Depolymerization of Actin Filaments by Profilin: Effects of Profilin on Capping Protein Function

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SUMMARY

Profilin interacts with the barbed ends of actin filaments and in vivo is thought to facilitate actin polymerization. This conclusion is based primarily on *in vitro* kinetic experiments utilizing relatively low concentrations of profilin (1-5 µM). However the cell contains actin regulatory proteins with multiple profilin binding sites that potentially can attract millimolar concentrations of profilin to areas requiring rapid actin filament turnover. We have studied the effects of higher concentrations of profilin (10-100 µM) on actin monomer kinetics at the barbed end. Prior work indicated that profilin might augment actin filament depolymerization in this range of profilin concentration. At barbed-end saturating concentrations (final concentration ~40 µM), profilin accelerated the off-rate of actin monomers by a factor of four to six. Comparable concentrations of latrunculin had no detectable effect on the depolymerization rate indicating that profilin-mediated acceleration was independent of monomer sequestration. Furthermore we have found that high concentrations of profilin can successfully compete with CapG for the barbed-end and uncap actin filaments, and these effects could be explained by a simple equilibrium model of competitive binding. In contrast, neither gelsolin nor CapZ could be dissociated from actin filaments under the same conditions. These differences in the ability of profilin to dissociate capping proteins may explain earlier *in vivo* data showing selective depolymerization of actin filaments after microinjection of profilin. The finding that profilin can uncap actin filaments was not previously appreciated and this newly discovered function may have important implications for filament elongation as well as depolymerization.
INTRODUCTION

Profilin is a multifunctional actin regulatory protein that binds actin monomers with a 1:1 stoichiometry. Profilin-actin complexes fail to form nuclei and in the absence of preformed actin filaments will not assemble into actin filaments. However, profilin-actin complexes can readily add to free barbed ends of actin filaments at rates similar to free actin monomers (1). For this reason, profilin is widely regarded as a protein that promotes actin filament assembly (1-7). The \textit{in vivo} effects of profilin on the actin filament cytoskeleton, however, have proved enigmatic and are not readily explained by the known functions of profilin. Genetic experiments in which both profilin isoforms in \textit{Dictyostelium} were deleted result in an increase in F-actin by about 70\% (8). Microinjection of profilin in Swiss 3T3 cells causes extensive depletion of filamentous actin, but cortical F-actin is selectively preserved (9). Similarly microinjection of profilin into normal rat kidney cells induces actin filament disassembly, selectively sparing actin in the cortex and contractile ring. While monomer sequestration by profilin could be responsible for depletion of F-actin, the pattern of loss of actin contrasted with the non-specific depletion of filaments observed when other monomer sequestering agents, DNase I and vitamin D binding protein, were employed (10), implying that monomer sequestration was not entirely responsible for these \textit{in vivo} observations. Others have shown similar differential effects on actin filament populations, with reduced density of parallel actin bundles in CHO cells overexpressing profilin (11).

Another unique characteristic of profilin is its ability to bind to poly-L-proline. A number of actin regulatory proteins contain profilin binding sequences of the type XPPPPP (X = G, A, P, S) (12). For example Vasoactive Stimulatory Phosphoprotein (VASP) contains 16-20 profilin binding sites, and N-WASP contains 12 profilin binding sites. These proteins localize to regions of high
actin filament turnover and are capable of attracting concentrations of total profilin in the mM range (13). While the relationship between free and total profilin in vivo has not been determined, there are data that suggest that the cellular content of polymerization competent profilin-actin complex is limited (14,15), raising the possibility that high concentrations of actin-free profilin are attainable locally. Previous in vitro studies examining the effects of profilin on actin filament kinetics have utilized profilin concentrations in the range of 0.5-5 µM. Cognizant of the ability of cells to locally generate much higher concentrations of profilin, we have examined the effects of profilin concentrations an order of magnitude higher (10-100 µM, final concentrations) than previously studied. Using an experimental design that simplifies kinetic analysis, we have found that profilin accelerates the off-rate of the barbed end in a concentration dependent fashion. Secondly we have discovered that high concentrations of profilin can competitively dissociate the barbed-end capping protein, CapG. These findings add two hitherto unappreciated functions for profilin that may help to account for the multiple and complex in vivo changes in actin filament concentration and morphology attributed to raising and lowering cell profilin content.
EXPERIMENTAL PROCEDURES

