Published in final edited form as:

Phys Biol. ; 7(1): 16008. doi:10.1088/1478-3975/7/1/016008.

Actin Polymerization Overshoots Induced by Plus-end Capping

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Abstract

Transient polymerization beyond the steady-state has been experimentally observed in *in vitro* actin polymerization time courses. These “polymerization overshoots” have previously been described in terms of the time-dependent probabilities for binding distinct nucleotide hydrolysis states within subunits near the plus ends of actin filaments. We demonstrate a different type of overshoot dynamics where the plus-end contribution to polymerization steadily decreases relative to that of the minus end. This decrease occurs due to plus-end capping of an initial impulse of rapidly created actin filaments. We calculate the contribution of these dynamics to observed overshoot magnitudes using rate equations describing the concentration of polymerized actin. We find this contribution is strongly sensitive to both initial filament concentration and plus-end capping rate. We develop an analytic formula that describes the magnitude of the overshoot as a function of these two key parameters. The overshoots we describe could be observed by current experimental methods for studying the effects of severing and branching mechanisms upon actin polymerization in the presence of plus-end capping and rapid nucleotide exchange. We also present a plausible cellular mechanism that could greatly amplify these overshoots *in vivo*.

Keywords

non-equilibrium polymerization; capping protein; rate equations; actin dynamics; ATP hydrolysis; seed filaments

Introduction

Actin is a globular protein found in eukaryotic cells that can polymerize into dense networks of filaments and other structures such as bundles [1]. The extent of actin polymerization is regulated by a number of actin-binding proteins [2]. *In vitro* polymerization time courses are a useful tool for researchers studying the effects of isolated actin-binding proteins upon polymerization. During these types of polymerization experiments, some researchers have observed polymerization that overshoots the steady-state concentration [3,4,5,6,7]. An experimentally measured polymerization overshoot is shown in Figure 1 where it is seen that the maximum polymerized fraction is $\approx 30\%$ higher than the final fraction. We note here that many of the overshoots reported in the literature were assayed via pyrene fluorescence. Therefore, the actual overshoot in polymerized actin is likely *greater* than what was reported since it has been shown that the pyrene assay diminishes actin polymerization overshoots [8].

Actin molecules bind nucleotide in various states of hydrolysis: the adenosine triphosphate (ATP) state, the adenosine diphosphate (ADP) state, or at least one intermediate state [9,10].

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PACS numbers: 87.15.rp

[‡]The plus end is also commonly referred to as the “barbed” end while the minus end is referred to as the “pointed” end.

Since the higher-free-energy ATP state polymerizes more readily than the lower-free-energy ADP state, one may intuitively conclude that a contribution to overshoot dynamics results from the spontaneous lowering of free energy via nucleotide hydrolysis (ATP→ADP). In fact, it has been explicitly demonstrated that such polymerization overshoots occur in the absence of excess ATP [3]. These overshoots have since been described for more general conditions in terms of time-dependent changes in the hydrolysis state of the bound nucleotide within polymerized subunits located near the plus ends of actin filaments [11]. Actin filaments are polarized in the sense that they have distinct plus and minus ends[‡], each with differing monomer-filament binding properties [12]. In general, the plus ends of actin filaments are more dynamic than the minus ends [1]. The framework of describing polymerization dynamics in terms of changes in the probability of plus-end subunits binding a nucleotide in a particular hydrolysis state extends the description given in [3] to account for presence of excess ATP in solution which provides a continuing input of chemical energy that partially offsets the spontaneous lowering of free energy via hydrolysis.

