Role of Nucleotide Exchange and Hydrolysis in the Function of Profilin in Actin Assembly*

(Received for publication, January 31, 1996, and in revised form, March 18, 1996)

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Profilin, an essential G-actin-binding protein, has two opposite regulatory functions in actin filament assembly. It facilitates assembly at the barbed ends by lowering the critical concentration for actin polymerization and whether the acceleration of nucleotide exchange on G-actin by profilin participates in its function in filament assembly are the issues addressed here. We show that 1) profilin increases the treadmilling rate of actin filaments in the presence of Mg²⁺ ions; 2) when filaments are assembled from CaATP-actin, which polymerizes in a quasi reversible fashion, profilin does not promote assembly at the barbed ends and has only a G-actin-sequestering function; 3) plant profilins do not accelerate nucleotide exchange on G-actin, yet they promote assembly at the barbed end. The enhancement of nucleotide exchange by profilin is therefore not involved in its promotion of actin assembly, and the productive growth of filaments from profilin-actin complex requires the coupling of ATP hydrolysis to profilin-actin assembly, a condition fulfilled by Mg-actin, and not by Ca-actin.

Living cells undergo changes in shape and motile behavior by spatially and temporally controlled rearrangements of the actin cytoskeleton. In the physiological ionic conditions, F-actin is assembled at steady state in the cell medium. Changes in the F-actin/G-actin ratio, which occur in response to stimuli, are made possible by shifts in steady state, i.e. changes in the critical concentration for filament assembly. These changes are elicited by capping proteins and profilin (1, 2) and amplified by G-actin-binding proteins (3). A high level of capping of barbed ends maintains the high critical concentration of pointed ends in the cytoplasm. A steep energetic gradient is therefore created between the cell medium and the loci where uncapped barbed ends are nucleated at the plasma membrane. We understand that in this way capping of barbed ends in the cytoplasm is required for a more efficient local actin assembly. In support of this view, recent evidence indeed indicates that the level of motility in fibroblasts (4) and Dictyostelium (5) correlates with the level of barbed end capping. Similarly, the actin-based propulsive movement of Listeria results from the local creation and maintenance of new uncapped barbed ends at the bacterium surface, while filaments are capped in the bulk cytoplasm (6).

Profilin has unique properties among G-actin-binding proteins. Under physiological ionic conditions (Mg-actin, 0.1 M KCl), it binds G-actin tightly (K = 10⁷ M⁻¹ for vertebrate profilin 1) and participates in the establishment of the pool of unassembled actin when barbed ends are capped. In contrast, when barbed ends are uncapped, the participation of profilin-actin complex in filament assembly (7, 8) results in a decrease in critical concentration (2), i.e profilin then synergizes with the uncapping effect to promote more efficient barbed end actin assembly. We demonstrated that the profilin-induced decrease in critical concentration at the barbed ends could not be accounted for in the context of reversible actin assembly, and we proposed that the free energy of ATP hydrolysis associated with actin polymerization was used by profilin. However, no evidence was provided in that work (2) on the nature of the reactions that could support profilin function in barbed end assembly and the detailed pathways in which ATP binding and hydrolysis could be used. The property of profilin to increase the rate of nucleotide exchange on G-actin (9–12) might be involved. For instance, ATP hydrolysis and/or Pi release might be accelerated by profilin upon association of the profilin-actin complex to a filament barbed end. It was also suggested in a short review (13) that the enhancement of nucleotide exchange on G-actin itself might also be part of its function in the promotion of actin assembly at the barbed end.

The above issues are raised in the present work. We show that when the polymerization of ATP-actin is quasireversible (CaATP-actin), profilin does not promote barbed end assembly; hence, the coupling of ATP hydrolysis to actin polymerization is required in the function of profilin. Profilins from Arabidopsis thaliana promote assembly of MgATP-actin at the barbed ends, like vertebrate profilins, while being unable to enhance the rate of nucleotide exchange on G-actin, which demonstrates that the latter property is not used in the main biological function of profilin in living cells.

