The Interaction between ATP-Actin and ADP-Actin

A TENTATIVE MODEL FOR ACTIN POLYMERIZATION*

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The involvement of interactions between ATP-actin and ADP-actin in actin polymerization has been studied. It has been found that ATP-actin and ADP-actin can copolymerize and that the rate of nucleation is enhanced when both ATP-actin and ADP-actin are present in solution. The fact that the heterogeneous interaction between ATP-actin (T) and ADP-actin (D) is stronger than either of the homologous reactions, T-T and D-D, agrees with the kinetic data in the accompanying paper (Carlier, M.-F., Pantaloni, D., and Korn, E. D. (1985) J. Biol. Chem. 260, 6565-6571) which show that filament ends having the DT conformation are more stable than those having the TT conformation. These data are incorporated into a model for actin polymerization in ATP in which the kinetic parameters for polymerization depend on the nature of the nucleotide (ADP or ATP) bound to the three terminal subunits of the actin filament.

From recent reports (1-4), our current understanding of ATP-actin polymerization can be summarized as follows. ATP hydrolysis accompanies ATP-actin polymerization but takes place on the polymer as a first order reaction following polymerization (1-3). Consequently, the growing filament consists of ADP subunits in the interior and ATP subunits near the ends forming a "cap." The size of the ATP cap increases with the rate of actin addition to filaments. At high actin concentration, ATP-actin can polymerize fast enough, under continuous sonication, for the reversible polymerization of ATP-actin to reach equilibrium before significant ATP hydrolysis occurs on the F-actin (4). The critical concentration for reversible polymerization of ATP-actin was found to be 3 μM in 1 mM MgCl₂ (4), 8-fold larger than the 0.35 μM critical concentration (3, 5, 6) measured at steady state, i.e. under conditions where ATP hydrolysis affects polymerization. Results obtained in ADP established that sonication does not affect the thermodynamic parameters of the polymerization (4). Further measurements indicated that this higher critical concentration was primarily due to the 10-fold larger dissociation rate constant of ATP-actin from the ATP polymer at equilibrium than from the polymer at steady state.

These results led to the idea that the ATP cap has a minimal size at steady state and that this small cap confers a higher stability to the F-actin than the big ATP cap present on the reversible equilibrium polymer. In other words, the interaction between a terminal ATP-actin subunit and an adjacent interior ADP subunit in the filament would be very strong (4). Further experiments to test this hypothesis are presented in this report. The data show that ATP-actin and ADP-actin copolymerize and that enhanced nucleation is observed when heterogeneous interactions between ATP-actin and ADP-actin occur, as compared to either of the homologous interactions.

These observations emphasize the importance of the nature of the nucleotide (ATP or ADP) bound to the end of actin filaments in the elongation process. The different possible combinations of nucleotide-actin subunits at the filament ends are examined in a model for actin polymerization. From the consideration of the kinetic parameters of the elementary steps, the proportion of the different types of filament ends at steady state as well as in the elongation and depolymerization reactions can be derived.

MATERIALS AND METHODS

Chemicals—[α-32P]ATP, [γ-32P]ATP, and [3H]ADP were purchased from New England Nuclear. The specific radioactivities of the nucleotides were measured on the pure fractions eluted from a Synchropak X-300 high performance liquid chromatography column (Synchron). The specific radioactivities of the [32P]ATP extracted from the ATP-G-actin were the same. Nonradioactive nucleotides are described in the accompanying paper (4).

Actin—G-actin from rabbit skeletal muscle, the 1:1 complexes of ATP-G-actin and ADP-G-actin free of unbound nucleotides, and the NBD'-labeled actin were prepared as described in the accompanying paper (4). Pyrenyl-labeled actin was prepared as described by Kouyama and Mihashi (7). All actin preparations were stored on ice in buffer G consisting of 5 mM Tris·Cl, pH 7.8, 0.2 mM diethiothreitol, 0.1 mM CaCl₂, 0.2 mM ATP, and 0.01% sodium azide.

Polymerization Studies—The polymerization of pyrenyl- and NBD-labeled Ca²⁺-G-actin and Mg²⁺-G-actin was monitored by the increase in fluorescence as described (4). The nucleotide content of buffer G was modified for each experiment. The amount of polymerized actin at steady state was determined by sedimenting F-actin at 150,000 x g for 1.5 h and measuring the concentration of G-actin in the supernatant by the method of Lowry et al. (8) with actin as standard. This determination allowed the fluorescence data to be converted into concentrations of polymerized actin. Where indicated, incorporation of labeled nucleotides was measured by resuspending the pellet of F-actin in buffer G without nucleotides, after carefully washing the walls of the tube and measuring the radioactivity.

