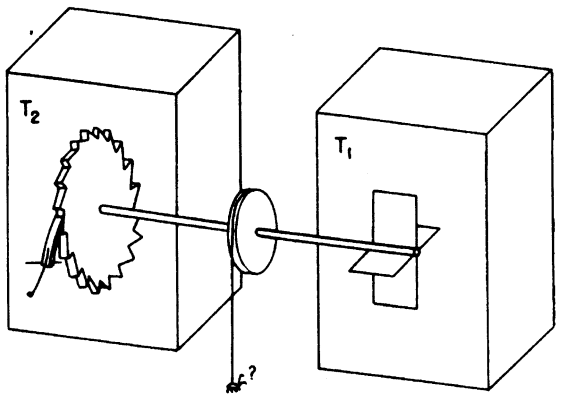


**Figure 5: Feynman's Brownian ratchet machine.** The machine consists of an axle with vanes on one end and a toothed wheel (the ratchet) on the other end. A pawl on a spring fits into the notches of the ratchet. The notches are asymmetric, allowing smooth movement in the clockwise direction but preventing backward slippage in the counterclockwise direction. Thermal motion of gas in the box containing the vanes will cause the axle to turn. If the temperature of the vanes ($T_1$) is greater than the temperature of the ratchet and pawl ($T_2$), useful work can be extracted to lift the bug that is dangling from the axle. From 'The Feynman Lectures on Physics,' volume 1, chapter 46.

to the axle, a clear violation of the second law of thermodynamics.

Of course the machine does not actually work under these conditions. The trick lies in ignoring the fact that thermal motion affects all parts of the system, the ratchet and pawl as well as the vanes. At a temperature where collisions with the vanes are sufficiently energetic to turn the ratchet step by step, the pawl is also jiggling around, and every so often the pawl will bounce out of its nook, allowing the ratchet to rotate backwards. If everything is at the same temperature, the number of forward steps will be balanced on average by the number of backward slips (51). But, Feynman's machine will work to turn primarily in one direction if the vanes and the ratchet are at different temperatures. If the vanes are hotter than the ratchet and pawl, the machine will turn forward and could indeed lift a weight, transducing the energy stored in the temperature gradient into mechanical force. If the ratchet and pawl are hotter than the vanes, the machine will turn backwards, but can still generate force. Feynman's example was designed to demonstrate that geometrical asymmetry, no matter how cleverly devised, cannot be used to generate force from thermal motion in the absence of an external source of energy (here, the external energy source is used to maintain the temperature gradient). But if such an external energy source is available, random thermal motion can be harnessed for unidirectional motion.

At the size scale of a cell or a protein assembly within a cell, Brownian motion is a major part of life. With thermal collisions constantly battering cellular components, it seems likely that the cell would have learned to harness some of this random motion in a directed way. Feynman's ratchet shows how random thermal motion can be harnessed with the help of an external energy source such as a temperature gradient. Large temperature gradients are essentially impossible to maintain over tiny cellular distances, but Feynman's concept of the Brownian ratchet can be readily extended to other energy sources such as electric fields and nonequilibrium chemical reactions that are available to cells (52). Brownian ratchets of all sorts share three basic characteristics. First, there is a discrete step or event that defines the steps of the ratchet; a potential of some kind with periodic energy minima in space or time. Second, there is random thermal motion in some component of the system, providing looseness somewhere so that the machinery can move both 'forward' and 'backward'. Third, critically, there is an energetic asymmetry, either in the periodic potential or in some other potential, that creates a preferred direction for the cycle to turn. In most interesting biological cases, the asymmetry is provided by a nonequilibrium chemical reaction. In our case, this nonequilibrium reaction is protein polymerization where monomer is in excess.

It is important to recognize here that Brownian ratchet models do not describe directed motion as being derived from thermal energy in a strict sense. Thermal motion is without direction. Directionality is bestowed by the asymmetric potential. For a cell, chemical energy must be put into the system in the form of generating and maintaining this asymmetric potential. For bacterial flagella, the asymmetric potential is manifest in the form of a transmembrane proton gradient. The cell uses ATP to pump protons out of the cytoplasm to maintain the chemical and electrical gradient that drives flagellar rotation. For polymerization-based force, the asymmetric potential is manifest in an excess of monomer.

