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A Role of Cofilin/Destrin in Reorganization of Actin Cytoskeleton in Response to Stresses and Cell Stimuli

Ichiro Yahara[1], Hiroyuki Aizawa[1], Kenji Moriyama[1], Kazuko Iida[1], Naoto Yonezawa[3], Eisuke Nishida[4], Hideki Hatanaka[2], and Fuyuhiko Inagaki[2]

[1]Department of Cell Biology, [2]Department of Molecular Physiology, The Tokyo Metropolitan Institute of Medical Science, Tokyo 113, [3]Department of Chemistry, Faculty of Science, Chiba University, Chiba 263, and [4]Institute for Virus Research, Kyoto University, Kyoto 606–01

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Various cellular events such as cell motility and division are directed by the actin cytoskeleton under the control of its regulatory system. Cofilin is a low molecular weight actin-modulating protein that was originally isolated from porcine brain (26) and is ubiquitously distributed in eucaryotes from the budding yeast to mammals (4, 14, 21). Cofilin binds to actin in both monomeric and polymerized forms in a 1:1 molar ratio and depolymerizes F-actin in a pH-dependent manner (26, 32). *In vitro* experiments indicated that cofilin binds and weakly severs F-actin at pH lower than 7.3 and depolymerizes it at pH higher than 7.3. The pH-dependent, functional alteration in cofilin may be physiologically significant because cytosolic alkalization is induced upon cell stimulation by serum or growth factors (22) and acidification to pH 6.9 is induced upon heat shock (9). Incubation of various cells in 5–10% DMSO or at elevated temperatures induces destruction of actin cytoskeleton and translocation of actin and cofilin to nuclei where they form rod-like structures (10, 12, 28). Although a nuclear translocation signal sequence has been identified in cofilin (1, 13, 19), stress-dependence of the translocation remains to be explained.

A body of evidence has indicated that a significant portion of cofilin and/or destrin/ADF an isoprotein of cofilin, in mammalian and avian cells is phosphorylated at a serine residue(s) (8, 16, 29, 31). Upon heat shock or treatment with DMSO, dephosphorylation of cofilin precedes the nuclear translocation of actin and cofilin (29). In addition, dephosphorylation of cofilin and/or destrin/ADF, has been detected upon stimulation of various cell types including T-lymphocytes, platelets, astrocytes, thyroid cells, parotid grand, and oocyte (2, 6, 8, 16, 30, 31). These results strongly suggest that phosphorylation and dephosphorylation of cofilin and destrin/ADF are critical events that lead to reorganization of the actin cytoskeleton and cell stimulation. Interestingly, phosphorylated cofilin was not detected in vegetatively growing cells of *Saccharomyces cerevisiae* or *Dictyostelium discoideum* (K.I. and H.A., unpublished observations). As will be seen below, phosphorylated cofilin is inactive in the interaction with actin (3, 23, 24). Thus it would be possible that growing cells of lower eucaryotes might not need to inactivate cofilin whereas a portion of cofilin is always inactivated in resting cells of higher eucaryotes.

In this review, we briefly summarize current progress on research on structure and function of cofilin and discuss a role(s) of cofilin in cellular responses.

Cofilin is an Essential Actin-modulating Protein for Lower Eucaryotes

Among numerous numbers of actin-binding proteins identified in *S. cerevisiae*, cofilin (14, 21) and *MYO2*-encoded unconventional myosin (15) are the only essential proteins for yeast cells to be viable. The lethality caused by disruption of the yeast *COF1* gene is complemented by the expression of porcine cofilin or destrin (14). The structural and functional conservation of cofilin in low and higher eucaryotes suggests that cofilin is a key regulatory protein of the actin cytoskeleton. In addition, *D. discoideum* has two cofilin genes, d*COF1* and d*COF2*, one of which, d*COF2* is not expressed under any condition and is therefore possibly a pseudogene. The disruptant of d*COF1* has never been isolated although those of d*COF2* were easily created, supporting the conclusion that d*COF1* is an essential gene in this organism (4).