As filaments age, hydrolysis of bound nucleotide occurs within polymerized subunits regardless of the rate of nucleotide exchange in the solution [13]. Because of the slower dynamics, however, subunits at the minus end have a longer time to undergo hydrolysis before a new ATP-bound monomer can associate. This makes the minus end more ADP-like, raising the minus-end critical concentration (the concentration of monomers such that net polymerization spontaneously ceases). The inequality of the critical concentrations at each filament end leads to the well-known phenomena of filament “treadmilling” in which the ATP-like plus-ends grow while the ADP-like minus ends shrink while the average filament length remains constant [14]. Without nucleotide exchange to maintain the higher-free-energy, ATP-like state at the plus ends, the polymer would spontaneously decay to an entirely ADP-bound state and treadmilling would cease. The overshoots modeled in [8,11] occur with a nonzero nucleotide exchange while that described in [3] occurs as the nucleotide exchange approaches zero. In both cases, the short-time polymerization dynamics must be much faster than hydrolysis. Otherwise, there could be no transient maximum above the steady-state polymerization determined by the relative contributions of ATP-bound actin versus ADP-bound actin.

The curtailing of polymerization via plus-end capping is a tenet of the Dendritic Nucleation Model of actin polymerization against a cellular membrane [2]. By mutating specific domains within plus-end capping proteins, one can vary their activity *in vitro*. This changes the rate at which capping proteins bind filaments and block further polymerization [15,16]. Increased capping protein activity decreases the fraction of uncapped plus ends, thus inhibiting polymerization. Since rapid polymerization dynamics are required for overshoot behavior, one may naïvely conclude that overshoots cannot occur in the presence of significant plus-end capping. The inclusion of plus-end capping into previous overshoot models has been shown to reduce the overshoot magnitude [11]. Still, overshoots in the polymerized actin concentration have been observed in living cells [17,18] where the extent of plus-end capping is believed to be significant [2]. Therefore, we feel it is important to elucidate all mechanisms that feasibly can lead to polymerization overshoots in the presence of plus-end capping. Changes in the plus-end capping state modify the relative contributions of the plus and minus ends to the net polymerization. In other words, the uncapped fraction is a means of switching between differing free-energy steady-states and could thus induce dynamic polymerization effects such as overshoots. In this work, we demonstrate how polymerization can increase to a transient maximum under conditions where the concentration of uncapped plus ends is steadily *decreasing* and the hydrolysis state of the nucleotide bound within those plus-ends is uniform.

[‡]The plus end is also commonly referred to as the “barbed” end while the minus end is referred to as the “pointed” end.

These overshoot effects could be observed during *in vitro* experiments using current methods and may be present in cells as well.

Theory

We model actin filaments in a solution of entirely ATP-bound monomers. All filament plus-ends are assumed to be ATP-bound while the minus ends can have a significant ADP-bound contribution which, as described in the Introduction, leads to a higher critical concentration. We assume that all minus ends are uncapped but plus ends can be either capped or uncapped. The polymerization is shifted from that dominated by minus ends to that dominated by plus ends via regulation of the fraction of uncapped plus ends. We assume that the rates of (un) capping are independent of time. The plus-end capping state may be modeled as the simple two-state system

of concentrations of filaments with capped (C) or uncapped (U) plus ends. At steady state, the net rate of filament uncapping must equal the net rate of filament capping: $k_{unc}^+ C = k_{cap}^+ U$. The steady-state uncapped fraction (ζ^+) is then given by

$$\zeta^+ \equiv \frac{U}{C+U} = \frac{k_{unc}^+}{k_{cap}^+ + k_{unc}^+}. \quad (1)$$

Effect of ζ^+ upon Polymerization

The rate equation describing the polymerization of an initial concentration of ATP-bound actin (G_0) is straightforward and appears in several textbooks [19,20]. Here, the effect of a fraction of the seed filaments being capped at the plus end is included. Thus, the change in the concentration of polymerized actin (F) with time is given by

$$\frac{dF}{dt} = (k_{on}^+ G - k_{off}^+) U + (k_{on}^- G - k_{off}^-) N, \quad (2)$$

where G is the concentration of monomeric actin available for polymerization. The monomer association and subunit dissociation rates are k_{on} and k_{off} , respectively, and the superscripts indicate the plus (+) or minus (−) end of the filament. Here, N is the total number of filaments and is employed as shown because all minus ends are assumed to be uncapped. The critical concentration is the concentration of monomeric actin such that the net rate of actin polymerization is zero. The critical concentration as a function of ζ^+ is computed from rearrangement of equations 1 and 2 with $dF/dt = 0$ [21]:

$$G_c(\zeta^+) = \frac{\zeta^+ k_{off}^+ + k_{off}^-}{\zeta^+ k_{on}^+ + k_{on}^-}. \quad (3)$$