ATPase Measurements—The hydrolysis of ATP accompanying actin polymerization was monitored by extraction of [32P]ATP using the method of Pollard and Korn (9).

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Copolymerization of ATP-Actin and ADP-Actin—Ca\textsuperscript{2+}-G-actin was equilibrated with various ratios of [\alpha\textsuperscript{32P}]ATP and [\textsuperscript{3}H]ADP, maintaining the total concentration of nucleotide constant, the actin was polymerized to steady state, and then the F-actin-bound ADP was analyzed for \textsuperscript{32P} and \textsuperscript{3}H to determine the amount of bound ADP that was derived from ATP and ADP, respectively. The data are summarized in Table I. The fraction of the F-actin-bound ADP derived from [\textsuperscript{3}H]ADP increased in proportion to the ADP/ATP ratio in solution, reaching a maximum of 0.38 mol/mol of F-actin when the 1:1 complex of [\alpha\textsuperscript{32P}]ATP-G-actin was equilibrated with 0.2 mM [\textsuperscript{3}H]ADP and no free [\alpha\textsuperscript{32P}]ATP. These results demonstrated that free ADP can partially displace G-actin-bound ATP and that ATP-G-actin and ADP-G-actin can copolymerize. It was expected, therefore, that when the 1:1 complex of ATP-G-actin was polymerized in the presence of ADP, less than 1 mol of ATP would be hydrolyzed per mol of actin polymerized. This was investigated in an experiment similar to the previous one except that the actin was 5% pyrenyl labeled, and [\gamma\textsuperscript{32P}]ATP replaced [\alpha\textsuperscript{32P}]ATP so that the rate of polymerization and ATP hydrolysis and the amount of ATP hydrolyzed and F-actin-bound ADP at steady state could be quantified. The results at steady state are shown in Table II. It was confirmed that the incorporation of [\textsuperscript{3}H]ADP into F-actin increased with the ratio of total [\textsuperscript{3}H]ADP to total [\gamma\textsuperscript{32P}]ATP in solution, and, as expected, the amount of ATP hydrolyzed varied inversely with the amount of [\textsuperscript{3}H]ADP incorporated into F-actin. The sum of ADP incorporated and ATP hydrolyzed was always very nearly 1 mol/mol of F-actin.

The time courses of polymerization and associated ATP hydrolysis are shown in Fig. 1 for the two extreme cases of Table II, the 1:1 ATP-actin complex polymerized in the presence of either 0.2 mM ATP or 0.2 mM ADP. The rate of polymerization was faster, and the lag due to nucleation was shorter, when both ATP and ADP were present than when the polymerizing solution contained only ATP. For the four samples listed in Table II, the maximum rate of polymerization was observed when the 1:1 ATP-actin complex was polymerized in the presence of free ADP only; polymerization was slower when only free ATP was added and the intermediate ratios of ADP/ATP gave rates of polymerization between the two extremes shown in Fig. 1. The same results were obtained when the G-actin was equilibrated with 50 \mu M MgCl\textsubscript{2} to convert Ca\textsuperscript{2+}-G-actin to Mg\textsuperscript{2+}-G-actin (4), before initiating polymerization.

The results in Fig. 1 were surprising because ADP-G-actin has been shown to polymerize only very slowly compared to ATP-G-actin (10) and with a very high critical concentration, 8.2 \mu M (3, 5, 10), compared to 0.35 \mu M for ATP-actin (3, 5, 6) under these conditions. Therefore, the addition of ADP might have been expected to decrease the rate of polymerization of ATP-actin and not to accelerate it.

At all times during the course of polymerization of the 1:1 complex of ATP-actin in 0.2 mM ADP, 0.65 mol of ATP was hydrolyzed per mol of actin polymerized, whereas 1 mol of ATP was hydrolyzed per mol of actin polymerized when only ATP was present in the solution (Fig. 1, inset). Also, about 20% less polymer was formed at the initial steady state in the sample containing 0.2 mM ADP than in the one containing 0.2 mM ATP (Fig. 1) and, in 0.2 mM ADP, the initial plateau was followed by a very slow depolymerization. Since only 65% of the original actin-bound ATP had been hydrolyzed when that initial plateau had been reached, the subsequent slow depolymerization must have been due to the very slow hydrolysis of the remaining 7 \mu M ATP. Hydrolysis of ATP would be expected to be very slow in the presence of a large excess (200 \mu M) of ADP (11).

