Mini-Review

Accelerating on a Treadmill: ADF/Cofilin Promotes Rapid Actin Filament Turnover in the Dynamic Cytoskeleton

Julie A. Theriot

Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02160

Actin is among the most thoroughly studied of proteins. It was first identified half a century ago as the major component of thin filaments in muscle. Work in the 1960s and 1970s showed that actin is also present in nonmuscle cells as well as in plants and protozoa. The actin-based cytoskeleton appears to be ubiquitous among eukaryotes, and indeed the invention of the actin cytoskeleton may have been a key step in the earliest history of the eukaryotic lineage. A densely written summary of the most important known properties of actin fills a good-sized volume (23). But there are major discrepancies between the well-characterized in vitro behavior of purified actin and the apparent behavior of actin filaments inside of intact, living cells.

Two discrepancies have been particularly puzzling. First, the concentration of unpolymerized actin present in most vertebrate nonmuscle cells is ~100-fold higher than the concentration of monomeric actin that can exist at steady state in vitro (for review see 5). Second, the apparent rate of turnover of actin filaments in dynamic structures inside living cells is up to 100 times faster than can be accounted for by the in vitro turnover kinetics (for review see 28). The first puzzle has been solved by the recent identification of abundant small actin monomer-binding peptides, thymosin β4 and its close relatives, that sequester much of the actin monomer inside cells (for review see 17). The solution to the second puzzle may also lie with an actin-binding protein. Two articles in this issue of *The Journal of Cell Biology* (3, 21) lend support to the hypothesis that the rapid turnover of actin filaments inside living cells is largely promoted by a group of small (15–22 kD) actin-binding proteins that includes cofilin, destrin, depactin, actophorin, and actin depolymerizing factor (ADF)[1], collectively called the ADF/cofilin family.

Like actin itself, ADF/cofilin family members are found in all eukaryotes including mammals, birds, amphibians, flies, worms, echinoderms, plants, fungi, and protozoans (for review see 16). ADF/cofilin is an essential protein in all species where its necessity has been tested genetically. Unusual among actin-binding proteins, members of this family bind in a 1:1 stoichiometry to actin in both monomeric and filamentous forms, and they induce rapid depolymerization of actin filaments, which is enhanced at elevated pH . The actin-binding activities of various members of the ADF/cofilin family are inhibited by phosphorylation and/or by competitive binding of phosphoinositides; thus ADF/cofilins are good candidates for downstream effectors of several types of signaling cascades that cause rearrangements of the actin cytoskeleton. Consistent with a role in rapid filament turnover inside the cell, ADF/cofilin is typically concentrated at the dynamic actin-rich lamellipodium in locomoting cells. Members of this class of actin-binding proteins have recently been receiving increasing attention from investigators interested in cytoskeletal dynamics. Since an excellent and comprehensive review on the properties of this protein family was published a little over a year ago (16), several interesting new findings have been reported that greatly enhance our understanding of the roles of ADF/cofilin in regulating the actin cytoskeleton in living cells. In this brief review, I will concentrate primarily on these new findings.

Actin Filament Dynamics and Treadmilling Are Enhanced by ADF/Cofilin

Actin monomers polymerize in a head-to-tail fashion to form long helical filaments whose two ends are structurally and dynamically distinct. The on- and off-rates of actin subunit addition to one end, the barbed end, are substantially faster than the equivalent rates at the opposite end, the pointed end. The concentration of actin monomer where the on- and off-rates are balanced is called the critical concentration. Actin is an ATPase, and ATP is rapidly hydrolyzed after polymerization. Due to this constant energy consumption, the actin polymer exhibits several interesting nonequilibrium behaviors; most notably, it is able to maintain different critical concentrations at the two ends (19). At an intermediate actin monomer concentration, it is possible to reach a steady state where monomer associates at the barbed end at the same rate as monomer dissociates from the pointed end. This results in a steady-state

Please address all correspondence to Julie A. Theriot, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02160. Tel.: (617) 258-6036. Fax: (617) 258-7226. e-mail: theriot@wi.mit.edu

1. *Abbreviation used in this paper*: ADF, actin depolymerizing factor.

flux of subunits through the filament, generally termed treadmilling. The rate of actin filament treadmilling is limited by the off-rate of ADP-actin monomers at the pointed end. Since actin filament turnover inside living cells occurs up to two orders of magnitude faster than can be accounted for by this off-rate (for review see 28), researchers have long postulated the existence of a cellular factor that acts specifically to accelerate the pointed end off-rate and therefore the rate of actin filament treadmilling. Until now, no candidate factor had been shown to have this particular activity.