For finite $k_{cap}^+ > 0$, the steady-state ζ^+ is between zero and unity. At $\zeta^+ = 0$, all plus ends are capped and the critical concentration is that of the minus ends alone

$G_c^- = k_{off}^- / k_{on}^- \approx 0.6 \mu\text{M}$, all plus ends are uncapped and critical concentration is that of a combination of plus and minus ends

$$G_c^{+/-} = \frac{k_{off}^+ + k_{off}^-}{k_{on}^+ + k_{on}^-} \approx 0.2 \mu\text{M}. \quad (4)$$

Thus, $G_c^{+/-} \leq G_c(\xi^+) \leq G_c^-$ and the maximum possible concentration of polymerized actin is $G_0 - G_c^{+/-}$.

We have previously modeled actin polymerization using detailed stochastic-simulation methods [8] and more complex rate equations [11]. These methods improve accuracy if it is necessary to treat filament disappearance or changes in the hydrolysis state of ATP-bound nucleotide within filament ends. However, in the present case of constant filament number and fixed association and dissociation rates, 2 is equally accurate.

An Overshoot can be Caused by Plus-end Capping

We define the overshoot magnitude, if any, as the difference ΔF between the peak polymerization and steady-state value of a single time course. One readily shows that when k_{cap}^+ is either very large or very small, ΔF vanishes. If k_{cap}^+ is very small, most of the new (seed) filaments reach a steady-state of polymerization near the maximum possible value,

$G_0 - G_c^{+/-}$. Then ΔF must be small. Therefore as $k_{cap}^+ \rightarrow 0$, $\Delta F \rightarrow 0$. If k_{cap}^+ is very large, any initially uncapped filaments are instantly capped and polymerization proceeds monotonically to $G_0 - G_c^-$. Thus, $k_{cap}^+ \rightarrow \infty$ implies $\Delta F \rightarrow 0$ as well. If we now consider an intermediate value of k_{cap}^+ , polymerization can proceed from the active plus ends before they become capped. At the beginning of the polymerization time course, both the free actin concentration and fraction of uncapped filaments are greatest. Thus, the net polymerization rate is maximal. If the time required to nearly complete polymerization is less than the time required for capping to occur, polymerization will first proceed close to $G_0 - G_c^{+/-}$ but then ultimately drop to $G_0 - G_c(\zeta^+)$. Because ΔF vanishes at both large and small k_{cap}^+ , there should exist a critical value (k_{cap}^{+c}) that maximizes ΔF .

The finding of an overshoot parallels the overshoot-type behavior described in [8,11] and observed in many polymerization experiments [3,4,5,6,7] where the final polymerized actin concentration is lower than a transient maximum. We note, however, that those overshoots have been shown to result from time-dependent changes in the hydrolysis state of the bound nucleotide within filament plus ends while the effect we describe here occurs in the absence of such changes. The overshoot we describe results from a plus-end capping-state change that necessarily changes the relative contributions of the filament ends which each have distinct critical concentrations.

Calculation of the Overshoot Magnitude

We now calculate the ΔF that results from plus-end capping occurring at a constant rate (k_{cap}^+). We assume that all plus ends are uncapped at $t = 0$. The polymerization of an initial concentration of ATP-actin (G_0) from a fixed concentration of seed filaments (N) is given by 2. In this case, however, the concentration of uncapped filaments (U) is a function of time. Since the total number of filaments remains constant,

$$\frac{dU}{dt} = k_{unc}^+ (N - U) - k_{cap}^+ U. \quad (5)$$

We note that 5 is independent of the concentration of polymerized actin and thus may be solved immediately:

$$U(t) = N\xi^+ + N(1 - \xi^+) e^{-(k_{unc}^+ + k_{cap}^+)t}. \quad (6)$$

We then use this expression in 2. The resulting equation may be significantly simplified by making the variable substitution $\Phi \equiv k_{on}^+ (G_0 - F) - k_{off}^+$. Upon substitution and simplification, 2 becomes:

