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Actin Polymerization and ATP Hydrolysis

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F-actin is the major component of muscle thin filaments and, more generally, of the microfilaments of the dynamic, multifunctional cytoskeletal systems of nonmuscle eukaryotic cells. Polymeric F-actin is formed by reversible noncovalent self-association of monomeric G-actin. To understand the dynamics of microfilament systems in cells, the dynamics of polymerization of pure actin must be understood. The following model has emerged from recent work. During the polymerization process, adenosine 5′-triphosphate (ATP) that is bound to G-actin is hydrolyzed to adenosine 5′-diphosphate (ADP) that is bound to F-actin. The hydrolysis reaction occurs on the F-actin subsequent to the polymerization reaction in two steps: cleavage of ATP followed by the slower release of inorganic phosphate (P_i). As a result, at high rates of filament growth a transient cap of ATP-actin subunits exists at the ends of elongating filaments, and at steady state a stabilizing cap of ADP · P_i-actin subunits exists at the barbed ends of filaments. Cleavage of ATP results in a highly stable filament with bound ADP · P_i, and release of P_i destabilizes the filament. Thus these two steps of the hydrolytic reaction provide potential mechanisms for regulating the monomer-polymer transition.

ACTIN, WHICH IS ONE OF THE TWO MAJOR PROTEINS OF muscle, occurs in every eukaryotic cell, in which it can account for more than 20% of the total cell protein (*1*). In addition to being one of the more abundant proteins in nature, actin is also one of the most highly conserved proteins. From amoebas to humans, actins are about 95% identical in amino acid sequence (*2*); yeast and soybean actins (*3*) are about 85% and *Tetrahymena* actin (*4*) is about 75% identical to muscle actins. Many of the substitutions that do occur are chemically conservative (for example, aspartate for glutamate) and restricted to a few regions of the polypeptide chain (*2–4*). Both its widespread occurrence and the evolutionary stability of its primary structure suggest the fundamental biological importance of actin.

Actin is a bilobed, approximately pear-shaped molecule (*5*) that consists of a single polypeptide chain of 42,000 daltons (*1, 2*). Monomeric at nonphysiologically low ionic strength, pure G-actin polymerizes through reversible noncovalent associations (*1, 6*) into filaments of F-actin that contain thousands of protomers (*7*), when either the Mg^{2+} concentration or the ionic strength is closer to

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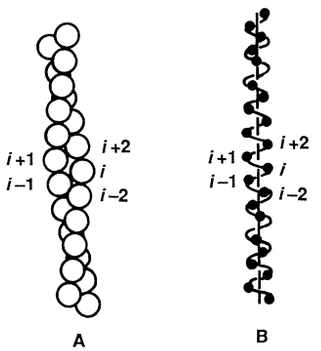

Fig. 1. Schematic representation of the F-actin filament. (**A**) The right-handed, double-stranded helix resembles the electron microscopic images and illustrates the potential subunit-subunit interactions. However, there is no convincing evidence that F-actin is a filament of two separate strands. (**B**) The left-handed, single-stranded helix illustrates an alternative, and presently preferred, interpretation of the structural data, but all of the potential subunit interactions are not shown. [Adapted from (*1*)]

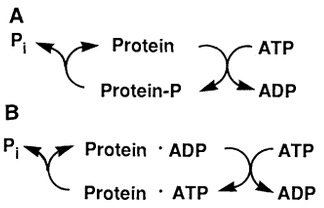

Fig. 2. (**A**) Covalent and (**B**) noncovalent regulation of proteins by an ATP hydrolytic cycle. Covalent regulation can involve phosphorylation (protein-P) and dephosphorylation of serine, threonine, or tyrosine residues. Noncovalent regulation involves association of ATP, hydrolysis of ATP to ADP, and displacement of the ADP by ATP.

physiological levels (*1, 8*). Addition and loss of monomers at the ends of the filaments continues after polymerization has reached steady state. In its electron microscopic image, F-actin has the appearance of a double-stranded, right-handed helix with the two strands crossing every 36 nm (Fig. 1A). However, detailed diffraction data have been interpreted as indicating a single-stranded, left-handed helix (Fig. 1B) with some controversy about the orientation of the subunits relative to the filament axis (*9*). The actin filament is polarized, as is most easily demonstrated by the electron microscopic images of filaments decorated with myosin (*10*), which, in the absence of adenosine 5′-triphosphate (ATP), binds to F-actin at a 45° angle pointing toward the "pointed" end and away from the "barbed" end of the filament.