Materials-Rabbit skeletal muscle Ca\(^{2+}\)-actin was prepared from frozen muscle (Pel-Freez, Rogers AR) in buffer G (5.0 mM Tris HCl, 0.2 mM ATP, 0.2 mM dithiothreitol, 0.1 mM CaCl\(_2\), and 0.01% sodium azide, pH 7.8). Pyrenyl-labeled actin on cysteine 374 was prepared with 0.7 to 0.95 mole of label/mol of protein using the method of Kouyama and Mihashi (16). Latrunculin A was purchased from Biomol (Plymouth Meeting, PA). Human profilin, gelsolin, and CapG were produced as recombinant proteins as previously described (13,17). CapZ was purified from rabbit skeletal muscle to greater than 80% purity as assessed by SDS-PAGE using the method of Casella et al. (18), and because its concentration is imprecisely known, the stated concentrations are approximate, but all the data displayed here come from a single preparation, so relative concentrations are precise.

Depolymerization Assays- In samples that did not employ a calcium sensitive capping protein (CapG or gelsolin), a 10 µM stock of actin (10 % pyrenyl-labeled) was converted to Mg\(^{2+}\)-actin by the addition of 125 µM EGTA and 50 µM MgCl\(_2\). After 10 m, MgCl\(_2\) and KCl were added to final concentrations of 2.0 mM and 40 mM, and the actin was given 60 m to polymerize to make an F-actin stock solution. The procedure was identical for samples containing calcium sensitive capping proteins except that the actin was not converted to Mg\(^{2+}\)-actin prior to polymerization. Depolymerization rates were measured after 33 to 100-fold dilution of the F-actin stock solution into identical buffer containing the stated amounts of capping proteins with or without latrunculin A. The initial fraction of G-actin in these samples is ~1% (19). In some experiments, capping proteins were added at the time of preparation of the F-actin stock, and the results of these assays were compared with the addition of capping protein at the time of dilution. In both types of
experiment, the stated concentration of capping protein is the final concentration after dilution. Polymeric actin concentration was determined as a function of time after dilution by the measurement of pyrene fluorescence in 300 μl samples in a spectrofluorimeter with excitation 365.6 and emission 386.6 nm. The initial rate of depolymerization was estimated by a linear fit to data corresponding to the first 15% decrease in pyrene fluorescence intensity. This method is justified on an experimental basis by excellent reproducibility and a theoretical basis because a linear rate of depolymerization is expected providing that the filaments are long enough that the filament number does not change during the initial time interval used for the calculation.

**Steady-State Fluorescence Measurements**—A 15 μM stock of actin (4 % pyrenyl-labeled) was polymerized by addition of MgCl₂ and KCl to final concentrations of 2.0 mM and 40 mM, respectively, and the actin was given 20 to 60 m to polymerize. F-actin was then diluted to 10 μM in profilin and gelsolin or CapG. The samples were incubated for 20 to 24 h at 22°C, and steady-state readings were obtained in a spectrofluorimeter with excitation 365.6 and emission 386.6 nm.

**Global fitting of depolymerization data**—The fitting procedure is an approximation based on several assumptions. Equations similar to those previously used to describe the interaction of profilin with both G- and F-actin (2) can be written for any other barbed-end capping protein:

\[
A + F_{(n-1)} \overset{k_c}{\underset{k_d}{\rightleftharpoons}} F; \quad K_{cc} = \frac{k_d}{k_c} \quad (\text{Eq. 1})
\]

\[
C + A \overset{k_c}{\underset{k_d}{\rightleftharpoons}} CA; \quad K_d \quad (\text{Eq. 2})
\]

\[
CA + F_{(n-1)} \overset{k_c}{\underset{k_d}{\rightleftharpoons}} FC; \quad K_{elong} = \frac{k_e}{k_c} \quad (\text{Eq. 3})
\]

\[
C + F \overset{k_c}{\underset{k_d}{\rightleftharpoons}} FC; \quad K_{cap} = \frac{k_c}{k_e} \quad (\text{Eq. 4})
\]