MATERIALS AND METHODS

Proteins—Actin was purified from rabbit muscle and isolated in the CaATP-G-actin form as described (14). Profilin was isolated from bovine spleen by poly-L-proline affinity chromatography as described (14). Actin was pyrenyl-labeled as described (15). Gelsolin was a generous gift from Dr Yukio Doi. Thymosin β4 (Tβ4) was purified from bovine spleen as described (2). Recombinant A. thaliana profilin 1 (vegetative form) and profilin 3 (floral form) were expressed in Escherichia coli and

1 The abbreviation used is: Tβ4, thymosin β4.
purified by poly-L-proline chromatography as described,2 followed by anion exchange chromatography (Resource Q, 6 ml, Pharmacia Biotech Inc.) using a linear gradient of NaCl in 20 mM Tris-Cl , pH 7.5. The purified profilins were then concentrated using centricon 10 (Amicon) and exchanged into the desired buffer.

Fluorescence Measurements—Actin polymerization was monitored by pyrene fluorescence, using a Sper F-actin spectrophotometer. A maximum amount of 1% pyrenyl-actin was present in all samples to minimize the bias in the data due to the fact that profilin does not bind to pyrenyl-labeled actin.

Polymerization of CaATP-actin was carried out in a Ca-F-polymerization buffer made up by adding 1 mM CaCl2, and 0.1 mM KCl to G-buffer (5 mM Tris-Cl, pH 7.5, 1 mM dithiothreitol, 0.2 mM ATP, 0.1 mM CaCl2, 0.01% NaN3). Polymerization of MgATP-actin was carried out in a Mg-F-buffer (2) made up by supplementing G-buffer with 2 mM MgCl2 and 0.1 mM KCl (physiological ionic conditions).

All experiments were performed using G-actin in which Cys374 was checked to be thoroughly reduced, to be sure that all of the actin was able to bind profilin with high affinity.

Steady-state fluorescence measurements of F-actin in the presence of Tjø, or profilin were carried out as described (2) following overnight incubation at room temperature in the dark.

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**RESULTS**

Rate of Filament Growth from MgATP-G-actin Subunits in the Presence of Profilin—The following experiment was designed to understand how the kinetic parameters for barbed end elongation where affected by profilin. The concentration of MgATP-G-actin complex, which participates in filament assembly, was kept constant at 0.5, and 1 μM in three series of experiments, while the concentration of free G-actin was varied (see "Materials and Methods"). Data, displayed in Fig. 1, show that in the presence of profilin-actin, MgATP-G-actin undergoes net positive barbed end growth at concentrations below the critical concentration measured in the absence of profilin in the control J (c) plot. The critical concentration of MgATP-G-actin was decreased at least 5-fold in the presence of 0.5 or 1 μM profilin-actin, in agreement with steady state measurements (2). This piece of data testifies that the decrease in critical concentration observed previously (2) was truly a decrease in ATP-G-actin steady state concentration. The slope of the J (c) plot was not modified in the presence of profilin-actin, which demonstrated that the rate constant for association of G-actin to barbed ends is not affected by the participation of profilin-actin in barbed end assembly. On the other hand, the extrapolated value of the dissociation rate constant of G-actin from filament ends (ordinate intercept of the J (c) plot) is decreased by profilin.

Profilin Increases the Treadmilling Rate of ActinFilaments—Treadmilling of actin filaments has been shown by Wegner (18) to result from the difference in critical concentra-

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2. H. E. M. Christensen, C. T. Tan, S. Ramachandran, U. Surana, C. H. Dong, and N. H. Chua, submitted for publication.
Profilin increases the turnover rate of actin filaments. A solution of 10 μM [3H]ADP-F-actin prepared as described under "Materials and Methods" was split in two samples supplemented or not with the indicated amount of profilin. A chase amount of ATP (0.2 mM) was applied to each sample at time 0. Aliquots were centrifuged at different times over a period of 8 h. The linear increase in [3H]ADP in the supernatant was measured and normalized to 1 for the control run in the absence of profilin. The increase in turnover rate in the presence of profilin is plotted as percentage increase of the rate measured in the control. Different symbols refer to independent experiments. The inset represents typical time courses of [3H]ADP increase in the supernatant in the absence (○) or in the presence (●) of 5 μM profilin.