$$\frac{d\Phi}{dt} + N(k_{on}^+ \xi^+ + k_{on}^-) \Phi + N k_{on}^+ (1 - \xi^+) e^{-(k_{unc}^+ + k_{cap}^+)t} \Phi = N\lambda \quad (7)$$

where $\lambda = k_{on}^+ k_{off}^- - k_{on}^- k_{off}^+$. We note that although 7 is a linear ordinary differential equation, the presence of non-constant coefficients implies that a closed-form solution, $\Phi(t)$, may not be readily accessible. In fact, solution of this equation via a computer algebra system yields an unwieldy expression involving the integration of nested exponentials. However, we need the solution at only two times. The first is at $t = \tau$, which we define to be the time where the maximum polymerization occurs. The second is $t \rightarrow \infty$, corresponding to the steady-state polymerization. At both of these times, $d\Phi/dt = 0$. From 7,

$$\Phi_\tau = \frac{\lambda}{(k_{on}^+ \xi^+ + k_{on}^-) + k_{on}^+ (1 - \xi^+) e^{-(k_{unc}^+ + k_{cap}^+)\tau}} \quad (8)$$

and

$$\Phi_\infty = \frac{\lambda}{k_{on}^+ \xi^+ + k_{on}^-}. \quad (9)$$

The magnitude of the overshoot, ΔF is then given by

$$\Delta F = \frac{\Phi_\tau - \Phi_\infty}{k_{on}^+} = \frac{\lambda(1 - \xi^+) e^{-(k_{unc}^+ + k_{cap}^+)\tau}}{(k_{on}^+ \xi^+ + k_{on}^-)^2 \left(1 + \frac{1 - \xi^+}{k_{on}^- / k_{on}^+ + \xi^+} e^{-(k_{unc}^+ + k_{cap}^+)\tau}\right)} \quad (10)$$

It is important to point out that the polymerization overshoots described by 10 are intrinsically non-equilibrium phenomena. If the actin being considered were an equilibrium polymer, G_c^+ would equal G_c^- , which would imply that $\lambda = 0$ and thus $\Delta F = 0$. Additionally, we note that our expression for ΔF has the appropriate limits. As $\tau \rightarrow \infty$, *i.e.*, as polymerization becomes so slow that filament elongation cannot occur before capping, $\Delta F \rightarrow 0$. If $\tau \rightarrow 0$, polymerization is so rapid as to instantaneously achieve the maximum value possible but then drop to the steady-state value for that particular k_{cap}^+ . Thus, as $\tau \rightarrow 0$, $\Delta F \rightarrow G_c(\xi^+) - G_c^{+/-}$. As $\xi^+ \rightarrow 1$,

$\Delta F \rightarrow 0$. This is because $\zeta^+ \rightarrow 1$ implies that no filaments are capped so the final polymerization is the maximum possible polymerization, $G_0 - G_c^{+/-}$.

Critique of the Model

The model of the overshoot magnitude we present in 10 rests upon three key assumptions. The first is that after the initial, sudden creation of filaments, the filament concentration remains constant. Since both the rates of *spontaneous* nucleation [4] and severing [22] are very low, we may assume that no new filaments are created during the rapid elongation phase immediately after filament creation. Also, since polymerization during this time is rapid, we may assume that the newly created filaments are not likely to completely depolymerize. Taken together, these facts imply that the filament concentration is reasonably constant over the polymerization time course we model here. The second assumption is that the rate of plus-end capping is constant. Since the number of capping proteins available in real cells [23] is much greater than the number of free filament ends located in a localized region near the cell membrane [17], we feel the assumption of a reservoir quantity of capping protein is valid *in vivo*. As discussed in a later section, depletion of capping protein effects could become significant *in vitro*, however. Lastly, we neglect the effect of the hydrolysis of the plus-end subunits. This effect can be important in some cases, depending upon the values of k_{cap}^+ and N . We discuss the relative contribution of plus-end nucleotide hydrolysis to the overshoot for experimentally accessible parameter values in the Discussion section.

