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REVIEW ARTICLE

Control of Actin Dynamics in Cell Motility

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Actin polymerization plays a major role in cell movement. The controls of actin sequestration/desequestration and of filament turnover are two important features of cell motility. Actin binding proteins use properties derived from the steady-state monomer–polymer cycle of actin in the presence of ATP, to control the F-actin/G-actin ratio and the turnover rate of actin filaments. Capping proteins and profilin regulate the size of the pools of F-actin and unassembled actin by affecting the steady-state concentration of ATP–G-actin. At steady state, the treadmilling cycle of actin filaments is fed by their disassembly from the pointed ends. It is regulated in two different ways by capping proteins and ADF, as follows. Capping proteins, in decreasing the number of growing barbed ends, increase their individual rate of growth and create a ''funneled'' treadmilling process. ADF/cofilin, in increasing the rate of pointed-end disassembly, increases the rate of filament turnover, hence the rate of barbed-end growth. In conclusion, capping proteins and ADF cooperate to increase the rate of actin assembly up to values that support the rates of actin-based motility processes.

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Keywords: actin polymerization; treadmilling; profilin; ADF; capping proteins

Introduction

It is now well accepted that cell locomotion and, more generally, changes in cell shape in response to stimuli are powered by actin polymerization (Condeelis, 1993). Physical analyses show that actin polymerization can provide a protrusive force sufficient to overcome the resistance of the cell membrane (Cortese *et al.*, 1989; Mogilner & Oster, 1996). In recent years, two aspects of the involvement of actin polymerization in motility have given rise to intense investigations.

First, a shape change of the cell in response to stimuli often necessitates a massive polymerization of actin filaments. The stimulation of blood platelets, neutrophils or chemotactic amoebae, is rapidly followed by a large increase in the cellular amount of F-actin. The increase in the F-actin pool correlates with an identical decrease in the pool of ''sequestered actin'', i.e. G-actin in complex with proteins that prevent actin from polymerizing, such as thymosin-β4 and profilin. The mechanism of control of the F-actin/G-actin ratio in cells is a key issue in motility.

Another fascinating feature of cell motility is the use of rapid actin filament turnover to generate movement. Continuous assembly of actin filaments at the leading edge of locomoting cells builds up the protrusive filopodial or lamellipodial extensions of the cytoplasm that determine the direction of movement. While net polymerization occurs at the front, net depolymerization occurs at the rear of the lamella. The rate of movement is 1 to 10 μm/min. For the advance of the lamellipodium to be driven by actin polymerization, the rate of filament growth at the leading edge would have to be as fast as 10 to 100 subunits per second. A key issue is to understand by which mechanism a cell can ''maintain high rates of net polymerization and net depolymerization simultaneously at different sites in its cytoplasm'' (Fechheimer & Zigmond, 1993). In such a steady regime of locomotion, the overall cellular F-actin content remains constant. The actin subunits coming from filaments depolymerizing at the rear of the lamella are recycled into new filaments assembled at the front in the seemingly rapid treadmilling process observed in locomoting keratocytes (Wang, 1985; Small, 1995). Early evidence for the autonomy of the lamella as a motile machine has been provided (Euteneuer & Schliwa, 1984).

Abbreviations used: $T\beta_4$, thymosin-β4; ADF, actin depolymerizing factor.

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Bacterial pathogens such as *Listeria monocytogenes* or *Shigella flexneri* (Higley & Way, 1997, for a review) mimic the dynamic behavior of actin filaments at the leading edge. They elicit their own propulsion in the cytoplasm by inducing actin polymerization at their surface. The movement can be monitored *in vitro* in acellular extracts (Theriot *et al*., 1994; Marchand *et al*., 1995), which provide a basis for identifying the cellular components of the motile machinery involved in actin nucleation at the plasma membranes and eventually reconstituting actin-based motile processes in a controlled medium.

In this short review, we will survey the principles of actin polymerization that are used by different actin binding proteins either to regulate actin desequestration, thus eliciting massive assembly of filaments, or to control actin filament turnover, thus mediating the forward movement of the leading edge.

The steady state of F-actin assembly in the presence of ATP

At the physiological ionic strength, in the presence of ATP, filaments (F-actin) coexist with monomeric actin (G-actin) at the critical concentration for polymerization. Because ATP hydrolysis is associated with actin polymerization, the critical concentration is not, in this case, a physical monomer–polymer equilibrium dissociation constant. It is the steady-state concentration at ATP–G-actin, C_{SS}, that is maintained in the medium *via* monomer–polymer exchange reactions. In the cell medium, actin is assembled under these steady-state conditions.