Only polymeric actin is known to have biological function. The most intensely studied example is the actin thin filaments of muscle, which, together with myosin thick filaments, provide the mechanochemical basis for contraction (*11*). However, muscle contraction is just one manifestation of the more general role of F-actin microfilaments in motile activities of all eukaryotic cells. Cell locomotion, cytokinesis, phagocytosis, platelet clot retraction, and ligand-induced clustering of cell surface receptors are a few examples of actomyosin-dependent mechanochemical activities of nonmuscle cells that are thought to be fundamentally similar to muscle contraction (*12*); proof of myosin's role is strongest for cytokinesis (*13*). In addition, actin filaments serve structural functions that probably do not involve myosin. Examples include the core bundles that support fine cellular projections such as the microvilli of the intestinal brush border and the stereocilia of the hair cells of the cochlea of the inner ear, the acrosomal process of sperm, and the cytoskeletal network that provides the structural organization and dynamic viscoelastic properties of the cytoplasm, which are important for the coordination of metabolic activities (*14*).

In muscle, the polymerization process is important only to provide and maintain the thin filaments required for contractile activity. In nonmuscle cells, extensive depolymerization and repolymerization of the cytoskeleton are likely to be continuing, regulated processes with actin filaments disappearing and reappearing at different times and places as they are needed for specific functions. Although the organizational state of actin in cells is greatly influenced by the interactions of G-actin and F-actin with other proteins (*1, 14, 15*), polymerization is the property of actin alone. Thus, it is essential to understand the mechanism of polymerization of pure actin in order to understand the dynamics of the complex microfilament systems in cells. Considerable progress has been made in the last few years, especially in understanding the role of ATP hydrolysis in the polymerization process, which is the subject of this article.

Monomeric G-actin contains one molecule of noncovalently bound ATP (*1, 8*), and it is hydrolyzed to F-actin–bound adenosine 5′-diphosphate (ADP) and free inorganic phosphate (P_i) during polymerization (*1, 8*) in a process that involves the transient formation of F-actin–bound ADP · P_i (*16*). ATP is not resynthesized when F-actin depolymerizes, but the ADP bound to the G-actin that dissociates from the ends of the filaments exchanges for ATP in solution and regenerates G-ATP-actin. Thus polymerization and depolymerization result in the continued hydrolysis of ATP; actin is an adenosinetriphosphatase (ATPase) (*1*).

Expenditure of metabolic energy by the hydrolysis of nucleoside triphosphates is not usual in noncovalent assembly processes in biology; for example, polymerization of hemoglobin and the more complex process of virus coat assembly have no such requirement. However, the formation of microtubules, which are the other major nonmembranous structural element of eukaryotic cells, is similarly accompanied by the hydrolysis of guanosine 5′-triphosphate (GTP) (*17*). In contrast to most other biological polymers, the major functions of both microfilaments and microtubules require spatially and temporally regulated depolymerization as well as polymerization. Nucleoside triphosphate hydrolysis may provide a regulatory switch for this interconversion of monomers and polymers.

The cyclical conversion of protein-bound ATP (or GTP) to protein-bound ADP (or GDP) is equivalent to covalent modification of a protein by a phosphorylation-dephosphorylation cycle (Fig. 2). In both cases the energy of nucleoside triphosphate hydrolysis is used to interconvert a protein between two conformations with different properties. Because reaction rates determine the ratio of the two conformations in a steady-state cycle, it is more susceptible to regulation than is an equilibrium reaction (*18*) and is commonly utilized to provide a regulatory switch. In this article we discuss recent data on the mechanism of ATP hydrolysis in actin polymerization that provide insights into the nature of the regulatory switch that ATP hydrolysis provides. Our primary purpose is to develop a model that is consistent with the data, can be tested experimentally, and can provide a basis for interpreting the effects of physiological and pharmacological stimuli on the cytoskeleton. Many of the ideas that will be presented are generally accepted but others depend on experiments performed in only one or a few laboratories. Significant disagreements will be mentioned.

Kinetics of Actin Polymerization and Accompanying ATP Hydrolysis

Although most of the physiologically important interactions between actin monomers and polymers probably occur at or near steady state, studies of the kinetics of the pre–steady-state polymerization process have provided the most useful information. The formation of polymeric F-actin from monomeric G-actin has usually been monitored by the increase in light scattering or by the increase in fluorescence of actin labeled with an appropriate fluorophore. The rate of elongation of existing filaments can be quantified in the same two ways and also by electron microscopic visualization of individual filaments, which has the distinct advantage of allowing independent measurement of the elongation rates at the barbed and pointed ends of the filaments.