Numerical Results

In order to verify the theoretical predictions of the preceding section, we created polymerization time courses over ranges of N and k_{cap}^+ values, with each range spanning several orders of magnitude. The individual time courses were generated by numeric integration of equations 2 and 5 with a time step of 5×10^{-4} s. The numerical values of the rate constants used in our calculation are given in Table 1. Each run began with a $1.2 \mu\text{M}$ concentration of G-actin added to a given concentration of free plus and minus ends (*i.e.*, zero-length seed filaments). Several polymerization time courses are shown in Figure 2 where it is seen that the overshoot magnitudes depend strongly upon both k_{cap}^+ and N . The arrows indicate the final polymerized fraction for time courses exhibiting the slowest dynamics. In Figure 2a, the curve for the slowest capping rate (solid) gives a small overshoot, which decays over a long time. In Figure 2b, the curve for the slowest capping rate (solid) gives a larger overshoot, which decays more rapidly. Time courses for the same conditions but much larger k_{cap}^+ values (dotted), yield clear overshoots, but with small magnitude. Time courses corresponding to intermediate values of k_{cap}^+ (dot-dashed) yield the largest overshoots, where polymerization near the maximum possible value decays to near the minimum possible value. These results illustrate plausible overshoot scenarios that do not require a change in the plus-end hydrolysis state.

When computing ΔF_{num} over the mesh of (N, k_{cap}^+) coordinate points, polymerization (numeric integration) was allowed to continue until a maximum in polymerization was reached. The steady-state polymerization was then calculated from equations 1 and 3, and ΔF_{num} was taken as the difference between the polymerization at the maximum and that at steady-state. This result is plotted in Figure 3a where, as predicted, for a large range of N values, there exists a critical k_{cap}^{+c} that yields a maximal polymerization response. From Figure 3a it is also seen that for many experimentally accessible combinations of k_{cap}^+ and N , there is no visible overshoot, while for other, equally accessible combinations, there is a large overshoot. For the entire range of N values, a diminishing overshoot magnitude is observed for low values of k_{cap}^+ . For higher

values of k_{cap}^+ , a precipitous drop in overshoot magnitude is seen to follow an approximately linear curve through the Nk_{cap}^+ -plane. This behavior is more clearly seen in Figure 4, which shows the two-dimensional projection onto the Nk_{cap}^+ -plane. For values of k_{cap}^+ greater than $\approx (0.016 \text{ nM}^{-1}\text{s}^{-1}) \times N$, the magnitude of the overshoot due to sudden changes in the uncapped fraction of plus ends is zero.

Approximation of the Time to Maximal Overshoot

To predict the result of a particular experiment, it is useful to have an explicit formula for the overshoot magnitude. Equation 10 is such a formula but in order to evaluate it we need an expression for τ , the time at which the maximum polymerization occurs during a single time course. From the time courses shown in Figures 2, that time appears to depend strongly on N but much less strongly on k_{cap}^+ . From the results of the numeric integration described above, we obtained the k_{cap}^{+c} that maximized the overshoot for each N . The observed value of τ (diamonds) at k_{cap}^{+c} is plotted versus N on a log-log scale in Figure 5. For constant values of $k_{cap}^+ = 0.001 \text{ s}$ (squares) and $k_{cap}^+ = 0.1 \text{ s}$ (triangles), τ versus N is also seen to follow a line of slope ≈ -1 . We thus assume that $\tau \propto 1/N$. This seems reasonable since, from 2, it is clear that the net polymerization rate depends linearly on N , and a faster polymerization rate intuitively implies a decreased time to maximum polymerization. We computed a theoretical overshoot magnitude (ΔF_{th}) by inserting $\tau \propto (1/N)$ into 10. We chose the constant of proportionality such that the fractional error between ΔF_{th} and the overshoot ΔF_{num} resulting from numerical integration of equations 2 and 5 is minimized. This was done by defining the fractional error over the entire range of N and k_{cap}^+ values to be

