Synergy between Actin Depolymerizing Factor/Cofilin and Profilin in Increasing Actin Filament Turnover*

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The mechanism of control of the steady state of actin assembly by actin depolymerizing factor (ADF)/cofilin and profilin has been investigated. Using Tβ4 as an indicator of the concentration of ATP-G-actin, we show that ADF increases the concentration of ATP-G-actin at steady state. The measured higher concentration of ATP-G-actin is quantitatively consistent with the increase in treadmilling, caused by the large increase in the rate of depolymerization from the pointed ends induced by ADF (Carlier, M.-F., Laurent, V., Santolini, J., Didry, D., Melki, R., Xia, G.-X., Hong, Y., Chua, N.-H., and Pantaloni, D. (1997) J. Cell Biol. 136, 1307–1322). Experiments demonstrate that profilin synergizes with ADF to further enhance the turnover of actin filaments up to a value 125-fold higher than in pure F-actin solutions. Profilin and ADF act at the two ends of filaments in a complementary fashion to increase the processivity of treadmilling. Using the capping protein CapZ, we show that ADF increases the number of filaments at steady state by 1.3-fold, which cannot account for the 25-fold increase in turnover rate. Computer modeling of the combined actions of ADF and profilin on the dynamics of actin filaments using experimentally determined rate constants generates a distribution of the different actin species at steady state, which is in quantitative agreement with the data.

Profilin and actin depolymerizing factor (ADF)/cofilin are essential actin-binding proteins that play a key role in the control of actin dynamics and actin-based motility processes. These two proteins fulfill their functions by interacting with actin in different ways.

Profilin binds ATP-G-actin specifically. Although profilin does not bind F-actin, the profilin-ATP-G-actin complex contributes to filament assembly, at the barbed end exclusively (1–4). When filaments are assembled at steady state in the presence of profilin, ATP-G-actin and profilin-ATP-G-actin can both be considered as polymerizable actin monomers undergoing monomer-polymer exchange at the barbed ends, therefore profilin lowers the steady-state concentration of ATP-G-actin (3). When all barbed ends are blocked by capping proteins, profilin only sequesters G-actin.

ADF/cofilin, on the other hand, specifically recognizes the ADP-bound form of both G- and F-actin. Hence, ADF too participates in filament assembly, but in a manner different from profilin; ADF-bound F-actin dissociates much faster than F-actin from the pointed ends of actin filaments, which results in a large enhancement of filament treadmilling (5). Actin-based motility of Listeria monocytogenes in the cytoplasm of infected cells, or the forward movement of the leading edge, which are supported by the treadmilling of actin filaments, are therefore enhanced by ADF (6). The effect of ADF on filament turnover is accompanied by the partial depolymerization of F-actin. The mechanistic Scheme I (see Fig. 1), which was proposed (5) to account for the effects of ADF on actin dynamics, implies the following. First, the steady-state pool of unassembled actin consists of ADF-G-actin and ATP-G-actin both in the free and ADF-bound states. Since ADF has a 100-fold higher affinity for ATP-G-actin than for ADP-G-actin under physiological ionic conditions (5, 7), a large proportion of ADP-G-actin is thought to be in complex with ADF, while ATP-G-actin is thought to be essentially free. Next, ADF is in rapid equilibrium with G-actin (7), so that nucleotide exchange, which is strongly inhibited on ADF-ADP-G-actin complex, occurs on free ADP-G-actin so as to regenerate ATP-G-actin which polymerizes better than ADP-G-actin. Finally, the establishment of a faster treadmilling by ADF implies that the steady-state concentrations of ADF-G-actin and ATP-G-actin increase until the fluxes of nucleotide exchange and polymerization onto barbed ends become equal to the large dissociation flux of ADF-ADP-actin from the pointed ends.

To assess the validity of Scheme I (shown in Fig. 1) and to model the effects of ADF on actin dynamics, it is necessary to elaborate methods allowing to measure the concentrations of the different G-actin species at steady state in the presence of ADF. In particular, the steady-state concentration of ATP-G-actin, [G₇], is an important parameter, since it directly controls the treadmilling flux, i.e. the rate of subunit addition to barbed ends at steady state, which equals k₋¹[B][G₇] – Cᵥ[β]), where k₋¹ is the rate constant for association of ATP-G-actin to barbed ends, [B] is the concentration of non-capped barbed ends, and Cᵥ[β] is the critical concentration for actin assembly at the barbed ends.

Once the steady-state parameters for actin assembly in the presence of ADF alone are known, the complexity of the system can be increased by adding profilin in addition to ADF, and using the same methods, to determine how the dynamics of actin filaments are affected by the presence of the two proteins, and discover which new regulatory properties arise from the cumulated effects of the two proteins.

The present work focuses on the above issues. We show that ADF causes a 3-fold increase in the steady-state concentration of ATP-G-actin, which accounts for the enhancement of treadmilling within Scheme I (see Fig. 1). We find that profilin synergizes with ADF to increase the turnover of filaments. In
the presence of both ADF and profilin, the rate of treadmilling is increased 125-fold. The ADF and profilin dependence values of the rate of treadmilling are quantitatively reproduced by computer modeling of Scheme II (shown in Fig. 9), using the experimental values (4, 5, 7) of the rate parameters for interaction of ADF and profilin with actin. In vivo consequences of the synergy observed in vitro between ADF and profilin are discussed.