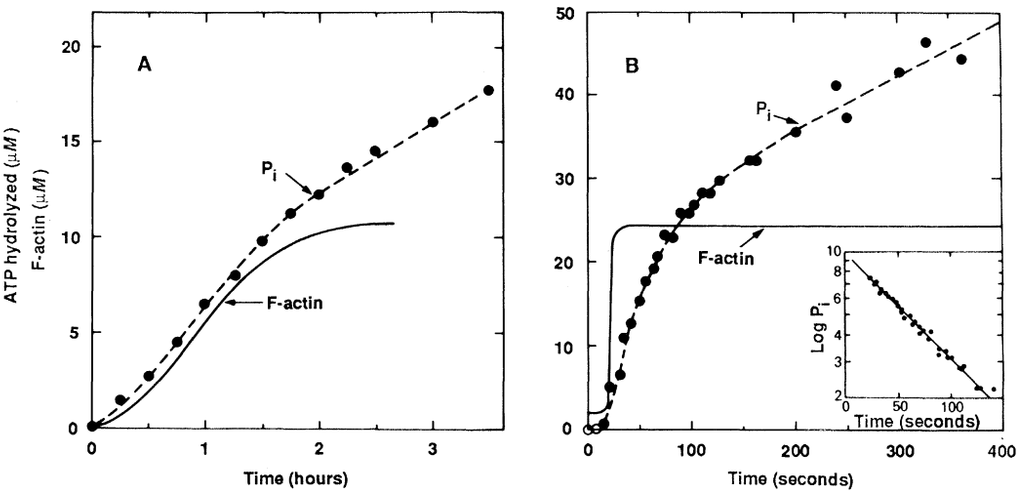

Fig. 3. Time courses of spontaneous polymerization of actin (solid lines) and accompanying hydrolysis of ATP (dashed lines) without (**A**) and with (**B**) continuous sonication. Note the difference in time scales in (A) and (B). When polymerization is very fast, ATP hydrolysis lags significantly behind. The first-order hydrolysis of ATP on the F-actin is shown in the inset. Data in (B) are from (*32*).

The interpretations of the most recent data (*19–23*) for the polymerization of actin are consistent with and significantly extend the model proposed originally by Oosawa and co-workers (*8*) for the formation of helical polymers; that is, polymers in which any subunit i interacts with subunits $i + 2$ and $i - 2$ in addition to the adjacent subunits $i + 1$ and $i - 1$ (Fig. 1A). The polymerization of Mg-ATP-actin [actin with ATP bound to the nucleotide-binding site and Mg^{2+} bound to the high-affinity cation-binding site (*24*)] is characterized (Fig. 3A) by a lag phase, in which monomers associate into small, thermodynamically unstable nuclei [probably trimers (*19–23, 25*)], followed by a rapid phase, in which the nuclei grow into long filaments with little accumulation of oligomers of intermediate sizes. Elongation occurs at both ends of the growing filaments (*26, 27*), more rapidly at the barbed ends than at the pointed ends. Elongation stops when the concentration of monomers decreases to the critical concentration, which is the monomer concentration at which the rate of loss of monomers from filament ends equals the rate of addition of monomers.

The time course of cleavage of ATP closely parallels the formation of F-actin under most conditions of polymerization (Fig. 3A). When polymerization has reached steady state, ATP hydrolysis continues at a slower, constant rate until all of the available ATP has been hydrolyzed. The continued hydrolysis of ATP when polymerization is at steady state is probably the sum of at least two independent processes: (i) one that is associated with the continued addition and loss of actin monomers at filament ends and (ii) one in which ATP is exchanged for ADP on internal actin subunits in the body of the filament followed by hydrolysis of the ATP (*28, 29*).

When in the polymerization process is ATP hydrolyzed? Polymerization can be separated from ATP hydrolysis (*30*) by (i) reducing the rate of ATP hydrolysis by lowering the temperature (*31*) or (ii) increasing the rate of polymerization, either by increasing the monomer concentration (*32*), or by increasing the filament concentration by continual sonication (*33*). In a typical experiment in which actin was polymerized with sonication (Fig. 3B), only about 15% of the ATP bound to F-actin subunits had been hydrolyzed when polymerization was complete (at about 20 seconds; note the difference in time scales in Fig. 3, A and B). The ATP that remained on the filaments was then hydrolyzed by a first-order reaction (Fig. 3B, inset), which continued until approximately one ATP was hydrolyzed for every F-actin subunit and was followed by ATP hydrolysis at the rate typical of the steady state. These, and similar (*30–32*) results prove that ATP hydrolysis occurs on the F-actin subsequent to the polymerization step.