Actin filaments have a structural polarity, with a barbed end, at which subunits associate rapidly, and a pointed end, which has much slower dynamics. The structural polarity is in itself sufficient to account for the polarized dynamics of actin filaments. In addition to the kinetic difference between the two ends, the fact that ATP hydrolysis is associated with polymerization generates a difference in critical concentration between the barbed and the pointed ends. The critical concentration at the pointed end, C_C^P, can be experimentally determined by blocking the barbed end with capping proteins, and it appears higher than the steady-state monomer concentration C_{SS} measured when both ends contribute to the monomer–polymer exchanges. Hence, at steady state, pointed ends undergo constant disassembly, which is exactly compensated by an equal flux of actin association to the barbed ends, a process called treadmilling or head-to-tail polymerization (Wegner, 1976), shown schematically in Figure 1. The intrinsic critical concentration at the barbed end, C_C^B, therefore, is lower than the experimentally determined steady-state concentration C_{SS}. However, because the monomer–polymer exchanges are more extensive at the barbed end, the value of C_C^B is close, and has often been considered equal, to that of C_{SS}.

The value of C_{SS} when both ends are free in solution can be written (Walsh *et al*., 1984):

$$C_{SS} = \frac{k_+^B C_C^B + k_+^P C_C^P}{k_+^B + k_+^P} \quad (1)$$

where k_+^B and k_+^P are the association rate constants of ATP–G-actin to the barbed and pointed ends, respectively, and C_C^B and C_C^P the critical concentrations at the barbed and pointed ends.

In equation (1), the numerator represents the rate of subunit dissociation from the two ends, the denominator represents the rate of subunit association to the two ends†. Under physiological ionic conditions, using measured values of $C_{SS} = 0.1\ \mu M$, $k_+^B = 10\ \mu M^{-1}\ s^{-1}$, $k_+^P = 0.5\ \mu M^{-1}\ s^{-1}$, $C_C^P = 0.5\ \mu M$ (Pollard & Cooper, 1986), it is easy to derive the value of C_C^B from equation (1). One finds $C_C^B = 0.08\ \mu M$.

The rate of treadmilling at steady state therefore is:

$$J_{SS} = k_+^B(C_{SS} - C_C^B) = -k_+^P(C_{SS} - C_C^P) \quad (2)$$

A value of $0.20\ s^{-1}$ is found. This flux is very slow, typically one to two orders of magnitude slower than the rapid turnover of actin filaments observed *in vivo*.

In the treadmilling cycle of F-actin at steady state (Figure 1), all rates are equal to the rate-limiting step. The cycle comprises dissociation of ADP-actin from the pointed end, ATP exchange for bound ADP on G-actin, followed by association of ATP–G-actin to the barbed ends. The rates of nucleotide exchange, k_e {ATP–G-actin}, which depend on the concentrations of ADP–G-actin and ATP–G-actin, cannot be rate-limiting in the cycle. Hence the rate-limiting step in the treadmilling cycle is the rate of subunit dissociation from the pointed ends. Consequently, factors affecting the rate of depolymerization from the pointed ends are expected to affect the turnover of actin filaments.

Equations (1) and (2) also show that the values of C_{SS} and J_{SS} are affected by capping proteins. When barbed ends are completely blocked by capping proteins, $C_{SS} = C_C^P$ and no treadmilling occurs.

† Since the barbed end undergoes net assembly at steady state, C_C^B represents the ratio of the dissociation rate constant of F–ADP–P_i subunits from the barbed ends (which is five- to tenfold lower than that of the F–ADP subunits) and of the association rate constant of ATP–G-actin (Carlier, 1991). In contrast, at the pointed end, C_C^P represents the ratio of the dissociation rate constant of ADP–subunits and of the association rate constant of ATP–G-actin.