In this issue of *The Journal of Cell Biology*, Carlier et al. (1997) demonstrate that ADF/cofilins can indeed perform this function (3). They have used bacterially expressed ADF1 from *Arabidopsis thaliana* and carefully examined its effects on the in vitro filament dynamics of rabbit skeletal muscle actin. Although plant ADF/cofilin family members share only ~30% amino acid identity with the vertebrate family members (10, 11), their actin-binding and -depolymerizing functions appear to be generally similar (11). Using a series of kinetic methods designed to measure separately the effects of ADF1 on the association and dissociation rates at the two ends of the actin filament, Carlier et al. have found that ADF1 accelerates the on-rate of actin monomer at the barbed end by 12-fold, without noticeably increasing the off-rate. At the pointed end, the on-rate is modestly enhanced, while the off-rate is accelerated by ~22-fold. Since actin filament treadmilling is limited by the pointed-end off-rate, we expect that ADF1 should accelerate the rate of treadmilling by approximately the same amount. Indeed, both the treadmilling rate (as measured by a pulse-chase experiment using fluorescently labeled nucleotide) and the steady-state actin ATPase are speeded up ~25-fold in the presence of saturating ADF1 (3). In other words, the difference between the critical concentrations of the barbed and pointed ends is greatly enhanced by the presence of ADF.

These results suggest that the actin/ADF complex can be thought of as a second type of polymer, dynamically distinct from the pure actin polymer. This is quite different from the effects of factors such as thymosin β4 that encourage actin filament depolymerization through sequestration of actin monomer; although sequestering factors can alter the final steady-state level of actin filaments, they cannot change the depolymerization kinetics of the polymer itself. ADF/cofilin family members bind more tightly to ADP-actin than to ATP-actin (3, 12), and thus the net depolymerizing ability of ADF is inhibited by nonhydrolyzable ATP analogs (21) or high levels of inorganic phosphate (14). Since the rate of ATP hydrolysis lags behind the rate of filament elongation when actin is polymerizing rapidly, the likelihood that an actin filament can be depolymerized by ADF increases as it grows older. This property may build a timing mechanism into the system that ensures that older actin filaments (e.g., at the rear of a lamellipodium) will be depolymerized more aggressively by ADF than nascent filaments (e.g., at the leading edge of a lamellipodium). In this way, the cell can maintain rapid turnover of the cytoskeleton as a whole while allowing efficient and rapid growth of new filamentous structures in particular locations (14). Further support for the model that ADF/cofilin exploits energy derived from ATP hydrolysis by actin to enhance filament treadmilling could be provided by an examination of ADF/cofilin effects on dynamics at the barbed end of filaments prepared from ADP-actin. ADP-actin is a true equilibrium polymer where the critical concentrations must be identical at the two ends (though the kinetics may still differ) (19).

Some of the conclusions reached by Carlier et al. will be controversial. In particular, the suggestion that ADF1 enhances the on-rate of monomers at the filament barbed end is difficult to reconcile with the observation that barbed-end elongation is a diffusion-limited reaction (4). It is hard to imagine how ADF binding to an actin monomer could increase the rate of its thermally driven collision with a filament end. The authors argue that the rate enhancement might be due to an electrostatic effect, where ADF binding enhances the dipole moment of the actin monomer in a way that steers the association reaction (3). Another possible interpretation of the rate enhancements of both polymerization and depolymerization is that ADF/cofilin increases the number of filament ends present in the in vitro reactions by severing the actin filaments. However, in contrast with a number of earlier reports, Carlier et al. do not find any evidence for ADF-induced actin filament severing using either EM or analytical ultracentrifugation, and their kinetic results are quantitatively inconsistent with a significant severing activity. Observations that have previously been interpreted as evidence for severing by other members of the ADF/cofilin family (including a rapid drop in pyrene-actin fluorescence and an acceleration of the initial phase of actin polymerization including "overshoot" kinetics) are also demonstrable using *Arabidopsis* ADF1, but they can be reinterpreted as effects due to the dramatic acceleration of the kinetics at the two ends of the actin filament (3). On the other hand, severing activity has been directly observed by videomicroscopy for two ADF/cofilin family members, *Acanthamoeba castellanii* actophorin (14) and human ADF (9), and the rapid drop in viscosity of actin filament solutions upon addition of ADF is most consistent with a severing mechanism. At present, although it is clear that ADF/cofilin family members enhance filament disassembly in vitro, neither of the two proposed mechanisms (filament severing vs acceleration of treadmilling) can satisfactorily explain all available data, nor are they mutually exclusive. Further refinement of the two rival models will require further experimentation, the rate of which will doubtless be accelerated by the novel and generally compelling treadmilling hypothesis proposed by Carlier et al.