MATERIALS AND METHODS

Proteins—Actin was purified from rabbit muscle acetone powder (8) and isolated as Ca-ATP-actin by gel filtration (9) on Sephadex-G200 in G buffer (5 mM Tris-Cl, pH 7.6, containing 0.2 mM ATP, 0.1 mM MgCl₂, 1 mM dithiothreitol, and 0.01% NaN₃). Mg²⁺ exchanging for bound Ca²⁺ on actin was performed as described (4). Thymosin β₁ was purified from bovine spleen as described (10). Vertebrate profilin I was also purified from bovine spleen by poly-L-proline chromatography (10). Recombinant Arabidopsis thaliana profilin I was expressed in Escherichia coli and purified by poly-L-proline chromatography as described (4). Recombinant ADFs (human ADF and A. thaliana ADF₁) were expressed in E. coli and purified by DEAE-cellulose and SP-trisacryl ion exchange chromatography as described (5). Protein concentration was determined spectrophotometrically using the following values of the extinction coefficients: ε₅₈₀ = 0.617 cm⁻¹ for actin; ε₁₄₈ = 0.015 μM⁻¹ cm⁻¹ for profilin; ε₁₄₈ = 0.89 cm⁻¹ for ADF₁, ε₁₄₈ = 0.63 cm⁻¹ for human ADF. The concentration of Th₁ was determined using the bicinechonic acid assay with bovine serum albumin as a standard.

Measurement of the Concentration of Unassembled Actin at Steady State—The concentration of unassembled actin was determined by a sedimentation assay. Actin was polymerized at pH 7.5 in the presence of 2 mM MgCl₂ and 0.1 mM KCl, and supplemented with ADF, profilin, or Th₁ as indicated. Samples containing no ADF were centrifuged at 400,000 × g, 20°C, for 30 min, after a 16-h incubation. Samples containing ADF were centrifuged similarly except within 15–30 min following addition of ADF, since the steady state is reached rapidly in the presence of ADF and the consumption of ATP is high (5), precluding long incubation times. It was verified that results identical to those presented here were obtained in the presence of an ATP-regenerating system (5 mM creatine phosphate, creatine kinase). The concentration of unassembled actin in the supernatants was determined by scanning the Coomassie Blue-stained SDS gels of electrophoresed samples (Arcus II, Agfa Corp., Orangeburg, NY) and comparing to actin standards electrophoresed on the same gel. The NIH Image analysis program was used.

Alternatively, the concentration of unassembled actin was determined using NBD-labeled actin and reading the fluorescence of NBD-actin in the supernatant and comparing with standards. This method can be used even in the presence of ADF, because once separated from F-actin by sedimentation, unassembled actin is essentially ATP-G-actin, which has a low affinity for ADF. Therefore, the possibility of quenching of NBD-G-actin fluorescence by ADF (5) is avoided.

Measurement of the Treadmilling Rate of Actin Filaments—The rate of filament treadmilling was estimated using two different methods (5), as follows. First, measurement of the rate of ATP hydrolysis that accompanies the association of ATP-G-actin subunits to the barbed ends at steady state yields the treadmilling rate. G-actin (15 μM) was equilibrated in G buffer containing γ-³²P-ATP (Amersham Pharmacia Biotech), and polymerized. When steady state was reached, the solution was split into several samples supplemented with the desired concentrations of ADF and profilin. The steady state rate of ATP hydrolysis was measured by removing 50-μl aliquots from the solution at intervals, for periods of up to 6 h, quenching the reaction in 10 mM molybdate, 1 N HCl and processing for extraction of the γ-³²P-labeled phosphomolybdate complex (5). Second, the turnover of filaments was estimated by measuring the rate at which fluorescently labeled e-ADF-F-actin filaments assembled from e-ATP-G-actin subunits became nonfluorescent ADP-F-actin filaments following addition of ATP in the medium, via the consecutive steps of dissociation of e-ADF-actin from the pointed end, exchange of ATP for bound e-ADF on G-actin (thus the fluorescence of e-ADF is 5-fold lower in the free than in the actin-bound state), and association of ATP-G-actin to barbed ends. The experiments were carried out at a concentration of 15 μM e-ATP-F-actin polymerized under physiological ionic conditions in the presence of 50 μM e-ADF, and supplemented with ADF and/or profilin, as indicated, 10 min before the addition of 0.5 mM ATP in the medium. The fluorescence of e-ADF was monitored at 20°C in a Spex spectrofluorimeter with excitation and emission wavelengths of 350 and 410 nm, respectively.