### TABLE I

Copolymerization of ATP-actin and ADP-actin: incorporation of nucleotides into F-actin polymerized in the presence of [\alpha\textsuperscript{32P}]ATP and [\textsuperscript{3}H]ADP

<table>
<thead>
<tr>
<th>Nucleotide added</th>
<th>ATP (\mu M)</th>
<th>ADP (\mu M)</th>
<th>ADP incorporated into F-actin (mol/mol of F-actin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[\alpha\textsuperscript{32P}]ATP</td>
<td>200</td>
<td>0</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0.65</td>
</tr>
</tbody>
</table>

### TABLE II

Copolymerization of ATP-actin and ADP-actin: ATP hydrolysis and exogenous ADP incorporation into F-actin

<table>
<thead>
<tr>
<th>Nucleotide added</th>
<th>ATP (\mu M)</th>
<th>ADP (\mu M)</th>
<th>ADP incorporated into F-actin (mol/mol of F-actin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[\gamma\textsuperscript{32P}]ATP</td>
<td>200</td>
<td>0</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0.65</td>
</tr>
</tbody>
</table>

### RESULTS

Dependence of the Increase in the Rate of Polymerization of Actin on the Ratio of ADP to ATP—In the experiments described in Fig. 1 and Tables I and II, the initial concentrations of ATP-G-actin was about 20 \mu M and the total free nucleotide concentration was 0.2 mM. Therefore, the maximum possible ratio of total ADP to total ATP that could be obtained was only 10:1. In order to determine the optimum concentrations of both ATP and ADP needed to obtain the maximum rate of polymerization, it was necessary to investigate a broader range of ADP/ATP ratios. This was accomplished by reducing the initial concentration of the 1:1 ATP-G-actin complex.
Copolymerization of ATP-Actin and ADP-Actin

**Fig. 1.** Time course of spontaneous polymerization and accompanying ATP hydrolysis for the polymerization of ATP-G-actin complex in the presence of either free ATP or ADP. The 1:1 complex of [γ-32P]ATP-G-actin (19.6 μM containing 8% pyrenyl-labeled actin) was incubated in buffer G but with either 0.2 mM ADP or 0.2 mM [γ-32P]ATP for 2 h at 20°C to allow nucleotide equilibrium. Then MgCl2 was added to 1 mM, and polymerization was monitored by fluorescence (solid line, in ADP; dashed line, in ATP). Hydrolysis of ATP was measured in the same samples (△, ADP sample; ○, ATP sample). These are two of the four samples in Table II. The fluorescence scale was converted into concentration of polymerized actin subunits by measuring the amount of G-actin in the supernatant after sedimenting the F-actin. Inset, the ATP hydrolyzed is plotted as a function of the actin polymerized.

**Fig. 2.** Effect of the ADP/ATP ratio on the rate of polymerization of actin. The 1:1 complex of ATP-G-actin (6.3 μM, 6.5% pyrenyl labeled) was incubated in buffer G with different concentrations of ATP and ADP and 10 μM GpG, with the total concentration of ATP plus ADP kept constant at 0.2 mM. Spontaneous polymerization was started by addition of MgCl2 to a final concentration of 1 mM. The ratios of total ADP to total ATP were 0, 6.8, 11.6, 17.2, and 31.7 in samples 1–5, respectively. Inset, the concentration of polymerized actin at time 1500 s versus the ADP/ATP ratio. Data for the ratio of 22.4 are not shown in the main figure. A.U., arbitrary units.

The amount of polymer formed at the time at which nucleation was completed or by the maximum rate of elongation. The maximum enhancement of the rate of polymerization was obtained with a ratio of ADP to ATP of 12–15, with polymerization being slower at both lower and higher ratios. These results indicate that the interaction between ATP-actin and ADP-actin subunits is preferred over the homologous interactions between either ATP subunits only or ADP subunits only.