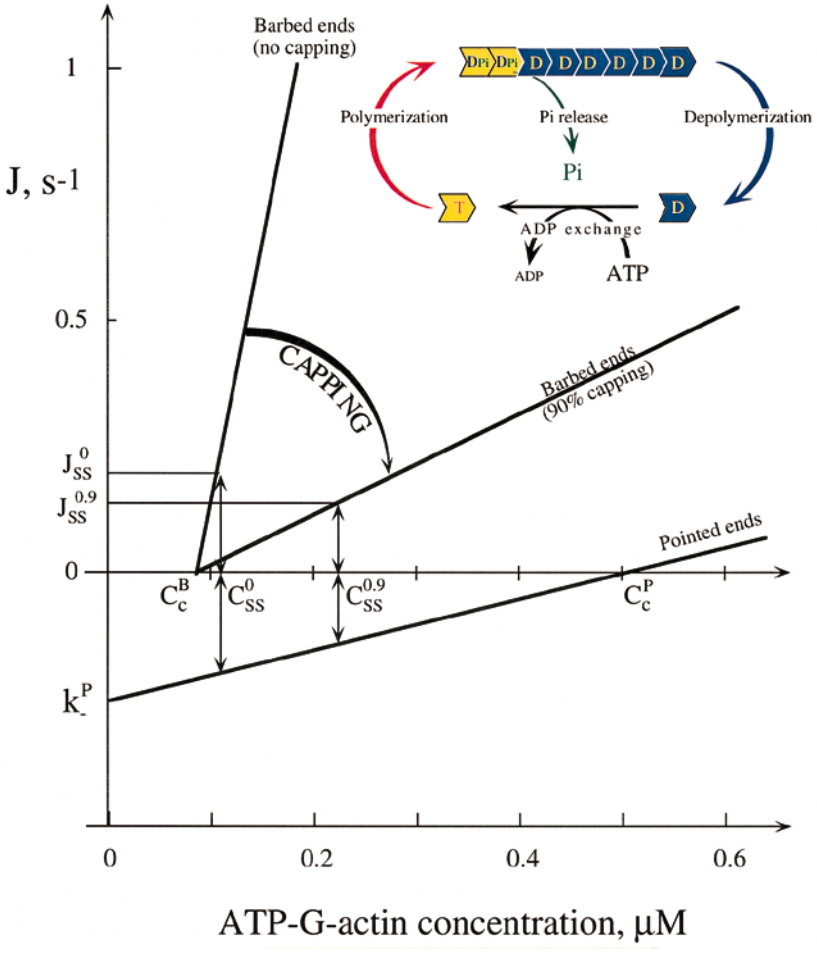

Figure 1. Treadmilling of actin filaments at steady state. Effect of barbed-end capping. The rates $J(c)$ of subunit association to the barbed and pointed ends are drawn *versus* the concentration c of ATP–G-actin. C_C^B and C_C^P are the intrinsic critical concentrations for actin polymerization at the barbed and pointed ends, respectively. C_{SS} is the steady-state ATP–G-actin concentration measured when both ends are free (no capping), at which point the net rate of assembly at the barbed ends J_{SS}^0 equals the net rate of disassembly at the pointed ends. When 90% of the barbed ends are capped, the slope of the $J(c)$ plot at the barbed ends is reduced tenfold, and the steady-state concentration of ATP–G-actin, $C_{SS}^{0.9}$ is higher than C_{SS}^0. The scheme is drawn using the following values of the kinetic, equilibrium and steady-state parameters for actin polymerization under physiological ionic conditions (Pollard & Cooper, 1986). $k_+^B = 10\ \mu M^{-1}\ s^{-1}$; $k_+^P = 0.5\ \mu M^{-1}\ s^{-1}$; $C_C^P = 0.5\ \mu M$; $C_{SS} = 0.1\ \mu M$. The value of C_C^B is calculated using equation (1). $C_C^B = 0.08\ \mu M$. Inset: treadmilling cycle of an average filament when both ends are free (Wegner, 1976).

Control of actin sequestration/desequestration

The critical concentration of ATP–G-actin at steady state therefore is buffered by the filaments to a value that is tightly controlled by the extent of capping. The concentration of sequestered monomeric actin, in the presence of a given total amount of G-actin binding protein, is solely determined by the value of the concentration of ATP–G-actin at steady state, C_{SS}, due to the effect of the law of mass action, and by the total concentration of G-actin sequestering protein, $[S_0]$, as described by the following equation:

$$[G_{seq.}] = [GS] = [S_0] \times \frac{C_{SS}}{C_{SS} + K_S} \qquad (3)$$

where K_S is the equilibrium dissociation constant of the GS complex. K_S is generally of the same order of magnitude as, and larger than, C_{SS} ($K_S = 2\ \mu M$ for thymosin-β4, $K_S = 0.2\ \mu M$ to $2\ \mu M$ for different profilins), hence the concentration of sequestered actin increases with C_{SS}. The larger the size of the reservoir of sequestered actin, the greater the extent of potential F-actin assembly upon lowering the value of C_{SS}. However, the rate of F-actin assembly when C_{SS} is lowered depends solely on the decrease in C_{SS}, not on the concentration of sequestered actin.