A yeast strain was created in which *COF1* is expressed under the GAL1 promoter (K.I., unpublished observations). This strain is unable to grow in media containing glucose or galactose as a sole carbon source but is able to normally grow in media containing mixtures of the two sugars. This result strongly suggests that the overexpression of cofilin in yeast cells causes cell arrest. Similar observations were also made by transient transfection of cofilin cDNA in cultured mammalian cells (K.I. and K.M., unpublished observations). Fur-

thermore, microinjection of anti-Xenopus ADF/cofilin antibody or unphosphorylated Xenopus ADF/cofilin to Xenopus embryos has been recently reported to inhibit cell division (2). All of these results suggest that appropriately controlled levels of active cofilin are required for cell growth.

Overexpression of Cofilin in *Dictyostelium* Cells

Confocal microscopic dissection of *D. discoideum* cells immunofluorescently labeled with anti-cofilin antibodies revealed that cofilin is codistributed with actin on ruffling membranes but not on adhesion plaques (4). This suggests that cofilin might be involved in dynamic but not in static actin structures. To investigate further functions of cofilin, biological phenotypes of *D. discoideum* cells in which the expression of *Dictyostelium* cofilin (d-cofilin) was increased several fold were examined (5). These cofilin-overexpressing cells were viable. The overexpression of cofilin caused co-overexpression of actin in these cells. In addition, the amount of F-actin but not of G-actin was increased in the overexpressing cells, suggesting that cofilin does not function in vivo as a simple actin-depolymerizing protein or monomeric actin-sequestering protein. The overexpression of cofilin enhanced actin bundles as observed by fluorescence microscopy. Consistent with this observation, it has also been reported that microinjenction or transient overexpression of cofilin or ADF/destrin induced actin bundles in cultured mammalian cells (24, 25). Furthermore, these cofilin-overexpressing cells exhibited enhanced cell motility, indicating that cofilin is an upstream positive regulator of cell motility.

It has been demonstrated that cofilin and its related proteins have the activity to sever actin filaments (7, 17, 27). Actophorin, a homolog of cofilin in *Acanthamoeba*, has been shown to transform latticework of actin filaments cross-linked with α-actinin into bundles by severing the filaments (18). d-Cofilin was shown also to cause a similar transformation of actin structures (5). On the basis of these results, it would be likely that the severing activity rather than the monomeric actin-sequestering activity is closely related to the in vivo function of cofilin.

Negative Regulation of Cofilin by Phosphorylation on Its Ser-3 Residue

A mixture of phosphorylated and unphosphorylated cofilin was subjected to binding to F-actin. Only unphosphorylated cofilin cosedimented with F-actin (24). Binding experiments to DNaseI beads indicated that phosphorylated cofilin did not bind to G-actin either (24). Although purified phosphorylated ADF/destrin did not depolymerize F-actin, treatment of the phosphorylated protein with alkaline phosphatase reactivated the ADF/destrin activity (3). All of these results indicate that cofilin and ADF/destrin are reversibly inactivated by phosphorylation. It should be noted, however, that phosphorylated cofilin binds PIP_2 as does the unphosphorylated form (24).

Phosphoamino acid analysis indicated that a serine residue(s) is phosphorylated in cofilin (3, 8, 16, 24, 29, 31). Peptide analysis of phosphorylated cofilin revealed that only the N-terminal peptide consisting of 21 amino acid residues is phosphorylated (24). This peptide contains two serine residues, Ser-3 and Ser-8. These two serine residues were separately replaced by Ala designated S3A and S8A mutants, respectively. S8A was phosphorylated but S3A was not when transfected to human 293 cells. On the basis of these results, we concluded that Ser-3 is the phosphorylation site of cofilin (24). This is consistent with the result by an other group on chicken ADF (3).