Enhancement of Nucleation by the Heterologous Interaction between ATP-Actin and ADP-Actin—That copolymerization of ATP-actin and ADP-actin is faster than polymerization of ATP-actin or ADP-actin alone could reflect differences in the rates of nucleation, elongation, or both. To compare the rates of nucleation of actin in the presence of ATP alone and ATP plus ADP, the 1:1 ATP-G-actin complex was incubated with either 0.2 mM ATP or 0.2 mM ADP, and a low concentration (0.2 mM) of MgCl2. Fig. 3a shows that while almost no change in the pyrenyl fluorescence occurred in the ATP sample for 40 min, there was a 2-fold increase in fluorescence of the ADP sample over the same time period with a nonlinear time course indicative of a nucleation reaction.

The rates of formation of nuclei in the two samples were more directly assessed by testing the ability of aliquots from each solution to act as seeds for the polymerization of 3 μM ATP-G-actin in buffer G containing 0.2 mM ATP and 1 mM MgCl2. The initial rates of elongation of G-actin subunits onto the seeds are a measure of the relative concentrations of F-actin nuclei in the samples (5, 12). The data in Fig. 3b show that an appreciable amount of nuclei were formed within 40
min in the ADP sample while no detectable nucleation occurred in the sample containing ATP. The results in Fig. 3, a and b, then, show that nucleation is facilitated by the interaction between ATP-actin and ADP-actin subunits when compared to the rate of nucleation with ATP-actin subunits alone.

To determine the reciprocal effect of ATP on the rate of nucleation of ADP-G-actin, a 1:1 ADP-G-actin complex (17.3 μM, NBD labeled) was polymerized by continuous sonication in the presence of 0.2 mM ADP and 1 mM MgCl₂, with and without 0.5 mM ATP. The length of the lag time before polymerization occurs is inversely related to the rate of nucleation (4). Sonication is required because the rate of spontaneous polymerization of ADP-actin, in the absence of ATP, is too slow to measure (10). Fig. 4 shows that the addition of the substoichiometric amount of ATP (0.5 μM ATP and 17.3 μM ADP-G-actin) caused a dramatic decrease in the lag time preceding the elongation process. As expected, the rates of polymerization after the lags were identical for the two samples because essentially only ADP-actin was polymerizing in both solutions and, once nucleation was completed, the concentration of filaments would have been the same in the two solutions under sonication (4). We conclude from this experiment that nuclei are formed more rapidly from the heterologous interaction of ATP-actin and ADP-actin molecules than from the homologous interaction between ADP-actin molecules only.

Effect of Different Concentrations of ATP and ADP on the Steady-state Critical Concentration of Actin—When actin is polymerized in the presence of 1 mM MgCl₂ and 0.2 mM nucleotide, the critical concentration is 0.35 μM with ATP (3, 5, 6) and 8 μM with ADP (3, 5, 10). Therefore, the critical concentrations of copolymers of ATP- and ADP-actin are expected to vary between 0.35 and 8 μM depending on the ATP/ADP ratio at steady state. Critical concentration curves obtained at different ATP/ADP ratios are shown in Fig. 5. In these experiments, the concentrations of MgCl₂ and nucleotides were higher than those used in the other experiments described in this paper so that, even at the lowest ATP/ADP ratio, the initial value of the ratio could be maintained throughout the incubation at all actin concentrations, despite the continued hydrolysis of ATP. This was facilitated by the fact that ADP inhibits competitively the hydrolysis of ATP by F-actin (11). That the initial ATP/ADP ratios were maintained is verified by the fact that all of the critical concentration curves are linear with respect to actin concentration and that none of the values changed between 4 and 18 h of incubation. The results in Fig. 5 show that the apparent critical concentration, C₅₀, is a decreasing function of the ATP/ADP ratio.

Wagner and Wegner (13) have shown that the equilibrium dissociation constant for the interaction between ADP and G-actin (Kₒ) is about 3-4 times greater than the constant (K₆) for the interaction of ATP and G-actin under conditions...
similar to those used in these experiments. On this basis, the partial critical concentrations of ATP-actin ($G_T$) and ADP-actin ($G_D$) can be calculated (Table III) as follows.

$$G_D = \frac{C_{\text{app}} [\text{ATP}]}{[\text{ATP}][D] + [\text{ATP}][D]}$$ (1)

$$G_T = \frac{C_{\text{app}} [\text{ADP}]}{[\text{ADP}][T] + [\text{ADP}][T]}$$ (2)

It was then found (Table III) that $G_D$ and $G_T$ are linearly correlated, as expected from the copolymerization model of Oosawa and Asakura (14),

$$\frac{G_D}{[D]} + \frac{G_T}{[T]} = 1$$ (3)

where $D$ and $T$ are the intrinsic critical concentrations for ADP-actin and ATP-actin, respectively.