The regulation of capping/uncapping of barbed ends is currently envisaged as one of the most plausible mechanisms for controlling the value of C_{SS}, hence the level of actin assembly. The molecular details of the regulation of capping/uncapping of barbed ends are, however, largely unknown. Uncapping of barbed ends could be elicited by PIP_2 binding to capping proteins such as gelsolin or the homolog of capZ in non-muscle cells (Hartwig *et al.*, 1995; Barkalow *et al.*, 1996; Nachmias *et al.*, 1996; Schafer *et al.*, 1996). The concentration of capping protein could, by itself, due to its low rate of interaction with barbed ends, regulate the extension of barbed ends from newly nucleated filaments (DiNubile *et al.*, 1995; Schafer *et al.*, 1996).

Other ways exist by which the critical concentration of ATP–G-actin can be controlled. A typical control of C_{SS} is effected by profilin, an essential G-actin binding protein (Pantaloni & Carlier, 1993). The profilin ATP–G-actin complex can productively associate with the barbed ends (Pring *et al.*, 1992). The net incorporation of an actin subunit from a profilin–actin unit is completed when profilin dissociates from the barbed end, following ATP

hydrolysis and P_i release (Perelroizen *et al.*, 1996). In this cycle, the profilin–actin complex acts as a pseudo-monomer, hence it can decrease the contribution of ATP–G-actin itself in the stabilization of filaments at steady state. It has now been demonstrated that the enhancement of nucleotide exchange on G-actin by profilin, first thought to be important for its biological function, does not play any role in the control of barbed-end actin assembly. Indeed plant profilins, which are unable to accelerate nucleotide exchange, lower the critical concentration like other profilins (Perelroizen *et al.*, 1996), and can rescue the motility defect caused by the deletion of endogenous profilin in yeast (Christensen *et al.*, 1996) and in *Dictyostelium* (Karakesisoglou *et al.*, 1996).

In conclusion, profilin is a complex, Janus-like protein. It is a very powerful G-actin sequestering agent when barbed ends are capped, due to its high affinity for actin ($10^7\ M^{-1}$ in mammalian cells). Equation (3) shows that since $C_C^P \approx 0.5\ \mu M$ under physiological ionic conditions (Pollard & Cooper, 1986), about 80% of the profilin is expected to be bound to ATP–G-actin when all barbed ends are capped, i.e. in the resting cells. When barbed ends are uncapped, upon cell stimulation, profilin–actin efficiently contributes to barbed-end filament growth. For this reason, profilin appears concentrated in regions of the cell where filament barbed ends are actively elongating (Buss *et al.*, 1992). In that sense, profilin can be considered to be a cellular marker of actin-based motile processes that develop at the leading edge, in the phagocytic cup or at the rear of *Listeria monocytogenes*.

More generally, any actin-binding protein able to participate in F-actin assembly is expected to affect the critical concentration for actin polymerization in some way. Thymosin-β4($T\beta_4$) is considered essentially a G-actin sequestering agent, which by definition binds exclusively to ATP–G-actin (Weber *et al.*, 1992; Cassimeris *et al.*, 1992; Carlier *et al.*, 1993; Safer & Nachmias, 1994). It is highly likely that $T\beta_4$, present at 500 and 300 μM in platelets and neutrophils, respectively, is responsible for a large part of the 150 μM unassembled actin in these cells in the resting state; the other main G-actin sequestering protein being profilin. However, *in vitro*, under some ionic conditions at least and a high concentration of $T\beta_4$, the $T\beta_4$–actin complex can be incorporated into F-actin, which affects the steady-state concentration of unliganded G-actin (Carlier *et al.*, 1996). The results of $T\beta_4$ overexpression experiments are not clear. In some cases, the amount of unassembled actin in cells was observed to decrease, total actin remaining constant (Sun *et al.*, 1996), which can be accounted for by the interpretation of the results obtained *in vitro*. In other cases, the concentrations of both polymerized and unpolymerized actin increased when $T\beta_4$ was overexpressed. These results are difficult to interpret because there is no available method to measure directly the concentration of free ATP–G-actin *in vivo*, or to monitor how it may be modified as an indirect consequence of the overexpression of one particular actin-binding protein.

Regulation of actin filament turnover by capping proteins and ADF/cofilin

As outlined above, the rate of treadmilling of F-actin *in vitro* is extremely slow, while *in vivo* filaments turn over at a much faster rate and appear especially dynamic in motile lamellipodia, where they turn over in a treadmilling-like process (Wang, 1985; Zigmond, 1993; Small, 1995). The actin-based propulsive movement of *L. monocytogenes* offers another example of rapid turnover, with filament half-lives of 30 to 60 seconds (Theriot *et al.*, 1992). The difference in the kinetics of filament turnover *in vitro* and *in vivo* suggests that cellular factors provide some regulation. Moreover, in the living cells different actin cytoskeletal structures do not turn over at the same rate. Stress fibers turn over much more slowly than actin filaments in the lamellipodium. Two actin binding proteins can regulate the turnover of actin filaments: capping proteins and ADF/cofilin.