To understand the detailed mechanism of ADF/cofilin effects on actin polymerization and depolymerization, we must also understand the structural basis of their interactions. A major step in this direction has come with the recent determination of the three-dimensional structure of mammalian destrin (8). The next phase must involve solving the structures of ADF/cofilins in complexes with filamentous and monomeric actin. In particular, it will be interesting to see whether the pH regulation of ADF/cofilin binding to actin filaments and the preference of ADF/cofilin for ADP-actin over ATP-actin reflect conformational changes in one or both proteins, and whether structural information can shed any light on the severing vs treadmilling controversy.

ADF/Cofilin Influences Actin-based Motility

In the cytoplasmic environment, the activity of any individual actin-binding protein is competed with and altered by the functions of scores of other actin-binding proteins also present. The net behaviors of complex mixtures of cytoskeletal proteins cannot generally be predicted from detailed knowledge of the properties of each component alone. Thus, the in vitro experiments described above cannot prove whether the enhancement of actin filament dynamics caused by ADF/cofilin is relevant to actin filament turnover in the cytoplasm or to actin-based motility; effects on motility must also be examined directly. Within the past few years, the actin-based movement of the intracytoplasmic bacterial pathogen *Listeria monocytogenes* has become a widely used model system for quantitative studies of actin-based motility. In this geometrically simple motile system, moving bacteria are associated with actin-rich comet tails made up of short, cross-linked host cell filaments (26). Actin filament elongation occurs only at the front of the tail, immediately adjacent to the bacterial surface, and the bacterial movement rate is tightly coupled to the filament elongation rate (22, 24). Loss of filaments from the tail occurs at a uniform rate throughout the entire structure and is apparently governed by host cell factors (24). The density distribution of actin filaments in the tail, and thus the apparent tail length, is a simple function of two variables: rate of filament elongation at the front (i.e., bacterial speed), and rate of filament depolymerization throughout the tail (20, 24). Thus, if the actin filament turnover rate is experimentally enhanced, the tails will become shorter. If filament turnover is slowed, tails will become longer. Since *L. monocytogenes* actin-based motility can be faithfully reconstituted in biochemically manipulable cytoplasmic extracts made from frog eggs (*Xenopus laevis*) (25) or from human platelets (27), the effect of ADF/cofilin on actin filament turnover can be directly assessed in this system.

In this issue of *The Journal of Cell Biology*, Rosenblatt et al. (1997) have examined the effects on *L. monocytogenes* motility of both lowering and raising the ADF/cofilin level in *Xenopus* egg cytoplasmic extracts. Immunodepletion of ~75% of the endogenous amount of XAC (*Xenopus* ADF/cofilin) results in extracts that can still support actin tail formation and bacterial motility, but the tails are about five times longer and contain ~15–20-fold more actin than tails formed in undepleted or mock-depleted extracts, while movement rate is essentially unaffected. This result is clearly consistent with a critical role for XAC in causing rapid actin filament depolymerization throughout the tail. Addition of wild-type XAC or chick ADF to approximately endogenous concentrations restores normal tail length, while an inactivated mutant XAC cannot rescue the phenotype. Addition of exogenous XAC to approximately three to six times the normal level results in dramatic shortening of the tails, accompanied by a modest (~20%) increase in the bacterial speed (21).

Similarly, Carlier et al. have checked the effects on *L. monocytogenes* motility of adding excess *Arabidopsis* ADF1 to human platelet extracts. In concentrated platelet extracts, addition of excess ADF has no effect on movement rate. However, in dilute extracts where actin monomer concentration might be rate limiting for actin filament elongation and therefore for bacterial movement rate, addition of exogenous ADF1 causes a dramatic enhancement of bacterial speed and a concomitant shortening of the actin tail (3). Although these results seem superficially to differ from those of Rosenblatt et al., it is important to realize that the experiments in the two papers cover a wide and poorly overlapping range of ADF/cofilin:actin ratios and that the rate-limiting step for bacterial movement is likely to be different in the two cases. Neither group directly measured the half-life of actin filaments in the tails, but all results are consistent with the hypothesis that the primary effects of ADF/cofilin on actin filament dynamics in tails associated with motile *L. monocytogenes* are identical to its effects on the dynamic behavior of pure actin, i.e., to increase the rate of actin filament turnover. Determining whether the turnover enhancement is due to the acceleration of treadmilling or to filament severing will require quantitative analysis of the length distribution of the actin filaments in the tails, determination of whether either the barbed or pointed ends (or both) are capped, and measurement of the rate of monomer exchange at the free ends.