where \(A\) is the actin monomer concentration, \(F\) is the concentration of filaments of \(n\) actin subunits (or \(n-1\) actin subunits when specifically indicated), \(C\) is the concentration of capping protein, and the constants are the respective equilibrium dissociation constants for which the energy square,
may or may not be satisfied depending on how the steady state condition compares thermodynamically with equilibrium. There is disagreement in the literature as to whether the energy square is satisfied for profilin, although the thermodynamic difference between the two possible pathways for addition of a subunit to F-actin is small in all reports (1,3,4,6). Qualitative interpretations of the depolymerization data in terms of a competitive binding model would be unchanged in the absence of a balanced energy square, however, the quantitative analysis would then be indeterminate, as any discrepancy in the results could be attributed to a deviation from thermodynamic equilibrium rather than a failure of the model. Also, the assumption of barbed-end capping by profilin is controversial in certain buffer conditions (20). When both profilin and another capping protein are present, Eqs. 2-4 are written for each protein with rate and equilibrium constants that are unique for each protein (for example, \( K_{Pcap} \) and \( k_{Pe-} \) for profilin, and \( K_{Ccap} \) and \( k_{Ce-} \) for the other capping protein). Typically, the rate constant for dissociation of capping protein from F-actin, \( k_{Ce-} \) (Equation 3), is slow, but this is not the case for profilin (6). To balance the energy square, a small value for \( k_{Ce-} \) must be accompanied by relatively weak binding to actin monomer or a corresponding small value for \( k_{Ce+} \). Thus, monomer binding by a capping protein may or may not be negligible.

Other assumptions can be related to these equations. We assume that the dissociation rate from the filament pointed end, \( k_{p-} \), is negligible where the sum of the barbed \( (k_{b-}) \) and pointed end \( (k_{p-}) \) dissociation rates is equal to \( k \) as defined in Equation 1. Under the conditions of our experiments, sequestration of monomeric actin by CapG is assumed insignificant. The concentrations of CapG used in our capping experiments were in the nanomolar range, orders of magnitude below the equilibrium dissociation constant of CapG for actin monomers \( (K_{Cd} \approx 1 \mu\text{M}) \). The concentration of free profilin is assumed to be much greater than the concentration of actin.
filaments. Also, the binding of profilin and CapG is assumed to be in rapid equilibrium with the barbed end with respective dissociation constants of $K_{Pcap}$ and $K_{Ccap}$ as in Equation 4. Finally, based on previous results (2,4,6), the elongation rate for addition of profilin-actin complex, $k_{P e+}$ is assumed to be approximately equal to $k_+$. The dissociation rate for profilin-actin complex is $k_{P e-}$.

Then, competitive binding between profilin and CapG for the barbed end results in a quadratic equation for the concentration of free barbed ends, $[F]$ as a function of the initial concentrations of profilin ($P_0$) and CapG ($C_0$), and the total concentration of filaments, $[F_0]$:

$$[F] = \left( \frac{([P_0] + K_{Pcap}) \cdot K_{Ccap} + ([C_0] - [F_0])}{K_{Pcap}} \right)^2 + 4 \cdot \frac{([P_0] + K_{Pcap}) \cdot K_{Ccap} \cdot [F_0])}{K_{Pcap}} \right]^{1/2} - \left( \frac{([P_0] + K_{Pcap}) \cdot K_{Ccap} + ([C_0] - [F_0])}{K_{Pcap}} \right)$$

This result is substituted into the rate equation:

$$\frac{d[A]}{dt} = k \cdot [F] + k_{P e-} \cdot ([F_0] - [F])$$

$$= ([F] \cdot (1 + P_0 / K_{d})) \cdot k. \quad (\text{Eq. 5})$$

and the initial rate of depolymerization is fit for all values of $C_0$ and $P_0$. Fitting parameters include the dissociation rate of actin from the barbed end in the presence or absence of profilin, the concentration of filaments, and the dissociation constants for profilin and CapG from the barbed end. Note that although Equation 5 includes $K_{Pd}$ for profilin and actin, given the stated assumptions including that of a balanced energy square, this is equivalent to fitting for $k_{P e-}$. 