Profilin Does Not Promote Actin Assembly at the Barbed End When the Polymerization of ATP-actin Is Quasireversible (CaATP-actin)—We have previously shown (2) that in the presence of ADP profilin was not able to participate effectively in assembly at the barbed end and to decrease the critical concentration but behaved strictly as an ADP-G-actin-sequestering protein. This finding was interpreted as evidence that ATP hydrolysis was involved in the function of profilin in barbed end assembly. If polymerization of ATP-actin was reversible, profilin would be expected to behave as a purely ATP-G-actin-sequestering agent. This prediction was tested in the following way. It is well known that the structural and functional properties of actin are dependent on the nature of the divalent metal ion (Mg2+ or Ca2+) that interacts with the β- and γ-phosphates of ATP bound to actin (for reviews, see Refs. 19 and 20). In particular, nucleation of filament is facilitated, and the critical concentration for filament assembly is lower, when Mg2+ (which is the physiologically actin-bound metal ion) is bound to ATP (21–23). The rate and the mechanism of ATP hydrolysis on F-actin are also different on Mg-actin and Ca-actin (24, 25), a conclusion supported by recent molecular modeling studies of the metal-nucleotide binding site on G-actin (26). Our earlier work showed that in the polymerization of MgATP-actin, ATP hydrolysis appeared closely coupled to the growth of filament in a large range of monomer concentration (24, 25). The nature of the nucleotide bound to the barbed end terminal subunits varied with the concentration (c) of MgATP-G-actin. Essentially ATP or ADP-P, was bound well above the critical concentration, while at steady state the proportion of terminal F-ADP subunits increased. As a result, the dependence of the rate of filament growth on monomer concentration (J (c) plot) curved downwards in the region of the critical concentration, as the proportion of rapidly dissociating terminal F-ADP subunits increased. In the presence of Pn, the J (c) plot was identical at high rates of growth but remained linear and extrapolated to a somewhat lower critical concentration because terminal F-ADP-P subunits dissociate more slowly than F-ADP subunits (27). Similarly, phosphate analogs BeF3− and AlF4− decreased the critical concentration at the barbed ends (28). In contrast, in the polymerization of CaATP-actin, ATP was hydrolyzed very slowly, and filaments grew with a large cap of ATP subunits even in a range of low G-actin concentrations (25). The J (c) plot displayed no appreciable change in slope at the critical concentration, as a result of the exclusive presence of F-ATP ends at the critical concentration (24). In other words, due to the strong uncoupling between actin assembly and ATP hydrolysis, the polymerization of CaATP-actin may be considered as quasireversible. Accordingly, the treadmilling rate of filaments assembled from CaATP-actin in the presence of only KCl appears to be very slow (29, 30). We confirmed this observation and further checked that this slow rate was not affected by profilin, in contrast with the result obtained for Mg-actin. Treadmilling is triggered by the difference in critical concentrations between the two ends of the filament, which is established because ATP hydrolysis is associated with actin incorporation into the filament (18). If the polymerization of CaATP-actin is quasireversible, critical concentrations are expected to be identical at the two ends, as is observed for the reversible polymerization of ADP-actin (31). Fig. 3 shows that this is indeed the case. At most 10% difference in critical concentrations at the barbed and pointed ends was measured for Ca-actin polymerized in the presence of 0.1 mM KCl, while in a parallel experiment carried out with Mg-actin under the same ionic conditions, the well known 5-fold difference in critical concentrations between the two ends was recorded. In an early work using electron microscopy to measure the rates of growth of actin filaments at the barbed and pointed ends, Pollard and Mooseker (32) also noticed that the critical concentrations were very similar at the two ends in 20 mM KCl and very different in 5 mM MgSO4 + 0.1 mM KCl.
Although these conditions were not, at that time, explicitly referred to as describing Ca-actin and Mg-actin assembly, respectively, the results obtained (32) are similar to the ones reported here. The ability of profilin to depolymerize F-actin was further investigated for Ca-actin or Mg-actin comparatively, with capped and uncapped barbed ends. The data, displayed in Fig. 4a, show that upon addition of increasing amounts of profilin to Ca-F-actin, only F-actin depolymerization due to “sequestration” of Ca-G-actin by profilin was observed, both with capped and uncapped barbed ends. The decrease in F-actin at steady state, i.e., the increase in the concentration of profilin-actin complex, (PA), was linear versus the total amount of profilin P₀, as described by the equation,