Measurement of the Concentration of ATP—The concentration of ATP was measured by removing 50-μl aliquots from the solution at intermediate times, quenching the reaction in 10 mM molybdate, 1 N HCl and processing for extraction of the γ-³²P-phosphomolybdate complex (5). Second, the turnover of filaments was estimated by measuring the rate at which fluorescently labeled e-ADF-F-actin filaments assembled from e-ATP-G-actin subunits became nonfluorescent ADP-F-actin filaments following addition of ATP in the medium, via the consecutive steps of dissociation of e-ADF-actin from the pointed end, exchange of ATP for bound e-ADF on G-actin (thus the fluorescence of e-ADF is 5-fold lower in the free than in the actin-bound state), and association of ATP-G-actin to barbed ends. The experiments were carried out at a concentration of 15 μM e-ATP-F-actin polymerized under physiological ionic conditions in the presence of 50 μM e-ADF, and supplemented with ADF and/or profilin, as indicated, 10 min before the addition of 0.5 mM ATP in the medium. The fluorescence of e-ADF was monitored at 20°C in a Spex spectrofluorimeter with excitation and emission wavelengths of 350 and 410 nm, respectively.

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Simulation of the Turnover of Actin Filaments in the Presence of ADF and Profilin Scheme II (Fig. 9)—The concentrations of the different G-actin species coexisting with actin filaments at steady state in the presence of ADF and profilin can be determined by computer modeling of the establishment of steady state through the following set of kinetic steps, which describe Scheme I. ADF and profilin are considered in rapid equilibrium binding with ATP-G-actin and ADP-G-actin. The concentration of barbed ends, $F_b$, is constant and is not affected by ADF or profilin. On the other hand, pointed ends can be unliganded ($F_p$) or ADF-bound ($F_pADF$), with different dissociation rate constants and $F_p + F_pADF = F_p$ constant. The concentrations of total actin (polymerized and non-polymerized) and of free ADF were initially set. The total concentration of ADF at steady state was derived as the sum of the concentrations of free and ADF-bound species. The concentration of polymerized actin was derived as the difference between total actin and the sum of all forms of G-actin at steady state. The molar fraction of ADF-bound F-actin was equal to the fraction of ADF-bound pointed ends, $[F_pADF]/[F_p] + [F_p'ADF]$.

The following set of rapid equilibria and kinetic steps were used in the modeling, where T, D, and P represent ATP-G-actin, ADP-G-actin, and profilin, respectively.

\[
\begin{align*}
D + ADF & \rightleftharpoons K_D DADF \\
T + ADF & \rightleftharpoons K_T TADF \\
D + P & \rightleftharpoons K_P DP \\
T + P & \rightleftharpoons K_P TP
\end{align*}
\]

1. Rapid equilibria.

\[
F_p + ADF \rightleftharpoons T-P \quad (h_k) k_{\text{DF}} \]

2. Kinetics of interaction of ADF with F-actin, in particular pointed ends.

\[
\begin{align*}
F_p + T & \rightleftharpoons F_p + T (k_{\text{FT}}) \\
F_p + T-P & \rightleftharpoons F_p + P (k_{\text{FTP}}) \\
F_p + TADF & \rightleftharpoons F_p + ADP (k_{\text{FADP}}) \\
F_p + D & \rightleftharpoons F_p + D (k_{\text{FD}}) \\
F_pADF + DADF & \rightleftharpoons F_pADF + DADF (k_{\text{FADPDADF}}) \\
F_p + T & \rightleftharpoons F_p + T (k_{\text{FT}}) \\
F_p + D & \rightleftharpoons F_p + D (k_{\text{FD}})
\end{align*}
\]

3. Association and dissociation processes at filament ends.

\[
\begin{align*}
D & \rightarrow T (h_k) \\
D-P & \rightarrow T-P (h_k') \\
DADF & \rightarrow TADF (k_{\text{FTP}})
\end{align*}
\]

4. Nucleotide exchange on G-actin.

\[
\begin{align*}
\text{Scheme II.}
\end{align*}
\]

The values of equilibrium and rate constants used in the modeling are listed in Table I.

The above reactions are used principally to derive the steady-state concentrations of D and T. Hence, for simplicity, the interaction of D with barbed ends could be omitted because D polymerizes with the same critical concentration at the two ends. Similarly, the interaction of T with pointed ends could be omitted because essentially the barbed ends contribute to the steady-state value of T. The simulated curves (Fig. 10) have been checked not to be significantly different when those reactions were omitted. However, the effect of profilin is accounted for in a more realistic fashion by considering the latter reaction.

RESULTS

ADF Causes an Increase in the Steady-state Concentration of ATP-G-actin—It has been established that the addition of ADF to an F-actin solution results in binding of ADF to F-actin, followed by partial depolymerization of F-actin (5, 7). The steady-state concentration of unassembled actin varies between 0.5 and 2.5 $\mu$M (for Arabidopsis ADF) and between 1 and 6 $\mu$M (for human ADF) as pH is increased from 6.8 to 8.2 (7). To quantify the concentration of ATP-G-actin, which actively participates in barbed end growth at steady state, use was made of thymosin $\beta_4$ as a G-actin sequestering protein that selectively binds ATP-G-actin (13). Addition of increasing amounts of $T\beta_4$ to F-actin results in depolymerization of F-actin as the $T\beta_4$-ATP-G-actin (TA) complex is formed. The increase in [TA] was quantitated by analysis of the gel patterns of unassembled actin present in the supernatants of sedimented samples of F-actin assembled in the presence of ADF and $T\beta_4$ (see “Materials and Methods”).