Hydrolysis of ATP on the F-ATP-actin polymer proceeds through the intermediate formation of F-ADP · P_i-actin subunits (F-actin subunits to which both hydrolysis products are bound), followed by the release of P_i into solution and the accumulation of the final product F-ADP-actin (*16*). At the barbed end of the filament, hydrolysis of ATP appears to be site-specific; that is hydrolysis at the interface between the string of ATP-actin subunits at the end of the filament and the ADP · P_i-actin subunit core is highly preferred to random hydrolysis within the string of ATP-actin subunits [adjustment of calculated curves to the data suggest that the rate constant is approximately 10,000 times larger (*34*)]. The much slower rate of polymerization at the pointed ends of filaments (see below) makes it difficult to evaluate the course of ATP cleavage at that end.

When polymerization is very rapid, the rate of addition of ATP-actin subunits to the filament ends will initially exceed the rate of site-specific ATP hydrolysis and the length of the string of ATP-actin subunits at the ends of the filaments will grow. As polymerization proceeds, the monomer concentration will fall and thus the rate of elongation will decrease until it becomes less than the rate of ATP cleavage on the F-actin. Then the strings of ATP-actin subunits will shorten as the interfaces between the ATP-actin and ADP · P_i-actin subunits move vectorially toward the filament ends. Because the rate of release of P_i into the medium [$k = 0.006$ sec^{-1} (*16*)] is much slower than the rate of site-specific cleavage of ATP on the filaments

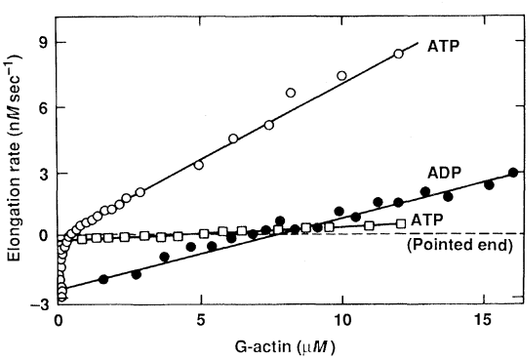

Fig. 4. The rate of elongation of F-actin (barbed end plus pointed end) as a function of G-actin concentration in the presence of ATP or in the presence of ADP, and for the pointed end in the presence of ATP (the barbed end was blocked with plasma gelsolin). The critical concentrations are the intercepts of the experimental lines with the dashed line that denotes a zero rate of elongation. The association rate constants are proportional to the slopes of the experimental lines, and the dissociation rate constants are proportional to the extrapolated value at zero actin. Data are from (*33, 44*).

[$k = 13\ \text{sec}^{-1}$ (*34, 35*)], F-ADP · P_i-actin subunits are a major long-lived ($t_{1/2} = 2$ minutes) transient species in the polymerization process (*16*). The relative proportions of terminal subunits of ATP-actin, ADP · P_i-actin and ADP-actin subunits in a population of filaments will depend on the relative rates of ATP cleavage, P_i release, and addition of new ATP-actin subunits, and thus will vary with the concentration of ATP-actin monomers. The nucleotide composition of the subunits at the tips of the filaments when the concentration of actin monomers is near or at the critical concentration is an important physiologically relevant issue.

Comparison of Actin Filaments Polymerized in ATP and ADP

The first information about the composition of the ends of actin filaments came from an analysis of the initial rate of filament elongation as a function of the concentration of G-actin in solutions containing either ATP or ADP. For a reversible, equilibrium polymerization process, that is, when the dissociation reaction is precisely the reverse of the association reaction, the rate of filament elongation can be expressed (*20*) as:

$$dF/dt = (k_+^B + k_+^P)Nc_1 - (k_-^B + k_-^P)N \quad (1)$$

where dF/dt is the rate of change in concentration of F-actin subunits; k_+^B, k_+^P, k_-^B, and k_-^P are association and dissociation rate constants at the barbed (B) and pointed (P) ends of the filaments; N is the filament concentration; and c_1 is the G-actin concentration. In Eq. 1, dF/dt is a linear function of c_1. When the experimentally determined initial rate of change of F-actin subunit concentration is plotted against the initial monomer concentration, a straight line is obtained with a slope $(k_+^B + k_+^P)N$ and an intercept at $c_1 = 0$ of $-(k_-^B + k_-^P)N$. By definition, the critical concentration c_c is the value of c_1 such that $dF/dt = 0$.