There are two published physical models of a Brownian ratchet mechanism for the polymerization motor (32,50). In both, the discrete step corresponds to addition of a single protein subunit to a filament tip. In both, the energetic asymmetry is provided by the chemical potential of protein polymerization, following the thermodynamic scheme of Hill and Kirschner (31). The difference between the two lies in where the thermal motion comes into the system. The first model, proposed by Peskin et al. in 1993 (32), envisions a stationary filament that encounters a moveable load (Figure 6A). As the load bounces around by thermal motion, it attempts to move both backward and forward. Backward diffusion is inhibited by the presence of the filament. Forward diffusion is uninhibited, and every so often the excursion distance is sufficiently large that a new protein subunit can intercalate between the filament tip and the load, ratcheting the position of the load one step further forward. This physical model allows calculation of the speed at which an object can be pushed by a polymerizing filament. Since it is Brownian motion of the load which opens the space for intercalation of a new monomer, the velocity is predicted to be directly proportional to the diffusion coefficient of the load object. If the load is immobile, the filament will not grow, and no force will be generated. For a ratchet of this sort operating

at optimum efficiency, the amount of force generated is limited only by the amount of free energy released by the polymerization reaction; a single actin filament is predicted to be capable of generating up to 9 pN of force (32).

This simple model is a very useful starting point for thinking about the polymerization motor. But much like Feynman's original perpetual motion machine, it ignores the fact that thermal motion must affect all components in the system, in this case, the filament as well as the load. In most biological situations, the load is much larger than the filament (the diameter of *L. monocytogenes* or *S. flexneri* is larger than an actin filament by two orders of magnitude), and thermal bending of the filament is much more likely to open a monomer-sized gap between the filament and the load than is diffusion of the whole load. Therefore, Mogilner and Oster described a revised 'elastic' Brownian ratchet model in 1996 (50), where the load is considered to be stationary, and the filament is subject to bending (Figure 6B). Here, the amount of force generated is predicted to depend on the stiffness (Young's modulus) of the filament and on the angle between the filament and the load, but does not depend on the diffusion coefficient of the load object. The relationship between force, velocity, and angle with this more realistic model is quite complex, and must be computed numerically

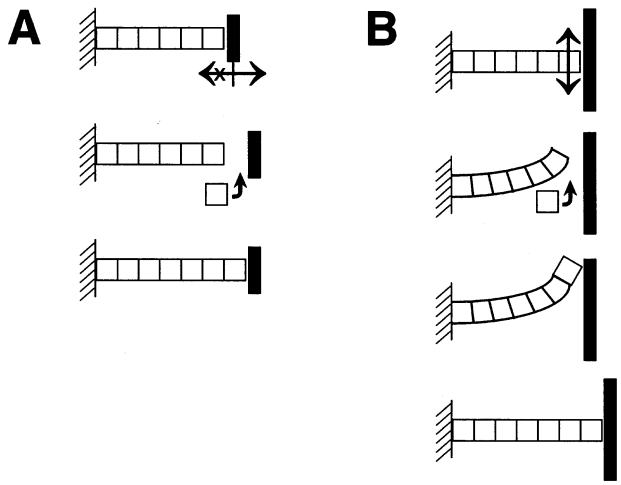


**Figure 6: Two Brownian ratchet models for the polymerization motor.** A) The filament is anchored at its left end; the right tip abuts a moveable load (black rectangle). The filament is immobile, but the load is subject to thermal motion. Diffusion toward the left is inhibited by the presence of the filament. Diffusion toward the right permits insertion of a protein subunit, ratcheting the diffusion of the load for unidirectional motion to the right. Velocity is dependent on the diffusion coefficient of the load (32). B) Again the filament is anchored at its left end, but now it is considered free to bend as a result of thermal motion. The load does not diffuse. When the filament bends far enough away from the surface, addition of a protein subunit is permitted. The elongated filament is under an elastic strain and exerts a spring-like force on the load. If the load is not immobilized this will push it toward the right. Velocity is dependent on the stiffness of the filament and on the angle between the filament and the load (50).

(50). Mogilner and Oster derived the velocities in four limiting cases where filaments are stiff or flexible, and the load force is high or low. Using parameters appropriate for actin, this model predicts that the maximum force that can be generated by a single actin filament is about 1.8 pN, and the velocity of polymerization under these conditions is adequate to explain the rapid movement of *L. monocytogenes* and protruding lamellipodia.