RESULTS

Profilin accelerates the rate of F-actin depolymerization- In agreement with the theoretical considerations of Pring et al. (2), and the experimental observations of Kinosian et al. for non-muscle actin (6), profilin accelerates actin filament depolymerization (Fig. 1). Lower concentrations of profilin resulted in lower magnitudes of acceleration, and a dose dependent acceleration in depolymerization by profilin was observed (see Fig 3). The concentration of actin monomer at the initial time point in the depolymerization data is very low, and therefore any monomer sequestering function of profilin should be negligible. In addition, we show that the observations of Kinosian et al. cannot be explained by monomer sequestration by comparing the effects of profilin with that of another monomer sequestering agent, latrunculin A. At high concentrations, latrunculin A alone had minimal effect on the time course of depolymerization. Also, monomer sequestration by latrunculin A did not alter the acceleration of depolymerization by profilin. From Fig. 1, the dissociation rate constant $k_e \cong 6 \cdot k$ when the capping protein in Equation 3 is profilin. That is, depolymerization is accelerated by a factor of about six when barbed-end saturating concentrations of profilin are present.

Profilin binds less tightly to pyrenyl-labeled actin monomers than to unlabeled monomers (21), creating a potential complicating factor in the interpretation of depolymerization data that are based on the direct measurement of the off-rate of pyrenyl-labeled subunits from F-actin. However, unlike a polymerization assay done in the presence of profilin, the effect of pyrenyl-labeling of actin on the depolymerization assay is easily predicted. Assuming that the $K_d$'s for pyrenyl and unlabeled actins are different, but that pyrenyl-actin randomly copolymerizes with unlabeled actin (22), then pyrenyl actin subunits can only dissociate when unlabeled subunits are also dissociating. In the worse case scenario, pyrenyl-actin subunits at the barbed end might not bind profilin, and would
therefore dissociate at the normal, uncapped rate ($k$ from Equation 1). In that case, 9 of every 10 subunits from a 10% pyrenyl-labeled filament would be dissociating at the rate of unlabeled subunits and the difference between observed dissociation rates in the presence and absence of profilin would still be 90% of what it would be if profilin bound identically to pyrenyl-labeled and unlabeled subunits. Thus, the effect of pyrenyl-labeling should be small. An increase to 50% pyrenyl-labeled actin in the depolymerization assay yielded similar results as those displayed in Fig. 1, suggesting that the magnitude of any artifact introduced by a difference in affinity is small (data not shown). Additionally, consistent with the pyrene fluorescence data, experiments using light scattering (with detection at 350 nm at a right angle to the incident light) also showed acceleration of depolymerization by profilin (data not shown).

Profilin prevents CapG from blocking the depolymerization of F-actin, and the data are qualitatively compatible with a competitive binding model- CapG prevents actin depolymerization as reported (23), but addition of sufficient profilin can reverse this effect (Fig. 2). Addition of latrunculin A shows that this result is not related to monomer sequestration. At 40 µM profilin, depolymerization data with or without CapG are superimposable. The observation that the depolymerization rates with or without CapG converge at saturating concentrations of profilin (Fig. 3) is consistent with competitive binding between CapG and profilin for the barbed end of actin filaments. Alternatively, these data are also consistent with the hypothesis that profilin and CapG form a hetero-complex at the barbed end that has the same dissociation rate constant as profilin alone bound at the same end. As depicted in Figs 2 and 3, the dissociation rate constant $k_e \cong 4 \cdot k$. We attribute the differences between this result and that of Fig 1 to limitations in the experimental method such that collection of data does not begin precisely at the time of dilution, but rather,
begins after the sample is mixed and placed in the fluorimeter. The uncertainty in this value is acknowledged in our stated conclusions.

Profilin has no effect on the depolymerization of filaments capped in advance by CapZ or gelsolin, but has similar time-dependent effects on the capping activity of these proteins. In contrast to the results with CapG, profilin has no effect on the depolymerization of filaments capped by gelsolin, even at substantially higher concentrations of profilin (Fig. 4A), and even though CapG and gelsolin are members of the same family of proteins that presumably cap filaments by similar mechanisms (24). The depolymerization rate for the control curve in Fig. 4A is relatively constant, a result that does not coincide with theoretical predictions of filament length that imply that all filament lengths should be equally represented at steady-state in the presence of gelsolin (25). Rather, these data are most simply interpreted as uniform depolymerization of filaments of similar length, and there are data in the literature that support this interpretation for low ratios of actin to gelsolin (e.g. 64:1) (26). In this case, competition by profilin would be expected to result in an increase in the rate of depolymerization as a function of time. This is not observed even at highest final concentration of profilin (98 µM).