The theory can be tested by adding F-ADP-actin seeds to solutions of G-ADP-actin because the elongation of ADP-actin is a truly reversible reaction, that is, G-ADP-actin adds to and dissociates from both ends of the filaments with the same reactions occurring when the concentration of G-ADP-actin is above and below the critical concentration. Thus when F-ADP-actin seeds are added to G-ADP-actin above its critical concentration, the seeds should elongate at an initial rate that is proportional to the G-actin concentration and, when the G-actin concentration is below the critical concentration, the seeds should depolymerize at an initial rate that is inversely proportional to the G-actin concentration. The G-actin concentration at which neither elongation nor depolymerization occurs is the critical concentration. The experimental data for ADP-actin (*33*) agree with this theory (Fig. 4). The rates of elongation at G-actin concentrations above the critical concentration fall on the same straight line as the rates of depolymerization (negative elongation rates) at G-actin concentrations below the critical concentration.

A very different result is obtained, however, when the same experiment is performed (*33*) with G-ATP-actin (Fig. 4). Above the critical concentration a straight line is observed as for ADP-actin, but sharp curvature occurs at the critical concentration and the slope of the line is different below the critical concentration. Also, the critical concentration in ADP is about 20 times as large as that in ATP even though (i) the F-actin consists essentially of ADP-actin subunits in both cases and (ii) the rate constant for the association of ATP-actin to filament ends is only about two to three times larger than that of ADP-actin. It was inferred from these and similar data that the low critical concentration of actin in ATP is due to the presence of a "cap" of slowly depolymerizing subunits at the ends of filaments (*33, 36*). According to this model, the association and dissociation reactions, and hence the shape of the elongation curve, will be different above and below the critical concentration because the compositions of the filament ends will be different. The same interpretation has been given to similar data for microtubule polymerization (*37*).

The first hypothesis (*35, 38*) was that the stabilizing cap consisted of one or two terminal ATP-actin subunits (*39*). Because there was evidence that the interactions between ATP-actin subunits were rather weak (*40, 41*), compared to the heterologous interactions between ATP-actin and ADP-actin subunits (*37*), it was proposed that the short ATP-actin cap stabilized the filament by virtue of strong interactions between the subterminal ADP-actin and the terminal ATP-actin subunits. However, this initial concept has been modified to accommodate recent data (*42*). Inorganic phosphate has been found to bind to F-ADP-actin subunits in a 1:1 ratio (*42*) and to lower the critical concentration of actin filaments in ADP (*42, 43*) by decreasing the rate of depolymerization by a factor of about 5 to 10 (*42*). In addition, the downward curvature of the rate of filament elongation as a function of G-actin concentrations in ATP (Fig. 4) is eliminated by the addition of P_i (*42*). These observations make it unnecessary to propose the existence of an ATP-actin and indicate that it is an ADP · P_i-actin cap that stabilizes actin filaments in low concentrations (near the critical concentration) of G-ATP-actin. If ATP-actin subunits are present at the ends of actin filaments, their kinetic behavior would have to be the same as that of ADP · P_i-actin subunits.

However, these experiments provide information only about events at the barbed ends of actin filaments because, as shown below, events at those ends are much faster than at the pointed ends. The properties of the pointed ends can be determined by similar experiments in the presence of proteins that block specifically the barbed ends of the filaments (*1, 15*) or by electron microscopic measurements of the elongation rates at the two ends of individual filaments (*26, 27*). Experiments performed in two laboratories with the barbed end–blocking protein gelsolin (*44, 45*), provided no evidence for a stabilizing cap at the pointed ends of filaments near the critical concentration (Fig. 4); that is, the elongation curve followed the same straight line above and below the critical concentration. Curvature did occur at G-actin concentrations greater than those plotted in Fig. 4, which indicates formation of an ATP-actin cap at the pointed ends under those conditions (*44*). However, Weber and co-workers (*46*), who used villin to block the barbed ends, and Pollard (*41*), who used the electron microscopic assay to measure the elongation rates, concluded to the contrary that an ATP-actin cap is present at the pointed end near its critical concentration. However, it is important that the four laboratories agree that when both ends of the filaments are free only the barbed ends will be stabilized by a cap at steady state (the likely physiologically relevant situation), because then the actin monomer concentration will be slightly above the critical concentration of the barbed end and very much below the critical concentration of the pointed end (Fig. 4).