**DISCUSSION AND MODEL**

The experiments presented in this paper were designed to understand the role of ATP hydrolysis in ATP-actin polymerization. Previous experiments (3) showed that there is an ATP cap at the ends of actin filaments at steady state, where the critical concentration is 0.35 μM. In the accompanying paper (4), we further showed that the reversible ATP-actin polymer (i.e. the equilibrium polymer before ATP hydrolysis occurs) has a much higher critical concentration of 3 μM; in this case the ATP cap is larger than at steady state. We concluded that the interactions between the actin subunits at the end of the filaments at steady state are different from the interactions between ATP subunits which occur in the equilibrium polymer at high actin concentration and that they confer a greater stability to the filament. These results indicated that the interface between ADP and ATP subunits, which is created by ATP hydrolysis on F-actin and which gets very close to the end of the filament as the steady state is approached, is probably very stable. This concept led us, then, to investigate more thoroughly how the heterologous interaction between ATP-actin and ADP-actin might differ from the homologous ATP-actin:ATP-actin or ADP-actin:ADP-actin interactions.

The experiments described in this paper provide more support for the proposed strong interaction between ATP-actin and ADP-actin, since they demonstrate both copolymerization of ATP-actin and ADP-actin, and more rapid nucleation when ATP-actin and ADP-actin interact than in the homologous interactions between either ATP-actin or ADP-actin alone. The observation that ATP-actin nucleates and polymerizes much more rapidly than ADP-actin (10) may be explained by the fact that ATP-actin is hydrolyzed to ADP-actin, thus providing a means for the heterologous reaction to occur. We infer from this that the T-D interaction is the preferred reaction in both the nucleation and elongation process. Presumably, therefore, when ATP-actin and ADP-actin copolymerize, an ordered DTDT polymer is transiently formed by the alternate additions of ADP-actin and ATP-actin subunits. This is suggested by the data in Fig. 1 which show that the ratio of ATP and ADP incorporated into the polymer is constant all along the polymerization curve.

It is interesting to note that, from the data thus far available, actin seems to behave differently from tubulin with regard to the interplay of nucleotides in polymerization. In contrast to the effect of ADP on actin polymerization, GDP is a potent inhibitor of nucleation and growth of microtubules, is not incorporated into microtubules polymerized in the presence of both GTP and GDP, and does not decrease the

**TABLE III**

Partial critical concentrations of ADP-G-actin and ATP-G-actin as a function of the ATP/ADP ratio at steady state.

<table>
<thead>
<tr>
<th>ATP/ADP</th>
<th>$C_{\text{app}}$</th>
<th>$G_D$</th>
<th>$G_T$</th>
<th>$(G_D/D_c) + (G_T/T_c)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td>μM</td>
<td>μM</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.35</td>
<td>0.35</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>0.125</td>
<td>0.43</td>
<td>0.09</td>
<td>0.32</td>
<td>0.93</td>
</tr>
<tr>
<td>0.05</td>
<td>1.7</td>
<td>1.41</td>
<td>0.28</td>
<td>1.12</td>
</tr>
<tr>
<td>0.02</td>
<td>2.5</td>
<td>2.3</td>
<td>0.20</td>
<td>0.93</td>
</tr>
<tr>
<td>0.001</td>
<td>5.97</td>
<td>0.03</td>
<td>1.02</td>
<td></td>
</tr>
</tbody>
</table>

The data for the apparent critical concentrations ($C_{\text{app}}$) were obtained from Fig. 5. The partial critical concentrations of ADP-G-actin ($G_D$) and ATP-G-actin ($G_T$) were calculated from these data according to Equations 1 and 2, assuming a ratio of 4 for the ratio of the dissociation constants for the interaction of ADP and ATP with G-actin (15) in the polymerization buffer. The value for $(G_D/D_c) + (G_T/T_c)$ (see text) was calculated using values of 0.35 and 6.4 μM for the intrinsic critical concentrations of G-actin in ATP ($T_c$) and ADP ($D_c$), respectively.