Roles for ADF/Cofilin in Living Cells

The experiments on *L. monocytogenes* motility described above are performed in cytoplasmic extracts and may not truly reflect the effects of ADF/cofilin on actin filament dynamics inside living, intact cells. The effect of raising or lowering cofilin levels on actin cytoskeleton dynamics has recently been examined in two unicellular eukaryotes and in two metazoans. The results suggest that ADF/cofilins are important in vivo for the acceleration of actin filament turnover during cell motility, cell growth, and cytokinesis.

Recent experiments in yeast (*Saccharomyces cerevisiae*) provide a good example of a quantitative correlation between effects of cofilin on actin dynamics in vitro and in vivo. Cofilin in budding yeast is localized to the cortical patches, small, rapidly moving, actin-rich structures usually concentrated at sites of cell surface growth (15). The turnover rate of filaments in the cortical patches can be indirectly measured by treating live cells with the actin monomer-sequestering drug latrunculin A and counting the number of patches left at later time points. The average half-life of actin filaments in these patches is normally short, <2 min. In yeast strains carrying temperature-sensitive mutants of cofilin, the actin filament half-life is lengthened by up to fivefold. The increase in actin filament half-life in vivo for two different temperature-sensitive alleles correlates extremely well with the magnitude of the defect in their ability to depolymerize actin filaments in vitro (Lappalainen, P., and D.G. Drubin, manuscript submitted for publication).

In the slime mold *Dictyostelium*, cofilin can be stably overexpressed at up to seven times its normal concentration. The overexpressing cells develop thick actin cables and exhibit dramatic membrane ruffling, and the average speed of their whole-cell locomotion is almost twice that of the control cells (2). The increase in cell speed upon cofilin overexpression is similar to the twofold increase in *L. monocytogenes* propulsion rate observed upon adding excess ADF1 to diluted platelet extracts (3), and the appear-

ance of thick actin cables is strikingly like the aggregation of actin filaments seen in vitro in the presence of a mixture of actophorin and actin-bundling proteins (α-actinin) (13). Surprisingly, the cofilin-overexpressing cells have a higher ratio of filamentous to monomeric actin than the controls, which is the opposite of what might be expected from the in vitro experiments. Interpretation of the phenotype is complicated by the fact that the cofilin overexpression also induces a threefold overexpression of actin (2) and possibly other cytoskeletal components.

In addition to its apparent role in accelerating the speed of cell locomotion and cortical actin filament turnover, ADF/cofilin also performs an important function during cytokinesis. Cofilin is localized at the cleavage furrow in several mammalian cell lines (18) and in early *Xenopus* embryos (1). The highly dynamic nature of the filaments in the cleavage furrow is presumably important for furrow function, since agents that inhibit either actin polymerization or actin depolymerization will prevent the completion of cytokinesis (for review see 6). The functional role of ADF/cofilin in the cleavage furrow has been experimentally demonstrated in both the frog and the fly. In *Xenopus*, endogenous XAC activity can be inhibited by microinjection of neutralizing antibodies into one cell of a two-cell stage embryo. Cytokinesis in the injected blastomere is blocked, while nuclear division proceeds normally. Microinjection of an unphosphorylatable XAC-containing fusion protein likewise inhibits cytokinesis (1). Thus, both XAC activity and normal regulation of that activity appear to be important during these early cell cleavages. Similarly, *Drosophila* cofilin (the product of the *twinstar* gene) plays an obligate role in cytokinesis in larval tissues (7). Unusually large foci of filamentous actin are found in cells from *twinstar* mutant embryos, consistent with the idea that cells lacking sufficient levels of cofilin can assemble actin filaments for purposes such as cleavage furrow formation, but cannot disassemble them in a timely manner (7).

Conclusion

ADF/cofilin family members are potent and crucial regulators of actin cytoskeleton dynamics whose function is to accelerate the actin filament turnover rate in living cells. The pace of research on these interesting proteins has likewise been accelerating over the past several years. Studies from a variety of organisms as well as careful quantitation of the effects of ADF/cofilin on actin filament dynamics in vitro support the hypothesis that these family members are the primary, if not the only, cellular factors responsible for the rapidity of actin filament depolymerization in dynamic cytoskeletal structures in all eukaryotes.

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