$$\text{error} = \sqrt{\frac{\sum_{m=1}^M (\Delta F_{th}(m) - \Delta F_{num}(m))^2}{\sum_{m=1}^M (\Delta F_{num}(m))^2}}, \quad (11)$$

where each m represents one coordinate combination, (N, k_{cap}^+) . Since the net polymerization rate depends upon G_0 (2), we expect τ to have some dependence on G_0 as well. We therefore repeated this error-minimization process for several experimentally accessible G_0 values. The results are shown in Table 2. We find that

$$\tau = \frac{(0.23 \text{ } \mu\text{M} \cdot \text{s}) \ln(G_0/0.23 \text{ } \mu\text{M})}{N} \quad (12)$$

approximates the time to maximal polymerization for various G_0 to within a fractional error of 2%. Figure 3b shows the results of the analytic theory for $G_0 = 1.2 \text{ } \mu\text{M}$. The surface plot of ΔF_{th} agrees with ΔF_{num} to within 4% total fractional error across the mesh. Since employing this approximation in 10 for various G_0 results in total fractional errors between ΔF_{th} and ΔF_{num} of no more than 6%, we take these results as evidence of the accuracy of the $1/N$ dependence of τ .

Discussion

By exploring the consequences of the non-equilibrium nature of the actin polymer in the presence of rapid nucleotide exchange, we have developed a theory describing polymerization dynamics due to a decrease in the plus-end uncapped fraction. We derived an expression for the height of the polymerization overshoots in terms of the capping rate and the filament

concentration. The overshoot mechanism we present is distinct from the previously established mechanism. Those earlier models required a time-dependent change in the hydrolysis state of the bound nucleotide within the plus-ends in order for any overshoot to occur. Furthermore, the inclusion of plus-end capping in those models reduces overshoot magnitudes while progressive increase of plus-end capping eliminates overshoots altogether [4,11]. The mechanism we present in this work is thus more consistent with the observation that polymerization overshoots do occur in *in vivo* experiments [17,18] where plus-end capping is believed to be both rapid and extensive [2]. To be clear, this overshoot-via-plus-end-capping mechanism still requires hydrolysis. Without hydrolysis, there cannot be differing critical concentrations which are necessary for different levels of polymerization. The overshoots we describe result from changes in the relative contributions of the thermodynamically different filament ends while those described previously result from chemical changes at only the plus end.

Relevance to In Vitro Experiments

We feel that measurement of the effects we describe, over some of the parameter range that we explore, should be experimentally feasible. The main difficulty lies in distinguishing the the capping contribution we model here from that resulting from a change in the hydrolysis state of uncapped plus ends described in [11]. A filament long enough to undergo multiple severing events likely is old enough such that it comprises mainly ADP-bound actin. Thus, newly created filament tips are ADP-like. Since the rate of polymerization at free filament ends is proportional to the concentration of free actin (2), and since the dynamics at the plus ends are so much faster than at the minus ends [1], it is likely that ATP-bound actin “caps” rapidly form at the end of the newly created filaments [25,26]. Thus, we envision a starting filament state of ADP-like minus ends with ATP-like plus ends. If the capping process occurs faster than the hydrolysis of the plus ends, one would expect that the contribution to the overshoot from the uncapping fraction, that we describe here, dominates the contribution from the change in plus-end hydrolysis state described in [11]. The time required for hydrolysis of the plus ends is at least $1/k_{hyd} \approx 3$ s [13]. This time required to hydrolyze a significant fraction of the plus ends can be increased by the continued addition of new ATP-monomers. If, for example, this time actually is ~ 10 s, we would expect that for capping rates substantially greater than 0.1 s⁻¹, the capping contribution to the overshoot is dominant. For a capping protein-filament association rate of $8 \mu\text{M}^{-1}\text{s}^{-1}$ [4], a capping protein concentration in excess of 15 nM would be sufficient. As seen in the projection of ΔF (Figure 4), a correspondingly high concentration of seed filaments (> 10 nM) also would be required. As the projection shows, our theory predicts a substantial overshoot for these parameters due to changes in the uncapped fraction.