The concentration of TA is determined by the concentration of ATP-G-actin, as described by the following equation.

\[
\frac{[TA]}{[T\beta_4]} = \frac{[G_T]}{([G_T]+K_D+K_T)} \quad (\text{Eq. 1})
\]

$[T\beta_4]$ is the total concentration of $T\beta_4$, $K_D$ is the equilibrium dissociation constant for binding $T\beta_4$ to ATP-G-actin and $[G_T]$ is the steady-state concentration of ATP-G-actin. According to Equation 1, [TA] increases linearly with $[T\beta_4]$. The value of $[G_T]$ at a given concentration of ADF is derived from the slope of the plot of [TA] versus $[T\beta_4]$ as described by Equation 2 below.

\[
[T\beta_4] = \frac{1}{m} \left( \frac{m_m}{m - m_m} \right) \quad (\text{Eq. 2})
\]

In the absence of ADF, the value of the slope was $m_0$, and the value of $[G_T^0]$, $[G_T^1]$, was derived from the conventional pyrenylactin fluorescence critical concentration plots carried out in parallel using the same actin solution. Equation 2 then can be rewritten as follows.

\[
[AD] = [G_T^1] - [G_T^0] = \frac{m}{m_0 - m_m} \quad (\text{Eq. 3})
\]

Data displayed in Fig. 2A show that upon increasing ADF concentration, the slope m of the plot of [TA] versus $[T\beta_4]$ increased, consistent with an increase in $[G_T]$ from 0.12 $\mu$M in the absence of ADF to a maximum of 0.27 to 0.35 $\mu$M. The increase was followed by a decrease at higher ADF concentrations. Similar results were obtained with Arabidopsis ADF1 and human ADF. The bell-shaped curve representing the change in $[G_T]$ versus ADF concentration (Fig. 2B) is strikingly similar to the curve representing the change in treadmilling rate (5), as expected within Scheme I.

Identical results (within 10%) were obtained when the concentration of unassembled actin in the supernatants was derived from fluorescence measurements of NBBD-labeled actin (see “Materials and Methods”).

In conclusion, the enhancement of the treadmilling cycle of F-actin by ADF is linked to an increase in both pools of ADP-G-actin and of ATP-G-actin. At pH 7.5, in the presence of ADF1, the total amount of unassembled actin is 1.7 $\mu$M (5), among which 0.27 $\mu$M is ATP-G-actin. Given the very high affinity (12.5 $\mu$M$^{-1}$) of ADF for ADP-G-actin (5), most of the remaining 1.4 $\mu$M G-actin consists of ADF-ADP-G-actin. The rate of treadmilling, $k_{2B}[AD] - C_{CB}^B$, increases from a value of $k_{2B}^0 (0.12 - C_{CB}^0)$ in the absence of ADF to a maximum value of $k_{2B} (0.3 - C_{CB}^B)$ in the presence of ADF, where $C_{CB}^B$ is the critical concentration for actin assembly at the barbed end. Since this represents a 25-fold increase in treadmilling rate, the value of $C_{CB}^B$ can be derived: 25(0.12 - C_{CB}^0) = 0.3 - C_{CB}^0$, leading to $C_{CB}^B = 0.11 \mu$M, which is very close to the value of $[G_T] (0.12 \mu$M) when both ends are free, and corresponds to a treadmilling rate of $k_{2B}^B$ in the presence of ADF and $C_{CB}^B$ in the presence of actin, where $C_{CB}^B$ is the critical concentration for actin assembly at the barbed end. Since this represents a 25-fold increase in treadmilling rate, the value of $C_{CB}^B$ can be derived: 25(0.12 - C_{CB}^0) = 0.3 - C_{CB}^0$, leading to $C_{CB}^B = 0.11 \mu$M, which is very close to the value of $[G_T] (0.12 \mu$M) when both ends are free, and corresponds to a treadmilling rate of $k_{2B}^B$ in the presence of ADF and $C_{CB}^B$ in the presence of ADF, using a value of 10 $\mu$M$^{-1}$ for $k_{2B}$ (Table I). A 3-$\mu$M-long filament turnovers in about 3 h in the absence of ADF, and in 6 min in the presence of ADF, in agreement with previous observations (5). Obtaining the same turnover rate by fragmentation would require filaments to be severed in 25 fragments. As will be explained under “Discussion,” fragmentation would not change the value of $[G_T]$.
To better understand this property of profilin is fully revealed by the presence of ADF, it is necessary to quantitate the on-flux of actin assembly onto barbed ends, i.e. to measure the concentrations of the polymerizing species ATP-G-actin (G₇) and profilin-ATP-G-actin (G₇P), in the presence of ADF. As developed in the previous section, Tθₐ was used as a G-actin binding protein that sequesters ATP-actin specifically and behaves as an indicator of the changes in [G₇]. Fig. 4 shows that the addition of profilin to F-actin lowers the value of [G₇], both in the presence and in the absence of ADF. This result demonstrates that profilin and ADF act in an independent fashion. Indeed, irrespective of the presence of ADF, the participation of profilin-actin to barbed end assembly lowers the contribution of ATP-G-actin itself. In the presence of 10 μM ADF, the value of [G₇] decreased from 0.27 μM to 0.11 μM upon increasing profilin concentration from 0 to 2 μM. Profilin is in rapid equilibrium with ATP-G-actin and the value of the concentration of profilin-actin complex, [G₇P], is given by the following equation.