From these data, it is not possible to conclusively determine if fundamentally different mechanisms explain the experimental differences between CapG and gelsolin. The off-rate of gelsolin is slow, with ½ time for release reportedly in the range of $7 \cdot 10^3$ s (27), although our own data suggests that it is somewhat faster. Since profilin has no chance to compete with pre-existing capped ends until the capping protein releases from the filament, competition under these circumstances becomes experimentally undetectable except with prolonged observation. While the duration of the observations in Fig. 4A would likely permit detection of competition based on the reported value of $t_{1/2}$ for gelsolin, the observed results may be explained by (1) a longer than
expected $t_{1/2}$, (2) a sufficiently high equilibrium dissociation constant of profilin for the barbed end that the amount of profilin does not achieve saturation, or (3) non-competitive or independent binding. In contrast, the dissociation rate constant for CapG can be estimated as in ref. 28 from an estimate of the association rate and the equilibrium association constant. From data not shown, equilibration for the association reaction occurs in less than 15 s in 4 nM CapG, corresponding to an upper limit on $t_{1/2}$ for dissociation of CapG of 160 s. This upper limit is within an order of magnitude of the value for $t_{1/2}$ necessary if competitive binding is a plausible explanation for the convergence of data for the time course of depolymerization of filaments with or without CapG under conditions of saturation by profilin.

If CapZ, an ubiquitous heterodimeric, barbed-end capping protein, was allowed to reach a steady-state interaction with actin filaments prior to depolymerization, then similar to the results with gelsolin, profilin was unable to augment the depolymerization rate (Fig. 4B). The dissociation rate of CapZ from barbed ends is reportedly $4 \cdot 10^4$ s$^{-1}$ (29,30) or half-time of $\sim 2 \cdot 10^3$ s, and therefore, as for gelsolin, the data shown in Fig. 4B should be sufficient to detect competitive binding by profilin if it was present. In other samples, depolymerization rates of filaments capped by CapZ plus and minus profilin were collected for up to $3 \cdot 10^3$ s and these data did not reveal evidence that saturating amounts of profilin cause the rate of depolymerization to increase as a function of time.

The results of depolymerization assays were dependent on when filaments were capped by gelsolin or by CapZ (Figs. 4B and 4C). In contrast to the results when capping protein was allowed to pre-equilibrate with F-actin, if the capping protein was mixed with F-actin at the time of initiation of depolymerization, then profilin did alter the depolymerization rate. In this case, a short time interval with rapid depolymerization was followed by a return to the same slow rate observed in the
absence of profilin. For both gelsolin and CapZ, higher ratios of capping protein to actin lessen the
duration and rate of rapid depolymerization. The simplest explanation for these results is that
profilin depolymerizes actin during the initial period of time before CapZ is bound, but after this
initial period, profilin cannot affect depolymerization kinetics. Results in Fig. 4C show that gelsolin
and CapZ have similar effects when added to F-actin at the same time as profilin. Difference curves
for the depolymerization rates obtained in the presence and absence of profilin are superimposable
for gelsolin and CapZ. The actual depolymerization curves for gelsolin and CapZ differ only
because the gelsolin curves have steeper slope after ~100 s due to the combined severing and
capping activity of gelsolin, resulting in more pointed ends from which subunits can dissociate (27).

Steady-state data and a global fit to the depolymerization data are consistent with competitive
binding of profilin and CapG for the barbed ends of filaments- The steady-state data are
representative of 3 independent assays completed with three different actin preparations (Fig. 5). In
all cases, the data for 0 µM profilin are indistinguishable for CapG or Gelsolin, yet in all cases,
there is significantly more F-actin in the CapG containing samples at high concentrations of profilin
relative to those samples with gelsolin. Qualitatively, the data are consistent with competitive
binding in that increasing profilin concentration reverses the capping effect of CapG, thereby
lowering the critical concentration and increasing the amount of F actin. Quantitatively the data are
more difficult to assess because the lower critical concentration probably originates both from the
loss of capping by CapG and by direct effects of profilin on the critical concentration of uncapped
barbed ends (3). Neither the dose response of the critical concentration to profilin nor the
dependence of these effects on the fraction of uncapped ends have been previously determined.