For other capping protein concentrations, or for potentially greater accuracy, the distinct contributions of capping and hydrolysis may be separated as follows. Since we model only the polymerization that occurs after the appearance of newly-created uncapped filament ends, polymerization should be stimulated via seed filaments that are as short as possible. One means of accomplishing this quickly is via sonication of the existing filaments [27]. We stress that here, we describe only an initial burst of sonication and not continuous sonication during polymerization as described in [27]. By employing the polymerization data analysis technique described in [11], the concentration of free plus ends in a typical solution of such filaments may be reasonably estimated. With that concentration known and fixed, ATP-bound G-actin and capping protein consistent with the k_{cap}^+ values given in Figure 4 may be added to the seed filaments. A rate of nucleotide exchange consistent with that given in [11] should be maintained via the addition of excess ATP in buffer. If the concentration of seed filaments is much greater than the capping protein concentration, significant depletion could occur as capping protein associates with filaments. This would serve to reduce the net plus-end capping rate, which we have assumed to be constant. Therefore, it is desirable for the capping protein concentration

to be maintained in a drip-type fashion. The measured ΔF , however, will have contributions from both capping and hydrolysis of plus ends. In [11], the rate-equation theory describing overshoots resulting solely from changes in the plus-end hydrolysis state was extended to include the effects of plus-end capping. That theory, which was shown to agree well with sophisticated stochastic simulations, can predict the time course that results in the *absence* of uncapped minus-ends. The ΔF obtained from that theory may then be subtracted from the measured ΔF , thus yielding the contribution of the shift toward minus-end dynamics induced by a change in the plus-end uncapped fraction. This experiment could be repeated for several values of k_{cap}^+ to discover if that minus-end contribution follows the qualitative behavior presented in Figure 3: a steady increase followed by a sharp decrease in the overshoot magnitude with increasing plus-end capping rate.

Relevance to Biological Cells

The change in monomeric actin concentration which corresponds to the *in vitro* overshoot we describe above may yield a greatly amplified overshoot *in vivo*. Such an amplification mechanism could work as follows. It is well-known that actin forms a non-polymerizing 1:1 complex with β -thymosins [1]. It is believed that the function of this sequestration is to maintain the reservoir of ATP-bound actin necessary for rapid polymerization responses to external signaling [1,2]. For simplicity, we only consider one hydrolysis state of bound nucleotide and one type of sequestering protein. Since the total amount of actin in the system is constant, the actin can only be in one of three polymerization states: monomeric (G), filamentous (F), or complexed with sequestering protein (C). The difference between maximum polymerization—which we assume occurs at a time τ —and steady-state polymerization is then $\Delta F = F_\tau - F_\infty = G_\infty - G_\tau + C_\infty - C_\tau$. We assume that the dynamics associated with sequestering are much faster than the polymerization dynamics. Then, we may presume that at all times free β -thymosin (T) and G are in dynamic equilibrium with the C protein complex with the dissociation constant given by $K_d = G \cdot T/C$. Upon rearrangement of the previous two equations, one can obtain

$$\Delta F = -\Delta G + \frac{1}{K_d} (G_\infty T_\infty - G_\tau T_\tau). \quad (13)$$

This polymerization response may be estimated as follows for a cell. First, the concentration of β -thymosin is on the order of hundreds of micromolar [12]. Thus, even if all monomeric actin were complexed and then suddenly uncomplexed, the total concentration of sequestering protein remains unchanged (to within an order-of-magnitude). There are various types of β -thymosins but the K_d for the actin-thymosin complex has been measured to be ~ 0.1 – $1 \mu\text{M}$ [28]. As seen in Figure 4, $0.1 \mu\text{M} < \Delta F < 0.45 \mu\text{M}$, which corresponds to a $\Delta G \sim -0.1 \mu\text{M}$. We then can estimate the order of magnitude of the *in vivo* overshoot to be

$$10 \mu\text{M} < \Delta F \sim -\Delta G \frac{T}{K_d} < 100 \mu\text{M}. \quad (14)$$

In short, the constraint of maintaining dynamic equilibrium between β -thymosin, free actin and the β -thymosin-actin complex actin forces uncomplexed actin onto existing filaments. This amplifies the change in critical concentration by a factor of $\sim T/K_d$. Thus there exists a plausible mechanism for the small overshoots that we describe to result in very large overshoots in polymerized actin measured in real cells.