Capping proteins increase the rate of barbed end assembly at steady state: the funneled treadmilling model

Recent reports point to the positive role of capping proteins (CapZ, CapG …) in the control of cell motility (Hug *et al.*, 1995; Sun *et al.*, 1995a; Witke *et al.*, 1995; Dufort & Lumsden, 1996). Cells having a higher content in capping protein appear to move faster. These results have thus far been considered as puzzling (Welch *et al.*, 1997a, for a recent review) within the conventional view that capping of barbed ends stabilizes filaments and abolishes the dynamics and treadmilling. Indeed, when barbed ends are partially capped, the value of C_{SS} increases with the capping up to C_C^P, and the treadmilling rate decreases down to zero. Let γ be the molar fraction of capped barbed ends. The steady-state concentration of ATP–G-actin is (Walsh *et al.*, 1984):

$$C_{SS}^{\gamma} = \frac{(1-\gamma)k_+^B C_C^B + k_+^P C_C^P}{(1-\gamma)k_+^B + k_+^P} \qquad (4)$$

The rate of disassembly from pointed ends at steady state is:

$$J_{SS}^{\gamma} = -k_+^P (C_{SS}^{\gamma} - C_C^P)$$

$$J_{SS}^{\gamma} = \frac{k_+^P k_+^B (1-\gamma)(C_C^P - C_C^B)}{(1-\gamma)k_+^B + k_+^P} \qquad (5)$$

This rate is exactly compensated by the overall rate of subunit association to the uncapped barbed

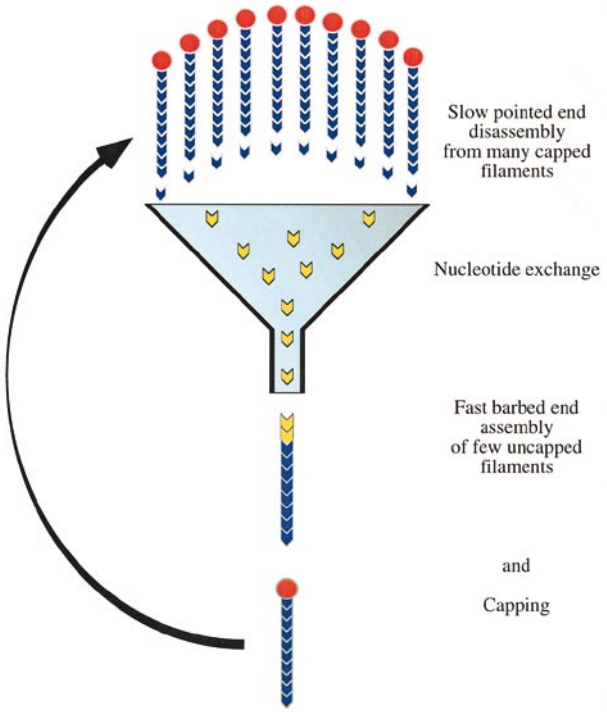

Figure 2. Capping of barbed ends increases the rate of barbed-end assembly at steady state: the funneled treadmilling process. The steady-state behavior of a population of filaments containing a large fraction of capped filaments is visualized. The overall barbed-end association and pointed-end dissociation fluxes are equal; however, individual barbed ends elongate faster than individual pointed ends shorten.