\[ [PA] = [P₀] \frac{A_c}{A_c + K_{dP₀}} \]  

where \( A_c \) represents the critical concentration and \( K_{dP₀} \) the equilibrium dissociation constant for the profilin-actin complex. Since the critical concentrations for assembly of Ca-actin are almost identical at the two ends, almost the same slopes were observed whether the barbed ends were free or capped. The derived value of \( K_{dP₀} \) for binding of profilin to CaATP-actin was 0.8 \( \mu M \).

In contrast, when the same experiment was done with Mg-actin, profilin only sequestered G-actin when barbed ends were capped by gelsolin. With uncapped barbed ends, no effective depolymerization of F-actin was observed upon addition of profilin, as previously demonstrated and explained by the profilin-induced decrease in critical concentration for assembly of Mg-actin at the barbed ends.

The interpretation of the above fluorescence data in the presence of profilin (Fig. 4a) in terms of reliable estimates of assembled and unassembled actin is questionable, since profilin does not bind to pyrenyl-actin (17). For that reason, one would logically expect that the fluorescence of samples of F-actin containing different concentrations of profilin be the same in all samples at equilibrium, because pyrenyl-labeled actin partitions between F-actin and free G-actin, while the profilin-actin complex contains only unlabeled actin (33). As discussed under “Materials and Methods,” the specific fluorescence of F-actin should increase as the concentration of profilin-actin complex increases. In fact, we checked that the fluorescence readings shown in Fig. 4a remained stable over a period of 12–24 h following the addition of profilin to F-actin, and increased thereafter very slowly, over a period of 2–3 days, to reach the expected constant fluorescence level at all profilin concentrations. We propose that the reason why pyrenyl fluorescence measurements truly reflect the amount of F-actin in the presence of profilin is the following. The addition of profilin to preassembled F-actin results in endwise depolymerization of pyrenyl-labeled filaments until steady state is reached, at which point the remaining filaments keep their initial intrinsic fluorescence, while the monomer pool consists of G-actin at the critical concentration (with a higher pyrenyl-labeling ratio than the filaments) and unlabeled profilin-actin complex. Since monomer-polymer exchange reactions are very slow, the labeling of filaments remains essentially unchanged for many hours. Over much longer periods of time (days), pyrenyl-actin redistributes over F-actin and G-actin. To check this interpretation of the data in Fig. 4a, a sedimentation assay of assembled and unassembled actin in the presence of profilin was carried out. Mg-actin was assembled at 6 \( \mu M \) in the presence or absence of gelsolin, and different amounts of profilin were added to the two series of samples. The samples were centrifuged at 400,000 \( \times \) g after 16 h of incubation. The amount of unassembled actin in the supernatant was evaluated by SDS-polycrylamide gel electrophoresis followed by Coomassie Blue staining. The data shown in Fig. 4b demonstrate that when barbed ends were capped, profilin caused actin depolymerization in proportion to the amount of profilin added. 80% of total actin was found in the supernatant in the presence of 10 \( \mu M \) profilin. This is consistent with a value of 0.66 \( \mu M \) for \( K_{dP₀} \) according to Equation 4, using \( C_C = 0.5 \mu M \). In contrast, when barbed ends were free, actin was barely detectable in the supernatant. Note that if profilin displayed a Mg-actin sequestering activity when barbed ends are free (\( C_{C} = 0.1 \mu M \)) one would expect to measure an amount of unassembled actin equal to \( C_{C} + [F]C_{G}^{\text{un}} + K_{competitive} \) in the supernatant. In the presence of 10 \( \mu M \) profilin, and using \( K_{dP₀} = 0.66 \mu M \), the amount of unassembled actin should be 0.1 + 10(0.1/0.76) = 1.42 \( \mu M \), which represents 24% of total actin in the experiment shown in Fig. 4b. Clearly the amount of actin in the supernatant is much lower than 5% of total actin, which demonstrates that \( C_{C}^{\text{un}} \) is lowered by profilin. In conclusion, the sedimentation data confirm that the fluorescence measurements in the presence of profilin truly reflect the amount of F-actin.