Acknowledgments

We would like to thank Shandiz Tehrani for generously sharing his experimental data. This work was supported by the National Institutes of Health under Grant R01-GM086882.

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Figure 1.

A polymerization time course assayed via pyrene fluorescence exhibits a maximum polymerization that is 30% higher than the final polymerized fraction. Such “polymerization overshoots” have been shown to be the result of the rapid polymerization of non-equilibrium actin [8,11]. These data were generously provided by Shandiz Tehrani of the Cooper Lab at the Washington University in St. Louis School of Medicine.

Figure 2.

Typical polymerization overshoots resulting from a change in plus-end capping. Arrows indicate the steady-state polymerized fraction of the relatively slower-evolving time courses (solid). Time courses were generated via numeric integration of equations 2 and 5 with $G_0 = 1.2$ μ M and rate constant values given in Table 1. A) $N = 10$ nM new filament ends. Solid/arrow: $k_{cap}^+ = 0.001$ s $^{-1}$; Dot-dashed: $k_{cap}^+ = 0.01$ s $^{-1}$; Dotted: $k_{cap}^+ = 0.1$ s $^{-1}$ B) $N = 100$ nM new filament ends. Solid/arrow: $k_{cap}^+ = 0.01$ s $^{-1}$; Dot-dashed: $k_{cap}^+ = 0.1$ s $^{-1}$; Dotted: $k_{cap}^+ = 1.0$ s $^{-1}$.

Figure 3.

The polymerization overshoot magnitude as a function of N and k_{cap}^+ . A) Results of numerically integrating equations 2 and 5. B) The formula given in 10 using $\tau \approx (0.38 \mu M \cdot s)/N$ as given by 12 for $G_0 = 1.2 \mu M$. The total fractional error (11) between the two surfaces is $\approx 4\%$.

Figure 4.

A projection of the ΔF shown in Figure 3 onto the Nk_{cap}^+ -plane. It illustrates for what experimental parameters significant contributions to the polymerization overshoot result from a sudden change in the plus-end uncapped fraction.

Figure 5.

The time (τ) at which maximum polymerization during a single time course is shown as a function of the concentration of newly created filament ends (diamonds) where k_{cap}^+ is set equal to the critical capping rate that maximizes the overshoot magnitude. The linear slope of approximately unity on this log-log scale implies that $\tau \propto (1/N)$. For comparison, the same time is shown for other capping rates: $k_{cap}^+ = 0.001 \text{ s}^{-1}$ (triangles); $k_{cap}^+ = 0.1 \text{ s}^{-1}$ (squares).

Table 1

Values of all rate constants used in our calculations

Symbol	Rate Constant	Value	Reference
k_{on}^+	Plus end monomer association	$11.6 \mu\text{M}^{-1}\text{s}^{-1}$	[24]
k_{on}^-	Minus end monomer association	$1.3 \mu\text{M}^{-1}\text{s}^{-1}$	[24]
k_{off}^+	Plus end monomer dissociation	1.4s^{-1}	[24]
k_{off}^-	Minus end monomer dissociation	0.8s^{-1}	[24]
k_{cap}^+	Plus end capping	$10^{-5} - 10^1 \text{s}^{-1}$	[4]
k_{unc}^+	Plus end uncapping	$4 \times 10^{-4} \text{s}^{-1}$	[4]

Table 2Dependence of τ upon G_0

$G_0 = 1.2 \mu\text{M}$	$\tau = (\text{const } \mu\text{M}\cdot\text{s})/N$	Total error in ΔF
1x	0.36	4 %
2x	0.54	3 %
5x	0.75	4 %
10x	0.90	5 %
25x	1.1	6 %