\[
\frac{[G₇P]}{[G₇]} = \frac{[P]_{act} \cdot [G₇]}{[G₇P] + Kₐ}
\]  

(Eq. 4)

Using a value of 0.15 μM for Kₐ (3), a value of 0.85 μM can be derived for [G₇P] in the presence of 2 μM profilin and 10 μM ADF. The rate of barbed end growth at steady state then results from the summed association fluxes of ATP-G-actin and profilin-ATP-actin onto barbed ends. The decrease in [G₇] is largely overridden by the increase in [G₇P], so that the overall rate of growth increases. Since the parameters for profilin-actin association to barbed ends are not very different from those found for actin,² the rate of barbed end growth at steady state is: k₁B₀ [(G₇) + [G₇P] - C₅₀B] = 10.0.11 + 0.85 - 0.11 = 8.5 s⁻¹, a value 3.4-fold higher than the value calculated in the absence of profilin.

Although the overall concentration of ATP-bound G-actin was increased by profilin, the total concentration of unassembled actin in the presence of ADF was lowered when bovine profilin was added and remained unchanged when plant profilin was added (Fig. 5). We conclude that the enhancement of nucleotide exchange by profilin contributes to accelerate the transition from ADF-G-actin to ATP-G-actin, thus decreasing the size of the ADF-bound G-actin pool.

The combined effects of ADF and profilin on the concentrations of G-actin in the ATP- and ADP-bound forms at steady state are described in a histogram shown in Fig. 6. The data clearly show that the addition of profilin to ADF-F-actin results in an increase in the pool of ATP-bound G-actin at the expense of the pool of ADP-bound G-actin, consistent with the higher rate of barbed end assembly.

Concentration of Filament Ends in F-actin Solutions in the Presence or Absence of ADF—Proteins of the ADF/cofilin family were early thought to cause rapid depolymerization of actin filaments via a pH-dependent severing effect (14–17), suggested by the rapid drop in fluorescence of pyrenyl- or NBD-labeled F-actin following addition of ADF. This interpretation was later reappraised (5, 7) by kinetic experiments, which showed that upon addition to F-actin, ADF first bound cooperatively to F-actin with a major quenching of fluorescence. Then, partial depolymerization to a new steady state was reached.

result testifies that the effect of profilin on filament turnover is not only due to an increase in the rate of regeneration of ATP-G-actin from ADP-G-actin, but is mediated, in a large proportion, by the replacement of ATP-G-actin by profilin-ATP-G-actin which assembles onto barbed ends only. In confirmation of this conclusion, Fig. 3C shows that the effect of profilin was only observed with profilin-Mg-actin, not with profilin-Ca-actin which rapidly exchanges bound nucleotide, but does not productively associate to barbed ends (4).

The treadmilling rate, which is increased 25-fold by Arabidopsis ADF1, was further increased 5-fold by bovine profilin (3-fold by plant profilin), coming up to an overall 125-fold increase (75-fold increase with plant profilin), as compared with the value found for pure actin. Since plant profilins do not increase the rate of nucleotide exchange on G-actin (4), this effect testifies that the effect of profilin on filament turnover is not only due to an increase in the rate of regeneration of ATP-G-actin from ADP-G-actin, but is mediated, in a large proportion, by the replacement of ATP-G-actin by profilin-ATP-G-actin which assembles onto barbed ends only.
rapidly, via the ADF-enhanced depolymerization from the pointed ends. It was shown that binding of ADF to F-actin increases the twist of the filament (18). ADF also lowers the viscosity of F-actin solutions. The structural alterations parallel the decrease in thermodynamic stability of F-actin linked to ADF binding. Spontaneous fragmentation of filaments, which occurs to a low extent in standard F-actin solutions, as a result of brownian agitation, therefore is likely to be enhanced when ADF is bound to F-actin. To get an estimate as accurate as possible of the rate constants involved in the treadmilling of F-actin in the presence of ADF, it is necessary to quantitate the exact number of filaments in the absence and presence of ADF. Carrying out such measurement has thus far been an elusive problem, due to the many drawbacks linked to different methods.

We attempted to use CapZ as a non-severing, weakly nucleating, high affinity, barbed end capping protein (11), to measure the concentration of barbed ends in an F-actin solution and appreciate how it is affected by ADF at concentrations that cause the maximum enhancement of treadmilling. We first verified, by immunodetection of gelsolin in pellets of F-actin, that gelsolin binds to barbed ends and severs as well standard and ADF-fully decorated filaments. The binding of CapZ to F-actin was measured (see “Materials and Methods”) in the absence and presence of ADF. The data shown in Fig. 7 and summarized in Table II show that a 25% increase in filament number was established in the first minutes following addition of ADF and did not further increase with time. This result is in agreement with the observation that, following addition of ADF to F-actin, the steady-state ATPase rate of F-actin increases rapidly (within 5 min) and remains constant for hours, consistent with a constant number of filaments. All data therefore indicate that the fast turnover of filaments in the presence of ADF allows the rapid readjustment of the length distribution, which is usually a very slow process (19). This process can be observed in the presence of CapZ due to its known slow rate of reaction with barbed ends (11).