The difference between the effects of profilin in the assembly of Ca-actin and Mg-actin at the barbed ends is further illustrated in the presence of thymosin \( \beta_{s} \) in Fig. 4c. Profilin promoted assembly off the pool of \( T_{2} \)-Mg-actin complex, due to the decrease in critical concentration, as previously demonstrated (2), but only depolymerization of Ca-F-actin by profilin was observed in addition to the depolymerization elicited by \( T_{2} \).

In summary, the above results demonstrate that when ATP-actin polymerizes in a reversible fashion, profilin only shows a G-actin-sequestering activity. The same result had been obtained with ADP-actin (2). The new evidence with CaATP-actin confirms the view that ATP hydrolysis, and not simply ATP, is necessary to explain that profilin lowers the critical concentration at the barbed end, thereby promoting actin assembly off the pool of sequestered actin.

A consequence of the above results is that when Mg\(^{2+}\) ions are added to Ca-F-actin assembled at steady state (with free barbed ends) in the presence of profilin, repolymerization of actin occurs to a much larger extent than expected from the
Fig. 4. The complex of profilin with CaATP-G-actin does not productively participate in assembly at the barbed ends and does not affect the critical concentration. A, fluorescence measurements. Solutions of 3 μM F-actin (1% pyrenyl-labeled) assembled in Ca-F-buffer (circles) or Mg-F-buffer (squares) in the presence (open symbols) or absence (closed symbols) of 10 nM gelsolin were incubated for 16 h in the presence of profilin at the indicated concentrations. B, sedimentation measurements. Samples of 6 μM F-actin, assembled in Mg-F-buffer in the absence (A) or presence (B) of 15 nM gelsolin, were supplemented with profilin at the indicated concentrations, incubated for 16 h and centrifuged at 400,000 × g, 20°C, for 30 min. The supernatants were submitted to SDS-polyacrylamide gel electrophoresis. Note that the concentration of unassembled actin is insignificant when barbed ends are free, while a large amount of actin is depolymerized when barbed ends are capped. The rightmost lane shows the total amount of actin in each sample before centrifugation. C, profilin does not lower the critical concentration of CaATP-actin at the barbed end. Squares, a solution of F-actin (1.5 μM, 1% pyrenyl-labeled) assembled in Mg-F-buffer in the presence of 3 μM profilin was supplemented with profilin. Pyrenyl fluorescence was monitored after 16 h of incubation.