Polymerization Model

The data presented thus far are consistent with the model shown in Fig. 5 for the polymerization of Mg-ATP-actin. At concentrations of G-actin above the critical concentration, monomers assemble into an unstable trimer (the nucleus). Addition of one more monomer forms a tetramer that then elongates rapidly at both ends to form a long filament of F-actin. At an early stage, an ATP molecule on the oligomer is cleaved, forming an interface between an ATP-actin and

an ADP · P_i-actin subunit. This interface becomes the preferred site for subsequent hydrolysis, at least at the barbed end of the filament. At very high concentrations of actin monomer, the rates of elongation are faster than the rate of site-specific ATP hydrolysis on the filament, and the ATP-actin cap lengthens at both ends of the filament. As polymerization proceeds, the monomer concentration falls and the rates of elongation at the filament ends become less than the rate of site-specific ATP hydrolysis on the filament (which is independent of monomer concentration) and the segments of ATP-actin and ADP · P_i-actin subunits become shorter at both ends of the filament, although the filament continues to grow. As the monomer concentration approaches the critical concentration, the ATP-actin subunits disappear and the ADP · P_i-actin cap approaches a short, limited length at the barbed end (illustrated as two subunits in Fig. 5) and disappears at the pointed end. However, if P_i were present in the polymerization medium, P_i would bind to all of the F-actin subunits [dissociation constant K_d = 1.5 to 2 m*M* (*41, 42*)] maintaining them as ADP · P_i-actin (*42, 43*).

Effects of ATP Hydrolysis on the Kinetic Constants

The basis of the regulatory switch provided by ATP cleavage and P_i release is revealed by comparing the critical concentrations and the association and dissociation rate constants at the two ends of actin filaments in ADP and ATP. The critical concentration can be obtained directly from experiments such as those described in Fig. 4 (the G-actin concentration at which $dF/dt = 0$; see Eq. 1), and the absolute rate constants can be calculated from the slopes and intercepts of the experimental curves when the filament concentration (*N* in Eq. 1) can be estimated. The results of one set of experiments (*44, 47*) are shown in Table 1. Pollard (*41*) has obtained another complete set of data under somewhat different ionic conditions by electron microscopic measurements of the rates of growth at both ends of individual filaments; although quantitatively different, the data are qualitatively similar to those in Table 1 and lead to similar conclusions.

First consider the data for actin polymerized in the presence of ADP. The rate constants are larger at the barbed end than at the pointed end, but, as they must be for an equilibrium polymer such as F-ADP-actin, the critical concentrations at the barbed and pointed ends are the same. For a steady-state polymer, however, the critical concentrations can be different at the two ends of the filaments, and, as we see from Table 1, they are different for actin polymerized in the presence of ATP; the critical concentration at the barbed end is much lower than at the pointed end.

Recent data on the properties of F-ADP · P_i-actin (*42*) provide a better understanding of the mechanism by which the chemical energy of ATP hydrolysis is utilized to regulate the dynamics of actin filaments. Cleavage of the γ-phosphate bond of ATP bound to F-actin results in a transient F-ADP · P_i-actin filament, from which subunits dissociate slowly at both ends and which, consequently, has a low critical concentration. The subsequent release of P_i generates an unstable F-ADP-actin filament from which subunits would dissociate rapidly if the filament were not capped. However, at the critical concentration in ATP, the relative rates of actin monomer association and dissociation, ATP cleavage and P_i release maintain a stabilizing ADP · P_i-actin cap at the barbed end of the filaments. Subunit turnover is slower at the pointed end so that only ADP-actin subunits are present at that end of the filament at the critical concentration. Thus, the formation of ADP · P_i-actin subunits in the filament provides two regulatory switches: (i) the ADP · P_i-actin cap maintains a lower critical concentration at the barbed end than would exist in its absence, and (ii) the presence of the ADP · P_i-actin cap at the barbed end, but not at the pointed end, allows the two ends to have very different critical concentrations. That the regulatory switch is due to the presence or absence of ADP · P_i-actin subunits is supported by the observation that, in the presence of ATP, addition of P_i has no effect on the critical concentration of the barbed end, whereas the critical concentration at the pointed end decreases substantially to a value nearer that of the barbed end (*42, 43*).