The moderate increase in number of filaments induced by ADF, which is in agreement with EM and sedimentation velocity data (5), cannot be responsible for the large increase in turnover. The present results confirm that the main property of ADF in regulating actin dynamics is to increase the rate of depolymerization from the pointed ends (5). On the other hand, a 27% change in length can cause a 60% decrease in viscosity, which depends on the third power of the filament average length (20). The change in filament number has to be taken into account in simulation of the steady state of F-actin in the presence of ADF.

### Table I

| Parameter description                                      | Constant notation | Measured value | Ref.     |
|------------------------------------------------------------|-------------------|----------------|---------|
| Binding of ADF to ADP-G-actin                              | $K_D$             | 0.13 $\mu$M    | (5, 7)  |
| Binding of ADF to ATP-G-actin                              | $K_D$             | 0.13 $\mu$M    | (5, 7)  |
| Binding of profilin to ATP-G-actin                         | $K_D$             | 0.15 $\mu$M    | (3, 29) |
| Association of ADF to F-actin (and to pointed ends)        | $h$               | 0.15 $\mu$M    | (3, 29) |
| Dissociation of ADF from F-actin (and from pointed ends)   | $h$               | 0.04 s$^{-1}$   | (7)     |
| Association of ATP-G-actin to barbed ends                  | $h$               | 10 $\mu$M$^{-1}$s$^{-1}$ | (30, 31) |
| Association of ADF-G-actin to barbed ends                  | $h$               | 4 $\mu$M$^{-1}$s$^{-1}$ | (30, 31) |
| Association of profilin-actin to barbed ends               | $h$               | 0.8 s$^{-1}$    | (30, 31) |
| Association of ADF-ATP-G-actin to barbed ends              | $h$               | 7 $\mu$M$^{-1}$s$^{-1}$ | Submitted |
| Association of ADF-G-actin to pointed ends                  | $h$               | 0.23 $\mu$M$^{-1}$s$^{-1}$ | (30, 31) |
| Association of ATP-G-actin to pointed ends                  | $h$               | 0.6 $\mu$M$^{-1}$s$^{-1}$ | (30, 31) |
| Dissociation of ATP-G-actin from pointed ends               | $h$               | 0.35 s$^{-1}$   | (30, 31) |
| Association of ADF-ADP-G-actin to pointed ends              | $h$               | 3 $\mu$M$^{-1}$s$^{-1}$ | (5, 7)  |
| Dissociation of ADF-ADP-G-actin from pointed ends           | $h$               | 9 s$^{-1}$      | (5, 7)  |
| Dissociation of ADF from G-actin                           | $h$               | 0.2 s$^{-1}$    | (29)    |
| Dissociation of ADF from profilin-G-actin                  | $h$               | 21 s$^{-1}$     | (29)    |
| Dissociation of ADF from ADF-G-actin                       | $h$               | 0.01 s$^{-1}$   | Unpublished data |

and independent of the presence of CapZ, this result indicates that simple addition of ADF to gelsolin-capped filaments generates uncapped filaments. As a result, addition of ADF to a solution of gelsolin-capped F-actin promotes very fast turnover in the population, since the number of pointed ends (58 nm in the present case) is far greater than the number of barbed ends (6–8 nm in the present case). As pointed out earlier (6), the growth of the very few barbed ends (13%) is then fed by the rapid depolymerization of all pointed ends, in a biased, “funneled” treadmilling process. We have verified, using the fluorescence assay described in Fig. 3B, that rapid turnover of filaments is actually observed under such conditions (data not shown).

The time dependence of the increase in filament number following addition of ADF was investigated. The amount of F-actin-bound CapZ was measured at different times from 15 min to 2 h following addition of ADF. Data displayed in Fig. 8 show that the increase in filament number was established in the first minutes following addition of ADF and did not further increase with time. This result is in agreement with the observation that, following addition of ADF to F-actin, the steady-state ATPase rate of F-actin increases rapidly (within 5 min) and remains constant for hours, consistent with a constant number of filaments. All data therefore indicate that the fast turnover of filaments in the presence of ADF allows the rapid readjustment of the length distribution, which is usually a very slow process (19). This process can be observed in the presence of CapZ due to its known slow rate of reaction with barbed ends (11).
parameters listed in Table I, and a steady-state Scheme II (shown in Fig. 9), which is an extension of Scheme I incorporating the effect of profilin. The resulting dependence values of the calculated concentrations of the different G-actin species on total ADF and profilin concentrations are displayed in Fig. 10.

The calculated curves show the same trend as, and in most cases a satisfactory quantitative agreement with the experimental curves, which argues in favor of the proposed Scheme II. The main features of the calculated curves that are supportive of Scheme II are (i) the increase, followed by a decrease, in ATP-G-actin concentration, as ADF concentration is increased; (ii) the accumulation of a large amount of ADF-ADP-G-actin; (iii) the severalfold increase in the ATP-bound G-actin when profilin is added to F-actin in the presence of ADF, and corresponding decrease in ADF-ADP-G-actin.