Increasing concentrations of profilin reverse capping by CapG and increasing CapG
concentrations reverse acceleration of depolymerization by profilin (Fig. 6). Saturating levels of
CapG are easily achieved that show convergence of the data in the presence or absence of profilin, consistent with competitive binding. Because of the relatively lower affinity of profilin, convergence at saturation with profilin is more difficult to prove, but the data are also qualitatively consistent with the interpretation of competitive binding. From a quantitative perspective, all data for depolymerization, either at fixed profilin or at fixed CapG concentrations, can be fit relatively well simultaneously using a single set of binding parameters (Fig. 6). The robust fit over a wide range of experimental variables is based on several assumptions that are outlined in the methods section. Parameters used to fit these data included the dissociation rate of actin from the barbed end in the presence (1.72 s\(^{-1}\)) or absence (0.32 s\(^{-1}\)) of profilin (\(k_{e-} \equiv 5.4 \cdot k_{..}\)). These values are generally in good agreement with previous reports (2,4,6) although the relative acceleration of depolymerization rate by profilin was predicted to be greater than 500-fold for Acanthamoeba actin (2). The concentration of filaments, [F] was 0.83 nM, and to our knowledge, this represents the first attempt to calculate [F] by this method. The dissociation constants for profilin (\(K_{Pcap}\)) and CapG (\(K_{Ccap}\)) from the barbed end were 2.2 \(\mu\)M and 0.033 nM, respectively. Different preparations of CapG were used to obtain the data in Figs. 3 & 6, with a 8 fold higher affinity noted for the CapG used in Fig. 6. We do not have an explanation for the variation between preparations, but note that the fit to the data accommodates relatively large variations in \(K_{Ccap}\) and that all data were consistent with the interpretation of competitive binding.
DISCUSSION

Depolymerization of actin by profilin- Concentrations of profilin within the cytoplasm of mammalian cells have been estimated to range from 10 µM in less motile cells such as endothelial cells (31) to 60 µM in highly motile cells such as polymorphonuclear leukocytes (32). Furthermore, studies of actin-based motility of *Listeria monocytogenes* (33,34) as well as *Shigella flexneri* (35,36) have emphasized the potential importance of actin-regulatory proteins that can attract VASP and N-WASP to regions of rapid actin filament turnover. As a consequence of the multiple profilin binding sites on VASP and N-WASP, these protein complexes can attract concentrations of profilin that approach the millimolar range (13). To understand how profilin functions in the living cells in vitro actin kinetics need to be performed at profilin concentrations likely to be found in the cell. Experiments with such high concentrations of profilin, however, are problematic because profilin sequesters significant concentrations of monomeric actin under these conditions. Initial attempts at assembly and disassembly experiments using spectrin-4.1 nucleated filaments to limit monomer exchange to free, barbed filament ends resulted in rapid destabilization of the spectrin-4.1 nuclei at these high profilin concentrations (10-40 µM).

Our results show that the actin subunit off-rate in the presence of saturating concentrations of profilin is four to six times that observed in the absence of profilin. This corresponds to the measured ratio of dissociation rate constants for profilin-actin to that for actin alone. This result was first predicted by Pring et al., but could not be confirmed using *Acanthamoeba* actin (2). We speculated at that time that the reason it could not be confirmed was that the terminal nucleotide on the barbed end of F-actin was different during depolymerization and elongation. However, Teuber and Wegner later suggested that the terminal nucleotide exchanges at a rate of 20 s\(^{-1}\) (37), a rate that is significantly higher than the subunit dissociation rate in either the presence or absence of profilin,
and the barbed-end terminal subunit would therefore be expected to be ATP under both elongation and depolymerization conditions. In retrospect, the explanation for the failure to detect enhanced depolymerization was either that the profilin concentration was not sufficiently high or that the observation is not valid for *Acanthamoeba* proteins. The first experimental data confirming that profilin can increase depolymerization rates was provided by Kinosian et al. for non-muscle actin (6). Under conditions in which free profilin is assumed to cap the barbed end of filaments, the dissociation rate of profilin-actin from the barbed end has been indirectly calculated at 5.5 to 550 times the dissociation rate of actin from uncapped filaments (2, 4, 6).