The Ability of Profilin to Increase the Rate of Nucleotide Dissociation from Actin Is Not Required for Its Function in Actin Assembly—In showing that the nature of the nucleotide bound to the barbed end terminal subunits is important in profilin function, the above results suggest that when the profilin-actin complex associates to a barbed end, profilin might modify the rates of nucleotide exchange or hydrolysis or of Pi release on that terminal subunit, which might mediate the function of profilin in actin assembly. It is therefore important to establish whether the property of profilin to decrease the critical concentration for actin assembly at the barbed end is or is not linked to its property to enhance the rate of nucleotide dissociation from actin. We have been helped, in this issue, by a systematic search among different profilin species in nature. Profilins are ubiquitous proteins in eukaryotes from protozoa to vertebrates. They seem to share structural and functional properties regarding activity binding, although the sequences show a moderate degree of similarity. Three large categories of known profilins can be distinguished, the lower eukaryote profilins (amoebae, molds, yeasts, and others), the vertebrate profilins, and the plant profilins. The degree of sequence homology within each class is at least 70%, while it is only 25–40% between profilins from different classes. While profilins from Acanthamoeba castellanii (9) and from vertebrates (10–12) have been shown to increase the rate of nucleotide exchange on G-actin, no corresponding information exists regarding plant profilins. In the plant Arabidopsis thaliana, four profilin species have been found, two vegetative forms (profilins 1 and 2) and two floral forms (profilins 3 and 4). The sequences of profilins 1 and 2, on the one hand, and of profilins 3 and 4, on the other hand, show 89 and 91% identity, respectively. The vegetative and floral forms are 71–75% identical. The compared sequences of profilins from bovine spleen, A. castellanii, birch pollen, and A. thaliana are shown in Table I. Arabidopsis profilins show 90% homology with birch pollen profilin. The alignment shows that plant profilins are closer to amoeba than to vertebrates and are characterized by an insertion of 3–6 amino acids after position 15, i.e. between helix 1 and strand 1, opposite to the actin-binding region in the three-dimensional structure.

We have assayed A. thaliana profilins 1 and 3 for their binding to G-actin, Mg-G-actin sequestration (with capped barbed ends), promotion of MgATP-actin assembly at the barbed ends, and effect on the rate of nucleotide exchange on G-actin. In low ionic strength buffer, formation of the plant profilin-actin complex was associated with a quenching of tryptophan fluorescence, quantitatively identical (25% quenching of actin fluorescence with both profilins 1 and 3, data not shown) to the one observed upon interaction of bovine spleen profilin with G-actin (14). From the profilin concentration dependence of the fluorescence change (data not shown), equilibrium dissociation constants of 1.8 and 2.3 μM were derived for small difference in critical concentration for assembly of Ca-actin (0.5 μM) and Mg-actin (0.1 μM) (data not shown). This result is quantitatively explained by the exchange of Mg<sup>2+</sup> for bound Ca<sup>2+</sup> on G-actin, which leads to different steady state conditions and to a change in the function of profilin from sequestering to assembly-promoting agent. This enhanced polymerization has previously been observed by others (34, 35) and was attributed to a dissociation of the profilin-actin complex by Mg<sup>2+</sup> ions. In fact Mg<sup>2+</sup> ions do not decrease the affinity of profilin for G-actin, as shown here (Fig. 4a) as well as in previous works (12, 14). The present interpretation hence provides a satisfactory account of the observed reaction, consistent with the above differences in polymerization of CaATP-actin and MgATP-actin.
prolfin 1-actin and profilin 3-actin complexes, respectively. Hence, plant profilins have a 5-fold lower affinity than bovine spleen profilin for G-actin. The affinity of plant profilins for G-actin was also derived from the G-actin sequestration assay. Data displayed in Fig. 5 show that under physiological ionic conditions (2 mM MgCl₂, 0.1 M KCl) and when barbed ends are capped, plant profilins cause depolymerization of F-actin as described by Equation 4, with a lower efficiency than bovine spleen profilin. Kₚ values of 1.8 and 2.6 mM were derived for profilins 1 and 3, respectively (as compared with Kₚ = 0.45 mM found for bovine spleen profilin in a parallel assay). In contrast, Fig. 5 shows that when barbed ends were not capped and a pool of unpolymerized actin was created by Tₐ (20 mM) in solution, plant profilins, like vertebrate profilin, promoted actin assembly off the pool of Tₐ-actin complex, an effect mediated by the decrease in critical concentration at the barbed end (2). Therefore, despite their lower affinity for G-actin, plant profilins behave in a manner identical to that of bovine spleen profilin, regarding their different effects in actin assembly when barbed ends are capped versus uncapped. On the other hand, when the rate of nucleotide exchange was assayed under physiological ionic conditions by monitoring the kinetics of dissociation of bound eATP upon addition of ATP (12), no change in the first order rate constant was observed upon addition of saturating amounts of plant profilins, while a 50-fold increase from 0.04 s⁻¹ to 2 s⁻¹ was observed when the assay was carried out in parallel with spleen profilin. Although the profilin-actin interface is probably similar for bovine spleen and plant profilins, as judged from the tryptophan fluorescence data, plant profilins appear unique among profilins from different species in being unable to enhance the rate of nucleotide dissociation from monomeric actin. We suggest that these profilins are therefore unlikely to enhance P₁ or nucleotide dissociation from the terminal F-actin subunit as well. In conclusion, the ability of profilins to decrease the critical concentration for actin assembly at the barbed end is not linked to their effect on nucleotide exchange on actin.