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**FIG. 5** Change in the apparent critical concentration for actin polymerization as a function of the ATP/ADP ratio. The 1:1 complex of ATP-G-actin (ATP; 4.4% pyrenyl-labeled actin) was polymerized in buffer G and 1.5 mM MgCl₂ in the absence of free nucleotide. When the maximum fluorescence was reached, the solution was divided into several samples which were supplemented with different amounts of ATP and ADP (total nucleotide concentration = 0.5 mM). Dilutions of each sample were then made into the corresponding buffer. Fluorescence was read after 4 and 18 h. The ATP/ADP ratios are as follows: x, only ATP added; Δ, 1; ○, 0.125; ●, 0.05; ▽, 0.02; □, only ADP added.
amount of GTP hydrolyzed per polymerized tubulin (15-19).

The data in this and the preceding paper (4) allow us to propose the following model (20). From structural studies, the actin filament is most probably a two-start double-strand right-hand helix, in which lateral interstrand bonds are stronger than the bonds parallel to the axis (21). This is equivalent to a one-start left-hand helix with 2 subunits/turn (actually 2.16 subunits/turn) as illustrated in Fig. 6. The subunit at position n (Fig. 6) interacts strongly with neighbors at positions n – 1 and n + 1 and more weakly with subunits at positions n – 2 and n + 2. From a general point of view, whether these neighbors carry ATP or ADP may affect the rate of ATP hydrolysis on the subunit at position n.

Sixteen possibilities could be considered. However, in the simple model that we consider here, we will deal only with a polymer growing in the presence of ATP and which consists essentially of ADP subunits, except for the subunits located at the ends. In such a polymer, only the three terminal subunits have unique environments. The last subunit (n = 1) interacts with two neighbors and has two unoccupied sites, the penultimate subunit (n = 2) interacts with three other subunits (n = 1, n = 2, n = 3), while the antepenultimate subunit (n = 3) interacts with four neighbors and, therefore, is similar to all of the more internal subunits. A symmetrical pattern occurs at the opposite end, except that the complementary areas are exposed. But, for simplicity, we consider only one end, say the barbed end, which is the fastest growing end, and, therefore, the main polymerizing unit in vitro. Since only ATP-actin monomers add to the filament and ATP hydrolysis on the filament is likely, in order of subunit addition, it is highly improbable that a T subunit will be interior to a D subunit. Therefore, we will consider only four possible conformations of filament ends, ...DDD, ...DDT, ...DTT, and ...TTT, which are named Fl, F2, F3, and F, respectively, according to their content of T subunits.

The fact that the plot of the rate of elongation of actin filament as a function of actin concentration exhibits discontinuities at 0.35 and 3 μM (3, 4) clearly indicates that the ATP and ADP composition of the filament ends depends on the G-actin concentration. Fig. 7 describes the relations between the four possible states of the filament ends. The relative proportions of Fl, F2, F3, and F depend on the concentration of ATP-G-actin. Equilibrium reactions exist between Fl and F, F2 and F3, and dissociation of D subunits from F2 represents the net depolymerization pathway of F-actin. Fl became the predominant species below 0.35 μM G-ATP actin. F2 and F3, in which D-T interactions take place, are the predominant species between 0.35 and 3 μM G-ATP-actin. F3 begins to be important above 3 μM G-ATP-actin, and, at the same time, the size of the ATP cap increases. Two different polymerization pathways A and B, are represented by the curved arrows in Fig. 7. The addition of a T subunit to an F2 filament generates an F3 end. Two possibilities then exist for the next transition; either ATP is hydrolyzed, which regenerates an F2 end but adds one D subunit to the filament (pathway A), or F3 binds another T subunit, thus remaining F2 but with the addition of one T subunit (pathway B). Pathway A represents a polymerization reaction with coupled ATP hydrolysis. Pathway B represents the reversible polymerization of ATP-actin uncoupled from ATP hydrolysis. The relative importance of these two pathways in the overall polymerization process depends on the concentration of monomeric ATP-actin. Pathway B is greatly favored at high actin concentration. The validity of this model must now be assessed and the value of the yet unknown kinetic parameters determined by quantitative fitting to the data for polymerization and ATP hydrolysis.

REFERENCES
Copolymerization of ATP-Actin and ADP-Actin