Very likely, the *in vivo* function of profilin is complex, and profilin may have activities that augment barbed end filament dynamics during both filament assembly and disassembly. The observation that a reduction in profilin suppresses the phenotype of capping protein mutants in *Drosophila* (7) can be interpreted as promotion of actin polymerization by profilin. However, in a more general sense, if profilin promotes filament dynamics at free barbed filament ends, then the absence of profilin may simply dampen the effects of the loss of regulated capping activity, thereby lessening the severity of the phenotype.

*Filament uncapping by profilin*- When filaments are capped by CapG, profilin enhances depolymerization by both uncapping and augmenting the actin subunit off-rate. Depolymerization data for filaments capped by CapG are consistent with competition between profilin and CapG for the filament ends. As expected with competitive binding, the data converge for samples with and without CapG at saturation by profilin. Like other postulated mechanisms, this novel method of uncapping actin filaments lowers the concentration of free actin at steady state (Fig. 5). Competitive binding at the barbed end could be explained by steric effects. The binding surfaces for gelsolin segment 1, and presumably segment 1 of CapG which possesses high homology to this
region of gelsolin, differ from profilin, however, both profilin and gelsolin segment 1 cannot occupy these binding sites simultaneously without some overlap of the ligands (38,39). The steric effect of intact gelsolin or CapG can only be greater relative to that of segment 1. Alternatively, profilin has notable allosteric effects on actin structure that could affect binding by gelsolin and CapG (19). More complicated alternatives to competitive binding could be postulated. However, the simple formation of a ternary complex of profilin, CapG and actin at the barbed end is unlikely. Such a ternary complex would be expected to exhibit similar behavior when profilin adds to CapG-actin filaments and when CapG adds to profilin-actin filaments, but this is not the case as demonstrated in Fig. 6 by the different end-points at saturating concentrations of profilin or of CapG. While the data presented here are consistent with a simple model of competitive binding, CapG may interact with more than one site on an actin filament during capping (24), so that the range of possible interactions with actin monomer and filaments are much greater than delineated in this model. Very likely the model is an imprecise description of the actual mechanism.

Similar uncapping was not observed for CapZ or gelsolin. This difference is likely to be the consequence of the slow off rates of both these capping proteins. This differential effect of profilin on capping protein function may help to explain the variation in sensitivity of different actin filament populations to raising and lowering the profilin concentration in vivo (9,10,11). The relevance of results showing rapid depolymerization of actin by profilin when filaments are diluted into gelsolin or CapZ is speculative. At concentrations of filaments and free capping protein-β2 (a homologue of CapZ) reported in neutrophil lysates (29), the half time for capping of a filament is less than a second and no more than 6 subunits can be expected to dissociate prior to capping in the presence of saturating profilin. On the other hand, if CapZ and/or gelsolin equilibrates with filaments in live cells, then the free concentration of capping proteins may be 3 orders of magnitude
less than observed in the neutrophil lysates, and capping of free ends would be slow enough to permit significant filament depolymerization by free profilin. Partial depolymerization of actin may be sufficient to cause extensive disruption of filament networks (40), and in concert with actin filament severing proteins may enhance actin gel solation (41), and high local concentrations free profilin could facilitate disassembly of specific actin filament populations. The resulting filament fragments could be capped before complete depolymerization, and transported to other sites of filament assembly. Much evidence supports the concept that filament assembly utilizes annealing reactions between such filament fragments (42,43).

Finally it should be kept in mind that following specific stimuli, profilin in the living cell could combine with other actin regulatory proteins to uncap not only CapG, but also gelsolin and CapZ. For example, proteins that are polyvalent for profilin such as VASP may sufficiently increase the local concentration of profilin to facilitate uncapping. This is a particularly interesting hypothesis, given the “anticapping” function recently assigned to Ena/VASP proteins (44). Under the appropriate conditions uncapping by profilin could not only affect actin disassembly, but could also serve to dramatically increase actin filament elongation and allow the generation of directional actin-based propulsive forces.