**DISCUSSION**

The present results bring new support to the view that profilin lowers the critical concentration for actin assembly at the barbed end and thereby enhances assembly at this end. Direct kinetic evidence is provided for active growth of filaments taking place at concentrations of MgATP-G-actin well below the critical concentration, when profilin-actin complex is present even in low amounts (0.5 μM). The presence of profilin-actin complex however does not modify the rate constant for association of MgATP-G-actin to the barbed ends. As a consequence of the decrease in critical concentration at the barbed ends, the treadmilling rate, which reflects the energetic difference between the two ends, is increased (25–30%) by profilin.

We had proposed (2) that ATP hydrolysis associated with actin assembly was involved in the function of profilin. This proposal relied on the idea that an isoenergetic model for growth of actin filaments from either G-actin alone or G-actin + profilin could not theoretically account for the observed decrease in critical concentration, and 2) on the experimental evidence for the sole G-actin sequestering activity of profilin in the presence of ADP. The present results bring more experimental support to the view that ATP hydrolysis, rather than ATP binding, at the barbed ends of actin filaments at steady state plays a role in profilin function. Advantage was taken of the different properties of CaATP-actin and MgATP-actin (see Ref. 19, for review). The hydrolysis of ATP following incorporation of a CaATP-actin in the filament is so slow that its polymerization can be considered as quasireversible, as demonstrated by the almost identical critical concentrations at the barbed and pointed ends and the barely detectable treadmilling at steady state. The present data, showing that profilin behaves with respect to Ca-actin as a purely G-actin-sequestering protein whether barbed ends are capped or uncapped and therefore does not affect the treadmilling rate, bring more support to the role of ATP hydrolysis in the function of profilin in barbed end assembly.

How is ATP hydrolysis used by profilin in barbed end assem-

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**TABLE I**

Alignment of selected profilin sequences

| Bovine          | Ac.   | White Birch |
|-----------------|-------|-------------|
| AGWAYIDNLM----- | ADG-T  | CQDAAVIGKDSPSVWAAPGKTFVNI |
| Ac. 1           | -SWTVTVDTL----- | VGT-GAVYQAALGL-D-GNTWATSAFVAT- |
| White Birch     | MSWTVYDIDLACDSNASLASSIVGAY- | D-GSWAQSFSQPF- |
| ATROPE1         | MSWQYSVDHMCLVENN---HHTAAA-ILGQ-D-GSWAQSFKFQVL- |
| ATROPE2         | MSWQYSDHMCLVEVNN---HHTHAA-IPFG-D-GSWAQSAFPQVL- |
| ATROPE3         | MSWQTVEDHMLCDVGDGGQHHTAAA-IVGAY-D-GSWAQSANSFPQF- |
| ATROPE4         | MSQWYVEDHMLCDVGDGGQHHTAAA-IGH-D-GSWAQAASFPQF- |

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ably? To examine whether profilin accelerated hydrolysis or Pi release on a terminal actin subunit, following profilin-actin association to the filament barbed end, we have shown that plant profilins, which do not affect the rate of nucleotide exchange on actin, still lower the critical concentration at the barbed end. Therefore, the main physiological function of profilin does not necessitate a faster rate of metal/nucleotide dissociation from actin, a conclusion opposite to previous models (13, 36, 37).