As expected from the low affinity of ADF for ATP-G-actin, the calculated steady-state concentration of ADF-ATP-G-actin is extremely low. As a result, the contribution of the association flux of ADF-ATP-G-actin at steady state is negligible as compared with that of ATP-G-actin, despite the high value of the association rate constant $k_{+B}$, as derived from initial rates of filament growth (Table I and Ref. 5). Consequently, the simulated behavior shown here with the rate parameters re-
ferring to plant ADF \(_1\) turns out to be almost identical with human ADF or other ADF/cofilin variants (yeast cofilin, acto-pholin), which display slower barbed end association rates of their complex with ATP-G-actin.\(^3\)

**DISCUSSION**

The present results allow one to understand quantitatively how the dynamics of actin assembly at steady state is controlled by ADF and profilin. The concentration of ADF-G-actin was assumed to be much smaller than that of ADF-ADP-G-actin. In the presence of ADF alone, the concentration of ADF-ADP-G-actin was calculated as the difference between total unassembled actin (1.8 \(\mu\)M) and the measured concentration of ATP-G-actin (0.27 \(\mu\)M). In the presence of both ADF and profilin, the measured amount of total unassembled actin was 0.9 \(\mu\)M (Fig. 5), the concentration of free ATP-G-actin was 0.11 \(\mu\)M, and the concentration of profilin-ATP-G-actin was such that (ATP-G-actin + [profilin-ATP-G-actin] - C,\(^5\)) was 5-fold higher than in the absence of profilin, leading to [profilin-ATP-G-actin] = 0.7 \(\mu\)M. The concentration of ADF-ADP-G-actin was then calculated as the difference between total unassembled actin and total ATP-bound actin, giving 0 \(\mu\)M. Hence, the amount of ADF-ADP-actin is greatly depressed by profilin.

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**TABLE II**

| Experiment no. | Standard F-actin | F-actin + ADF | F-actin + ADF | F-actin + gelsolin | F-actin + gelsolin |
|---------------|-----------------|--------------|--------------|--------------------|--------------------|
| 1             | 9.6             | 9            | 6            | 1.7                | 1.7                |
| 2             | 10.4            | 16.3         | 15.1         | 4.8                | 11                 |
| 3             | 9.6             | 11           | 7.6          | 2.0                | 6.4                |
| 4             | 9.2             | 13           | 10.2         | 1.6                | 8.4                |
| Average       | 9.7             | 12.3         | 9.7          | 2.5                | 8.6                |

as a result from the more fragile structure of ADF-F-actin, the moderate 27% increase in filament number cannot explain the large increase in turnover.

The ADF-induced increase in the steady-state concentration of ATP-G-actin implies that, due to the law of mass action, the equilibria of ATP-G-actin with sequestering proteins are shifted toward complex formation, leading to an increased sequestration. Therefore, in vivo, the reported actin depolymerizing activity of ADF is due in part to its intrinsic property to partially depolymerize actin into ADF-ADP-G-actin, but also results from the increase in all complexes of ATP-G-actin with sequestering proteins like thymosin \(\beta_4\).

At high concentration of ADF (above half a molar equivalent to total actin), the steady-state concentration of ATP-G-actin...
and the rate of treadmilling both reach a maximum and begin to decrease. As outlined in a previous paper (7), this decrease is explained by the fact that, as the ADF-ADP-G-actin complex is stabilized by ADF binding, its association to filament ends becomes predominant over its dissociation into free ADF and ADP-G-actin. The pathway leading to ATP-G-actin association to barbed ends via nucleotide exchange then is depressed and the cycle rate is slowed down as a gradual shift takes place from the steady-state cycle situation with associated ATP hydrolysis toward the equilibrium situation in which monomer-polymer exchange reactions are fed by ADF-ADP-actin (see Fig. 10C). The implication of this result may be important in vivo, to understand the effects of overexpression of ADF or of GFP-tagged ADF. Depending on the degree of overexpression and the relative original concentrations of actin and ADF, an increase or a decrease in actin dynamics might be expected to occur.

The experimental demonstration of the synergy between ADF and profilin in the enhancement of filament turnover is a main point of this work. Profilin alone has a weak effect on filament turnover (4); however, the property of profilin-actin to participate in barbed end assembly is sufficient to explain the synergy between ADF and profilin. These two proteins act each at one end of the actin filament, in a complementary fashion. ADF increases the flux of depolymerizing subunits from the pointed ends, while profilin increases the flux of assembly onto the barbed ends at steady state. Profilin optimizes the directionality of treadmilling in two ways as follows. First, profilin drives the ADP-bound state of actin toward the ATP-bound state, thus preventing the reverse pathway (association of ADF-ADP-actin with filament ends). Second, profilin replaces ATP-G-actin, which can associate with the two ends of the filament, by profilin-ATP-G-actin, which associates only with the barbed ends. Even in the presence of profilin, the rate-limiting step in the treadmilling cycle is still the dissociation from the pointed ends. The ADF-induced increase in the rate constant for actin dissociation from the pointed ends is certainly at least 5-fold higher than the 25-fold increase found earlier (5). The measured rate of depolymerization from the pointed end was probably tempered by the reverse reaction of ADF-ADP-actin association to the pointed ends, which had