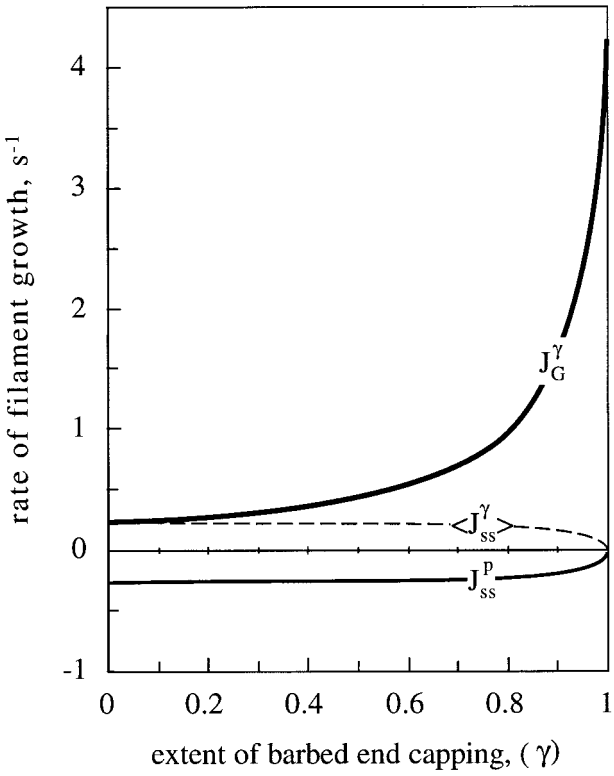

Figure 3. Effect of barbed-end capping on the rates of barbed-end growth and pointed-end shortening at steady state. The rate of barbed-end growth J_G^γ is calculated as a function of the molar fraction of capped barbed ends γ according to equation (6) (heavy continuous line). Light line and broken line represent the steady-state rates of pointed-end disassembly J_{SS}^P and the overall rate of barbed-end growth $\langle J_{SS}^\gamma \rangle = J_g^\gamma(1-\gamma)$, respectively, which are equal and of opposite signs.

ends. Note that, as outlined by Annemarie Weber (Walsh *et al.*, 1984), because barbed ends are more dynamic than pointed ends, when 90% of the barbed ends are capped ($\gamma = 0.9$), the value of J_{SS} is only 30% smaller than the value measured when both ends are free (Figure 1).

Although capping of the barbed ends slows down the overall treadmilling rate, it introduces a dramatic asymmetry in the individual rates of depolymerization at the pointed ends and polymerization at the barbed ends, as first noticed by Dufort & Lumsden (1996). The number of growing barbed ends decreases, but the rate of growth of the few uncapped filaments increases. The regular treadmilling flux of subunits is strongly ''funneled'' to actively feed the growth of the uncapped barbed ends. This is illustrated in Figure 2. To be specific, when a molar fraction γ of the barbed ends is capped, the slope of the J(c) plot at barbed ends is reduced to $(1-\gamma)$-fold its original value, so that the overall rate of assembly onto the remaining uncapped barbed ends equals J_{SS}^γ (equation (5)). But the net rate of growth J_G^γ per uncapped barbed end is $1/(1-\gamma)$-fold greater:

$$J_G^\gamma = \frac{J_{SS}^\gamma}{(1-\gamma)} = \frac{k_+^P k_+^B (C_C^P - C_C^B)}{(1-\gamma)k_+^B + k_+^P} \quad (6)$$

As an example, when 90% of barbed ends are capped, the rate of growth of each of the 10% of uncapped barbed ends is tenfold faster than the rate of disassembly from each pointed end. Therefore, although the overall treadmilling rate is 30% lower than in the absence of capping proteins, individual uncapped barbed ends grow $10 \times 0.7 =$ sevenfold faster than in the absence of capping. Figure 3 illustrates the changes in J_{SS}^γ and J_G as a function of γ, using the known vales of the kinetic parameters for actin assembly under physiological conditions. Note that close to the limit ($\gamma = 1$, i.e. all barbed ends capped), if a putative barbed end appeared in a very large population of capped barbed ends, it would grow at a rate equal to k_+^B. $(C_C^P - C_C^B) = 4.2$ subunits per second, that is 20-fold faster than the treadmilling rate observed when

both ends are free. Note that in the funneled treadmilling process, the mass of assembled actin is constant, but in contrast to the regular treadmilling model, the length of filaments does not remain constant: uncapped filaments increase in length, while capped filaments steadily shorten.

The above considerations show that capping of actin filaments increases the rate of actin assembly at the remaining barbed ends at steady state, through the mechanism described above. It is therefore expected that increasing the capping of filaments increases the actin-based motility of living cells and the rate of propulsion of *Listeria*, which are all powered by barbed-end assembly. In contrast, decreasing the capping of filaments is expected to slow down actin-based motility of cells. These results have indeed been obtained (Hug *et al.*, 1995; Sun *et al.*, 1995a) and the interpretation we propose is consistent with the basic principles of actin polymerization in the presence of ATP.

However, capping is not sufficient, quantitatively, to account for the very high rates of actin-based motility observed *in vivo* in some cases. Capping can theoretically increase the rate of barbed end assembly up to four subunits/second, leading to a maximum rate of movement of 0.8 μm/minute. *In vivo*, variable velocities in the range of 1 μm/minute (forward movement of the leading edge) to 40 μm/minute (locomotion of keratocytes) have been reported. The rate of *Listeria* propulsion varies from cell to cell also in the range of a few μm/minute up to 60 μm/minute. Therefore, other factors, in addition to capping proteins, are to be thought of as agonists of actin filament turnover.

Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover

A biochemical *in vitro* analysis of the interaction of ADF/cofilin with actin showed that this protein was able to increase the turnover rate of actin filaments by more than one order of magnitude, and to accelerate the actin-based motility of *L. monocytogenes* in platelet extracts (Carlier *et al.*, 1997). ADF therefore appears to be the best candidate for the cellular control of actin dynamics in motility. The *in vitro* experiments (Carlier *et al.*, 1997) show that this function of ADF is supported by a large increase in the rate constant for dissociation of actin subunits specifically from the pointed ends.

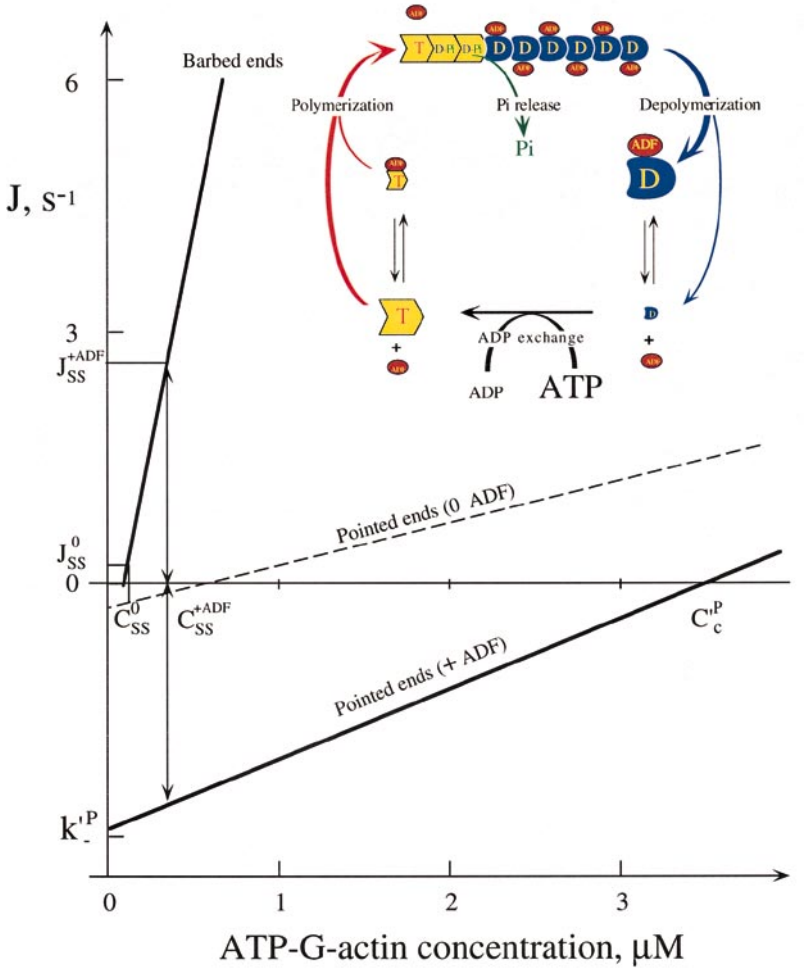

Figure 4. Enhancement of the treadmilling of actin filaments by ADF. The J(c) plot at the pointed end is modified by ADF. The dissociation rate constant and the critical concentration at the pointed ends are increased (Carlier *et al.*, 1997). The kinetic behavior is assumed to be unchanged. As a result, the rate of treadmilling J_{SS} is greatly increased. The J(c) plot at the pointed ends in the absence of ADF is drawn as a broken line for comparison. Inset: treadmilling scheme of an average ADF–F-actin filament (from Carlier *et al.*, 1997).

In vivo, ADF is known to be an essential protein, whose deletion leads to lethal defects in centrosome translocation and cytokinesis (Gunsalus *et al*., 1995; Abe *et al*., 1996), while overexpression increases motility (Aizawa *et al*., 1996). ADF had first been thought to act as a G-actin sequestering protein and filament severing factor (Sun *et al*., 1995b; Moon & Drubin, 1995, for reviews). the more recent biochemical work (Carlier *et al*., 1997), however, shows that since ADF binds both G and F-actin with a high preference for their ADP-bound forms, it participates in actin polymerization and changes the kinetic parameters for filament assembly in an end-specific fashion. At steady state, the very rapid dissociation of ADF–ADP–actin from the pointed ends results in a large increase in treadmilling rate. The increased pointed-end off-rate is compensated by an equally increased rate of assembly at the barbed ends, elicited by an increase in C_{SS}. A steady-state pool of ADF–ADP–G-actin accumulates and feeds the pool of ATP–G-actin until the value of C_{SS} is reached. The treadmilling kinetic scheme of actin filaments in the presence of ADF is outlined in Figure 4.