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REFERENCES


**FOOTNOTES**

1The abbreviation used is: pyrenyl-labeled actin, actin labeled on Cys374 with N-(1-pyrene)iodoacetamide.
FIGURES

FIGURE 1. Effects of Profilin on the time course of actin filament depolymerization. Time course of depolymerization of 0.1 µM 10% pyrenyl-labeled Mg$^{2+}$ F-actin in presence (triangles) or absence (circles) of 40 µM profilin (closed symbols). To verify that sequestration by profilin is not responsible for the acceleration of depolymerization, the same experiment was repeated in presence of 15 µM latrunculin A (open symbols).

FIGURE 2. Time course of the depolymerization of CapG-capped actin filaments. Time course of depolymerization of 0.1 µM 10% pyrenyl-labeled Mg$^{2+}$ F-actin in the absence (down triangles) or presence of 8 nM CapG (squares) or 40 µM profilin (circles), or both CapG and profilin (open, up triangles). Inset, the same experiment was repeated in presence of 15 µM latrunculin A; the symbols have the same meanings.

FIGURE 3. Dose response of profilin on the initial depolymerization rate for filaments capped by CapG. Dependence of the initial depolymerization rate of 0.1 µM 10% pyrenyl-labeled Mg$^{2+}$ F-actin on profilin concentration in presence (closed circles) or absence (open triangles) of 8 nM CapG. Inset, the same experiment was repeated in presence of 15 µM latrunculin A and the results are depicted with identical symbols.

FIGURE 4. Effect of profilin on capping by gelsolin and CapZ. A, time course of depolymerization of 0.3 µM 10% pyrenyl-labeled Mg$^{2+}$ F-actin capped with gelsolin at ratio 1:100 in presence of 0 (closed squares), 40 (closed circles), or 98 (open triangles) µM profilin and 15 µM of latrunculin A. For these samples, gelsolin was incubated with F-actin prior to dilution. B, time-dependent effects of profilin on depolymerization in the presence of CapZ demonstrated by the time course of depolymerization of 0.3 µM 10% pyrenyl-labeled Mg$^{2+}$ F-actin. When F-actin stock is
made in the presence of CapZ and then diluted to a final concentration of 0 (open circles) or 40 µM (open triangles) profilin, the results are indistinguishable. The ratio of CapZ to actin is 1:120. In contrast, when CapZ is used at the same final concentration, but is not added until the time of F-actin dilution, then the data for 0 (closed circles) or 40 (closed triangles) µM profilin differ. When CapZ is added at the time of F-actin dilution, but at a lower concentration (50% of that for solid symbols), then the time dependence of depolymerization for 0 (+) or 40 (x) µM profilin is as shown, and the initial rate of depolymerization with 40 µM profilin is very similar in the presence (x) or absence (squares) of CapZ. C, difference plot of the time course of depolymerization when gelsolin (1:200, open squares; 1:10, closed squares) or CapG (1:120, closed circles) are added to F-actin at the time of dilution in 40 µM profilin relative to the same assay in the absence of profilin. The data for CapZ at a dilution of 1:120 are calculated directly from those pictured in B. The other data are calculated by the same method, but the original curves for the time-course of depolymerization are not shown. Ratios are of capping protein to actin subunits.

FIGURE 5. Steady state actin polymerization in presence of gelsolin or CapG. Steady state pyrene fluorescence of 10 µM 4% pyrenyl-labeled Mg²⁺ F-actin capped with gelsolin at ratio 1:50 (triangles) or CapG at ratio 1:25 (circles) in presence of indicated amounts of profilin. The ratios are of capping protein to actin subunits.

FIGURE 6. Global fit of the rates of F-actin depolymerization in presence of various amounts of profilin and CapG. Dependence of the absolute value of initial depolymerization rate of 0.1 µM 10% pyrenyl-labeled Mg²⁺ F-actin on CapG concentration in presence (circles) or absence (triangles) of 40 µM profilin. Inset, dependence of the absolute value of initial depolymerization rate of 0.1 µM 10% pyrenyl-Mg²⁺ F-actin on profilin concentration in presence of 0 (squares), 0.5 (circles) or 1.0 (triangles) nM of CapG. Because the prior results showed no measurable effect
from re-association of subunits, latrunculin A was not utilized. The solid lines represent the global fitting of all the data in the figure to a single set of equilibrium dissociation constants.
Fig. 5

Fluorescence (cps x 1000)

Profilin (μM)
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