![Fig. 8. ADF causes a rapid redistribution in length of the filaments.](image)

The experiment was conducted as detailed under Fig. 7. F-actin preincubated for 20 min with 56 nM CapZ was supplemented with or not with 10 μM ADF (arrow). The amount of F-actin-bound CapZ was determined after sedimenting the samples at the times indicated. Inset: immunoblot of the data shown in the main frame. a–c, CapZ standards (0.25, 0.5, and 1.0 pmol, respectively); d–f, F-actin-bound CapZ at times 15, 60, and 100 min following addition of ADF. d–f, controls without ADF; d∗–f∗, ADF-containing samples.

![Fig. 9. Scheme II for the combined effects of ADF and profilin on F-actin dynamics at steady state.](image)

This scheme is an extension of Scheme I incorporating the role of profilin (P) acting in synergy with ADF to increase the efficiency of treadmilling.
not then been appreciated properly. Taking into account the slight increase (27 ± 10%) in the number of filaments observed in the presence of ADF, the conclusion that emerges from our work is that ADF increases the rate constant of depolymerization from the pointed ends at least 50-fold.

Using two profilin species, only one of which enhances nucleotide exchange on G-actin, has been helpful to dissect the individual kinetic steps through which profilin can accelerate the treadmilling cycle. Formation of the polymerizable profilin-ATP-actin complex without increase in the rate of nucleotide exchange is sufficient to increase the rate of treadmilling 3-fold as compared with the level observed with ADF alone. A larger (5-fold) increase, however, is observed if profilin increases the rate of regeneration of ATP-actin from ADP-actin. Again, if ADF were simply a severing factor, bovine profilin could also increase the rate of treadmilling by increasing the rate of nucleotide exchange on ADP-G-actin. This effect has indeed been observed under sustained sonication (22). More specifically, we found that a 3-fold increase in steady-state ATPase rate was induced by profilin when the number of ends was increased 20-fold, corresponding to an average length of 0.3 μm, upon sustained fragmentation due to sonic vibration.4 Clearly, ADF does not sever filaments to that extent, yet the increase in rate due to profilin is larger. Moreover, the effect of plant profilin on the treadmilling rate cannot be explained by fragmentation, because as explained above, it requires the high ATP-G-actin concentration induced by ADF.

In conclusion, detailed measurements of rates of treadmilling as a function of filament number, and of the effect of ADF on filament number were required to quantitatively understand the mechanism of action of ADF.

CapZ has proved to be an interesting tool to measure the number of filaments in an actin solution and, in combination with gelsolin, to quantitate the spontaneous fragmentation in the absence and presence of ADF. It is remarkable that the average filament length derived from these measurements is 7.2 μm in the absence of ADF, which is the persistence length of filaments found in a previous study (23). This figure suggests that the spontaneous fragmentation-length redistribution processes that result from brownian agitation lead to the establishment of a filament length compatible with a rod-shaped polymer. The smaller average length of ADF-decorated filaments (5.7 μm) is compatible with electron microscopy data (5).

Although the technique using CapZ to measure the number of filaments does not suffer from the artifacts linked to electron microscopy, it has its own biases, as follows. The experiments cannot actually discriminate whether the 27% increase in filament number induced by ADF is due to enhanced spontaneous fragmentation due to the increased fragility of ADF-F-actin, or to enhanced nucleation by CapZ in the presence of ADF. The muscle capping protein is known to weakly nucleate, at G-actin at concentrations above the critical concentration (11). In the present experiments, the G-actin concentration is equal to the critical concentration, hence nucleation by CapZ in standard F-actin solutions is very unlikely. However, the situation may be different in the presence of ADF, because ADF-ADP-G-actin nucleates very easily in the vicinity of the critical concentration (7). Hence, at steady state in the presence of ADF, the nucleation activity of ADF, enhanced by CapZ, introduces a bias in the measurements of the concentration of filament ends. The measured 27% increase in filament ends may thus be an overestimate. Second, the presence of CapZ in the assay medium interferes with the process of establishment of the distribution in length of filaments, because blockage of the barbed ends that

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are created by fragmentation prevents the reverse reactions of monomer-polymer exchange leading to an increase in average length. This latter bias also leads to an overestimation of the steady-state concentration of filaments.

The assembled data impose a number of constraints that are included in the simulation of actin steady state in the presence of ADF and profilin. The simulated behavior and calculated concentrations of the different actin species are in good agreement with the experimental data, in support of the proposed model.

At optimum concentrations of ADF and profilin, it takes 1 min for a 3-μm-long filament to travel a distance equal to its length. This figure lets us anticipate that the rheological properties of actin gels may be affected by actin dynamics in the presence of ADF and profilin (24). Experiments are under way to address this question.

The synergy observed in vitro between ADF and profilin may be functionally relevant in the control of actin-based motility.

Both proteins are present in the lamellipodia of motile cells where monomeric actin exists in large amounts (25), are essential in pluricellular organisms, and are involved in developmental processes in which very active actin dynamics is involved (26–28).

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