The present work combined with older results concerning the mechanism of ATP hydrolysis associated with the polymerization of MgATP-actin leads to a model for profilin function, proposed in Scheme I. Nucleotide hydrolysis is highly favored on an ATP-F-actin subunit immediately adjacent to an ADP-F-actin subunit (25). The association of MgATP-G-actin-profilin complex to an F-ATP end has little chance to be followed by ATP hydrolysis; therefore, in this case profilin dissociation is not enhanced. In contrast, association of MgATP-G-actin-profilin to an F-ADP end will trigger ATP hydrolysis on the newly added subunit, which causes profilin dissociation, due to the lower affinity of profilin for ADP-actin than for ATP-actin (2, 14). In this model, the presence of terminal ADP-actin subunits at steady state and the coupling of ATP hydrolysis to association of profilin-actin to barbed ends are necessary for the net incorporation of actin in filaments from profilin-actin units. This is satisfied under physiological ionic conditions (MgATP-actin, 2 mM MgCl₂, 0.1 M KCl), in which ATP hydrolysis appears closely coupled to filament growth in a range of concentrations around the critical concentration. As a result, treadmilling is relatively efficient under these conditions and is enhanced by profilin. In contrast, ATP hydrolysis is largely uncoupled from the assembly of Ca-actin, and the fact that terminal subunits are only CaATP-F-actin at equilibrium does not allow any net incorporation of actin from profilin-actin units. Accordingly, treadmilling is low for Ca-actin and remains unchanged in the presence of profilin.

From a structural point of view, it is interesting to note that profilin-actin complexes made from different profilin species (amoebae, plant, vertebrates), which display different equilibrium dissociation constants, are equally able to participate in assembly at the barbed ends. Indeed, the different effect of these profilins on nucleotide dissociation is likely to be linked to slight differences in the structure of actin in the complex, i.e. a more or less pronounced opening of the cleft containing metal-nucleotide. The fact that despite these different structures all profilin-actin complexes equally well participate in barbed end assembly indicates that upon association of a profilin-actin unit to the barbed end, the constraints imposed by the actin-actin contacts in the polymer, especially at the interface between the bottom of subdomain 1 of the barbed end terminal subunit and the tops of subdomains 2 and 4 of the newly added subunit, are sufficient to counteract the profilin-induced deformation of the

**Fig. 5.** Plant profilins behave like vertebrate profilins in promoting assembly of MgATP-actin at the barbed ends and like sequestering actin when barbed ends are capped. A, capped barbed ends. F-actin (1.5 mM, 1% pyrenyl-labeled) was assembled in MgF-buffer containing 5 nM gelsolin, and supplemented with bovine spleen profilin (●), A. thaliana profilin 1 (○), or A. thaliana profilin 3 (▲) at the indicated concentrations. Pyrenyl fluorescence was monitored after 16 h incubation. B, free barbed ends. F-actin (1.5 mM 1% pyrenyl-labeled) was assembled in MgF-buffer containing 20 μM TpG, and supplemented with bovine spleen profilin (●), A. thaliana profilin 1 (○), A. thaliana profilin 3 (▲) at the indicated concentrations.
The very high homology between Arabidopsis profilins and birch pollen profilin indicates that most probably all plant profilins fail to enhance the rate of nucleotide exchange on G-actin. Interestingly, it has been recently shown that 1) birch pollen profilin can functionally replace mammalian profilin in living cells (35); 2) Arabidopsis profilins can replace yeast profilin; 3) the failure of profilin-deficient Dictyostelium cells to form a fruiting body (38) can be rescued by maize pollen profilin (39). These results, combined with the present data, demonstrate that the function of profilin in vivo is not mediated by its effect on nucleotide exchange on G-actin.

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