The enhancement of treadmilling due to ADF is independent of the effect of capping proteins. When barbed ends are partially capped, in the presence of ADF, the effects of ADF and capping accumulate to cause a large increase in the rate of barbed-end growth at steady state. This is due to the fact that the difference $C_C^P - C_C^B$ in the presence of ADF is increased by about one order of magnitude as compared to the value ($0.5\ \mu M - 0.08\ \mu M = 0.42\ \mu M$) obtained in the absence of ADF. The combined action of ADF and capping can then account for the rates of barbed-end growth of 10 to 100 subunits per second that are required to account for rapid movements of the leading edge of keratocytes or of *L. monocytogenes*.

It is noteworthy that ADF binding to F-actin is limited by P_i release (Carlier *et al*., 1997) implying that newly nucleated, barbed-end growing filaments made of F–ADP–P_i subunits at the front of the lamella cannot be depolymerized by ADF. These filaments are in addition more rigid than older F–ADP–actin filaments (Isambert *et al*., 1995). The action of ADF is therefore limited to the rear of the lamella, or of the actin tails of *Listeria*, where both capped and uncapped filaments depolymerize from their pointed ends.

The effect of ADF on treadmilling results in the establishment of a new steady-state concentration of unassembled actin (G-actin + ADF–G-actin) coexisting with ADF-decorated filaments. It will be of great interest to examine how controlled combinations of ADF with other G-actin binding proteins ($T\beta_4$, profilin …) can generate new features in actin dynamics *in vitro*, that will eventually help us to understand the *in vivo* complexity of actin-based motile processes.

In binding to F-actin subunits with a 1:1 molar ratio, ADF profoundly affects the structure and mechanical properties of actin filaments. The conformation change can be monitored by the quenching of fluorescence of probes covalently attached to the C terminus of actin (Carlier *et al*., 1997). A large drop in viscosity linked to ADF binding to F-actin reflects this large structural change. The decrease in viscosity was first attributed to the severing of filaments by ADF, but electron microscopy observations and sedimentation velocity data rather suggest that the flexibility and the distance between filaments in solution might be affected by ADF binding.

Further kinetic and thermodynamic studies of the effect of ADF on actin polymerization will no doubt shed light on the nature of the structural change in F-actin linked to its interaction with ADF, and reveal whether and how this change is involved in its biological function.

Conclusions and perspectives

The observations of actin dynamics in living cells strongly suggest that the polymerization of actin, which powers the movement of the lamellipodium or of *L. monocytogenes*, results from the steady-state ATPase cycle of actin. The ADF-enhanced funneled treadmilling process presented here shows that the known principles of actin polymerization at steady state are sufficient to account for the increase in the rate of barbed-end assembly induced by capping proteins and ADF.

This model points to the following crucial issues that need to be solved in the near future. First, how are the length and number of filaments controlled on a steady-state basis? Within the present model, if barbed ends elongate at a fast rate, uncapped filaments should grow indefinitely, while capped filaments are fated to disappear by endwise depolymerization. The steady state implies that the concentrations of capped and uncapped filaments, as well as the concentration of free capping proteins should be maintained constant. A possible regulation may be afforded: (1) by eventual capping of the growing barbed ends by capping proteins, which are continuously recycled following complete depolymerization of capped filaments; and (2) by on-going nucleation of new uncapped filaments. The molecular mechanisms responsible for maintaining a steady nucleation rate and extent of capping have not been explored.

The nature of the machinery that nucleates filaments able to grow from their barbed ends is not known, nor is the mechanism understood that allows these elongating barbed ends to escape the capping proteins present in the medium. One may speculate that some insertin-like factor bound to terminal subunits at barbed ends could allow association of G-actin but would compete with the binding of capping proteins. The rate of capping itself could be regulated through the availability of capping proteins, or the affinity of capping pro-

teins for barbed ends might be regulated by ATP hydrolysis on the terminal subunits of the filament.

The movement of *L. monocytogenes* in platelet extracts appears a well chosen model system in which to address these questions. Recently, a large complex of several polypeptides containing the two actin-related proteins Arp2 and Arp3 has been demonstrated to be involved in the initiation of actin assembly at the surface of *Listeria* (Welch *et al*., 1997b). How this complex establishes a structural and functional link between the bacterial protein ActA and the barbed ends of actin filaments remains to be elucidated.

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