Possible Physiological Consequences of ATP Hydrolysis

It is likely that the actin monomer concentration within the cell never varies appreciably from the critical concentration. Any increase in monomer concentration would be immediately buffered by filament elongation, and any decrease would be immediately corrected by depolymerization of filaments. Thus filaments will generally be at or near steady state, and formation of new filaments from G-actin will occur infrequently. However, if the difference in critical concentrations at the barbed and pointed ends observed for filaments of pure actin is maintained in the cell, filaments at steady state will not be static. The concentration of G-actin at steady state will be greater than the critical concentration at the barbed end and less than the critical concentration at the pointed end (Fig. 4 and Table 1). Thus, as was predicted (*20*) and demonstrated (*27*) for pure actin, on average, filaments will continue to grow at the barbed end while shortening at the pointed end ("treadmilling") although the total concentration of F-actin remains constant. The maximum rate of this process (from the data in Table 1) would be about 3.5 μm/hour (*48*), which is likely to be too slow to be of significance for any of the motile processes that were discussed at the beginning of this article. However, there is evidence (*49*) that the reaction rates might be significantly greater at the higher protein concentrations that occur in cells. Thus, the reaction might have more biological relevance than appears to be the case from the rate constants in Table

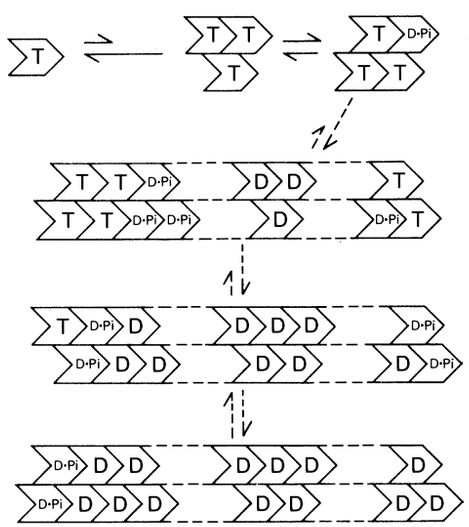

Fig. 5. Schematic illustration of the proposed model for the polymerization of MgG-actin in ATP. Each chevron represents an ATP-actin (T), an ADP · P_i-actin (D · P_i), or an ADP-actin (D) subunit. The final filament will consist of perhaps 1000 or more subunits and only those at the ends and in the middle are represented. The ATP-actin cap at both ends of the filament will be very long when the monomer concentration is high; at steady state the ATP-actin cap will have disappeared, and there will be a short ADP · P_i-actin cap at the barbed end but probably not at the pointed end (see text for details).

1. Indeed, there is one report (*50*) of possible treadmilling of actin filaments in cells at a rate of about 45 μm/hour.

The difference in critical concentrations at the two ends of the filaments could become even more important physiologically through the synergistic interaction of filaments with actin-binding proteins. When a protein that blocks the barbed end interacts with filaments in the test tube, the filaments depolymerize at the pointed end until the monomer concentration reaches the new, higher critical concentration at that end. For example, from the data in Table 1, the monomer concentration would increase from 0.36 to 4 μ*M*. In many motile cells, the total actin concentration is so high (100 to 200 μ*M*, if uniformly distributed) that this slight increase in monomer concentration, which is at the expense of polymer concentration, would have little effect on the concentration of filaments.

However, many cells contain high concentrations (100 to 200 μ*M*) of proteins [profilin and others (*1, 15*)] that bind actin monomers. In the presence of 100 to 200 μ*M* profilin [which binds actin monomers with a dissociation constant K_d of ~5 μ*M* (*15*)], an increase in the actin monomer concentration to 4 μ*M* would cause the formation of 45 to 90 μ*M* profilin-actin complex. As all of the actin bound in this complex would come from F-actin, the filament concentration would be reduced by about 50% in this example. Thus the difference in critical concentrations at the two ends of the actin filament, combined with the presence of barbed end–blocking proteins and monomer-binding proteins could provide a powerful mechanism for regulating the concentration of microfilaments.