These two different models are not mutually exclusive. In reality, it is possible that both elastic bending of the filaments and thermal motion of the load object (plasma membrane, endosome, bacterium, etc.) can cooperate to open up the gap that allows addition of a subunit to the growing filament. Quantitative comparisons, however, seem to suggest that filament bending makes the more substantial contribution. For objects being pushed rapidly by comet tails, speed does not increase for smaller objects as predicted by the Peskin et al. model (53). Similarly, changing the diffusion coefficient of the load object does not change the speed. The presence of an intermediate filament network in fibroblasts decreases the diffusion coefficient of bacterium-sized objects by a factor of four (54) but does not change the velocities of *L. monocytogenes* or *S. flexneri* (P. Giardini and J. Theriot, unpublished results).

These thought-provoking models do have significant limitations. An underlying assumption of both calculations is that the presence of the load force affects only $k_{on}$ and not $k_{off}$. The models are strongly dependent on the exact nature of the interaction between the filament tip and the object being pushed. They assume that the components remain completely unattached from one another and interact as smooth rigid bodies. These assumptions are oversimplifications of the real situation, but there is not yet enough quantitative experimental data in any relevant system to determine exactly how much these limitations affect the accuracy of the models' predictions.

## Strength in Numbers

Conceptually, the greatest limitation of the two Brownian ratchet models for the polymerization motor is that they are not easily generalizable to cases where multiple filaments are interacting with the same load object, which is almost always true in interesting biological situations. The interactions of multiple filaments have been treated theoretically in two specific cases: the multiple protofilaments cooperating in a microtubule pushing against a wall and multiple actin filaments surrounding a symmetric bead.

Naturally occurring cytoskeletal polymers are built from multiple protofilaments that associate with one another laterally in order to provide enhanced stability against thermal breakage; there are two protofilaments in an actin filament, and thirteen protofilaments in a microtubule. For a microtubule whose tip abuts a load object, the protofilaments that are too close to the object (less than one monomer width away) may not be

able to elongate, but they can 'subsidize' the growth of neighboring protofilaments which are further from the object by supporting a portion of the load (55). Calculations that take this into account provide a substantially better fit to the experimental data for force generated by elongating microtubules (4) than the simple single-filament models.

When actin filaments are nucleated all around the surface of a spherical bead, a substantial number of the beads spontaneously break symmetry and form comet tails that look and behave very much like the tails associated with *L. monocytogenes* and *S. flexneri* (53,56). This experimentally observed symmetry-breaking can be explained by a model where each actin filament is a simple elastic Brownian ratchet. The presence of the bead effectively couples the polymerization of different filament tips, such that filaments on the same side of the bead cooperate with one another, much like the multiple protofilaments in the microtubule, while filaments on opposite sides of the bead inhibit each other's growth. This arrangement allows for small stochastic fluctuations to be amplified, so that symmetry-breaking can occur in the system as a whole (A. van Oudenaarden and J. Theriot, manuscript submitted). Further generalization of multifilament systems beyond these two special cases will certainly be an important step in understanding how the polymerization motor contributes to motility in living cells.

## From the Polymerization Motor to Cell Motility

For a cell to maintain overall directionality in its movement, it must be able to regulate the functioning of the polymerization motor in space and time. In the case of actin-driven protrusions, regulation is performed through accessory proteins. The basic properties of the polymerization motor are consequences of inherent actin filament dynamics, but the cell controls the polymerization motor by altering filament kinetics in several ways. The site of actin-driven pushing force is determined by local activation of a nucleating complex (the Arp2/3 complex) that overcomes the kinetic barrier to the initial formation of a filament from soluble subunits (57). The efficiency of the nucleotide-driven turnover is enhanced by the action of depolymerizing factors such as cofilin, which preferentially bind to and destabilize D-form filaments (58). In protruding lamellipodia, these two types of dynamics-enhancing factors are spatially segregated, ensuring that the polymerization motor continues to push in a single direction (59). The lamellipodium structure as a whole undergoes treadmilling, with the total amount of polymer remaining constant and individual protein subunits continuously cycling through the filaments as ATP is continuously consumed.

In order to understand the dynamic biophysical basis of cell motility, it will be necessary to bring together this type of biochemical and structural description with further biophysical characterization of the polymerization motor. Classical molecular motors are also involved to some extent in most cases where the polymerization motor is functioning, including the lamellipodium and the mitotic spindle. Learning how polymerization forces and molecular motor forces interact in a living cell will continue to be a significant challenge to cell biologists over the next several years. The thermodynamic and physical theories outlined here give us a useful perspective for experimental design to address these complex issues; now let us start our motors!

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