All of these possibilities result from the difference in critical concentrations at the two ends of the filaments, which is a direct consequence of the ATP hydrolysis that is associated with actin polymerization. These reactions do not depend on the presence of an ADP · P_i-actin cap at the end of the filament. However, the large difference in the dissociation rate constants at the barbed ends of filaments in ADP and ATP is a direct result of the ADP · P_i-actin cap. If a filament should lose its ADP · P_i-actin cap (perhaps through several sequential dissociation events uninterrupted by an association event, or by an increase in the rate of P_i release from the terminal subunits), it will rapidly depolymerize because of the difference in the dissociation rate constants for ADP-actin and ADP · P_i-actin subunits. Because the system must remain at steady state, other filaments must grow to maintain a constant concentration of F-actin subunits. Thus actin filaments are likely to be in a dynamic situation at steady state. At any instant, most of them (those with capped barbed ends) will be slowly elongating while a few (those that have lost their barbed-end cap) are rapidly shortening; any given filament will oscillate between these two phases. These expected fluctuations in filament length (dynamic instability) have not been demonstrated for actin filaments but they have been dramatically recorded for microtubules (*51*), which have a similar stabilizing cap at steady state (*37, 52*); theoretical calculations (*53*) suggest that the length changes are not expected to be as large for microfilaments as for microtubules.

Table 1. Kinetic constants for the elongation of actin filaments in ADP and ATP. The data (*44, 47*) are for rabbit skeletal muscle actin in 10 m*M* tris Cl^-, *p*H 7.8, 0.2 m*M* dithiothreitol, 0.01% NaN_3, 1 m*M* $MgCl_2$, and either 0.2 m*M* ADP or 0.2 m*M* ATP. Data for the pointed end were obtained by measuring the elongation rates of filaments with blocked barbed ends and data for the barbed end were calculated by subtracting the pointed-end data from those for filaments with both ends free (Fig. 4). The data for filaments in ATP are the kinetic constants (k_- and k_+) near the critical concentration (c_c) at which point the barbed end is capped with ADP · P_i-actin subunits and the pointed end is not capped; the kinetic constants at the barbed and pointed ends in ATP may be different at actin monomer concentrations much higher than the critical concentration because of the presence of long caps of ATP-actin subunits at both ends [see text and (*40, 41*)]. A complete set of kinetic data obtained by electron microscopic measurements of filament growth rates has been published (*41*).

Nucleotide	k_- (sec^{-1})	k_+ ($\mu M^{-1} sec^{-1}$)	c_c (μ*M*)
	Pointed end		
ADP	0.4	0.05	8
ATP	0.4	0.1	4
	Barbed end		
ADP	6	0.75	8
ATP	0.14	1.4	0.1
	Both ends		
ADP	6.4	0.8	8
ATP	0.54	1.5	0.36

Concluding Remarks

It is generally accepted that polymerization of ATP-actin occurs through a nucleation elongation process and that the nucleus is a trimer. It is likely that short oligomers are stabilized by ATP hydrolysis. The ATP hydrolysis that accompanies elongation at both ends of the filaments occurs on the F-actin subsequent to the addition of monomer. At high monomer concentrations, the rate of filament elongation exceeds the rate of ATP cleavage and a long stretch of ATP-actin subunits and relatively long-lived ADP · P_i-actin subunits accumulates, probably at both ends. As the monomer concentration falls and the system approaches steady state, the length of the ATP-actin string shortens and then disappears but a short ADP · P_i-actin cap remains, at least at the barbed end of the filament at steady state. As a result of ATP hydrolysis, the critical concentration at the barbed end of the actin filament in ATP is lower than at the pointed end. This would result in partial depolymerization of the filament if the barbed end were blocked by a specific barbed end–blocking protein, and this depolymerization could be very extensive in the presence of an actin monomer-binding protein. However, depolymerization would be much less in the presence of P_i, which binds to F-actin subunits and reduces but does not eliminate the difference between the critical concentrations at the two ends of the filament. In addition, the dissociation rate constant for ADP · P_i-actin from the barbed end of an ADP · P_i-actin capped filament is very much smaller than the dissociation rate constant for ADP-actin from an uncapped filament. This creates a situation of potential dynamic instability tempered by P_i binding; filaments that lose their ADP · P_i-actin cap will rapidly depolymerize while filaments that retain their ADP · P_i-actin cap will necessarily elongate to maintain the system at steady state. Thus the cleavage of ATP and the release of P_i that accompany actin polymerization and the ADP · P_i-actin cap at the end of the filament provide several potential mechanisms for regulating microfilament dynamics in nonmuscle cells. Binding of P_i to actin subunits within the body of the filament may also affect other physiological properties of actin filaments in muscle as well as in nonmuscle cells. For example, it would be interesting to investigate whether the ability of P_i to cause muscle to relax (*54*) might be due to the binding of P_i to the actin thin filaments rather than or in addition to binding to the myosin thick filaments, as suggested (*55*). The major task now before us is to discover the relevance of what is known to occur in the test tube to filament dynamics in the cell.

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