S3D-cofilin may be structurally and functionally similar to phosphorylated cofilin because both the mutated cofilin and the phosphorylated protein possess negative charges in the third amino acid residues. It was confirmed that, like phosphorylated cofilin, S3D-cofilin does not bind to actin (24). Furthermore, the expression of S3D-cofilin was found not to rescue Δcof1 yeast cells whereas the wild type or S3A-cofilin did. This strongly suggests that the ability of cofilin to bind to actin is crucial for the viability of yeast cells.

Identification and Structural Analysis of Actin-binding Site of Cofilin

Peptide mapping of cross-linked products of cofilin and actin suggested that the region of cofilin containing Lys-112 and Lys-114 is involved in the binding to actin (34). Indeed, a synthetic dodecapeptide corresponding to the sequence from Trp-104 to Met-115 was found to inhibit the binding of cofilin to G-actin (34). Similarly, another peptide, DAIKKKL, corresponding to the sequence from Asp-122 to Leu-128 also weakly inhibited the binding to F-actin (33).

Recently, we determined the tertiary structure of destrin/ADF, an isoprotein of cofilin, by triple-resonance multi-dimensional nuclear magnetic resonance. It was unexpectedly found that the folding of destrin is similar to those of repeated segments commonly present in the gelsolin family (11) although there are no amino acid sequence homology between these proteins. According to the structure of gelsolin segment 1 and actin complex, the long α-helix (Gln-95 to Leu-112) binds in a cleft formed at the interface of actin subdomains 1 and 3 (20). Analogously, it would be possible that the long α-helix (Leu-111 to Leu-128) of destrin binds to actin in a similar fashion. This model is supported by the fact that both of the sequences identified as actin-binding sites (33, 34) are contained in the helix. Furthermore, this model may explain the Ca^{2+}-independent binding of

destrin to actin. Gelsolin Asp-109 intermolecularly chelates Ca^{2+} with actin Glu-167, providing molecular basis for the Ca^{2+}-dependent binding of gelsolin to actin (20). A cluster of lysine residues in destrin, Lys-121, Lys-125, Lys-126 and Lys-127, appear to correspond to gelsolin Asp-109. We suggest that these lysine residues might electrostatically interact with actin Glu-167.

It should be noted that the nuclear localization signal of destrin is localized in the opposite side of the actin binding helix. We have noted that unphosphorylated cofilin in cultured mammalian cells (ca. 50% of total cofilin) were not localized in nuclei (24, 28). In addition, translocation of S3A-cofilin into nuclei required heat shock or other stresses when expressed in cells (24). We have observed that cofilin mutants which lost the actin-binding activity were not localized in nuclei in cells incubated at elevated temperatures (K.M., unpublished observations). These results suggest that dephosphorylation of cofilin and/or the binding of cofilin to actin are not sufficient for translocation of cofilin to nuclei. Conformational changes in cofilin or the actin/cofilin complex may be further required for translocation into nuclei.

Summary

1. Cofilin is an essential actin-regulating protein widely distributed in all eucaryotes. The structure and function of cofilin are conserved during evolution.

2. Cofilin depolymerizes F-actin *in vitro* at alkaline pH and severs F-actin *in vitro* at pH lower than 7.3. Overexpression of cofilin in viable cells induced bundles of actin filaments suggesting that the severing activity rather than the actin-depolymerizing or monomeric actin-sequestering activity is physiologically significant *in vivo*.

3. The actin bundle formation induced by overexpression of cofilin is accompanied with an increase in cell motility of *Dictyostelium* cells.

4. In higher vertebrates, the actin-binding activity of cofilin is negatively regulated by phosphorylation on its Ser-3 residue. The actin-binding activity is essential for yeast cells to grow.

5. Stresses and various cell stimuli activate cofilin by inducing dephosphorylation of cofilin in resting vertebrate cells.

6. Cofilin has an nuclear localization signal sequence and translocates into the nucleus together with actin in response to various stresses. Functional roles of cofilin/actin in the nucleus remain to be elucidated.

7. Tertiary structure of destrin (cofilin) resembles that of gelsolin segment 1 and well explains its functions such as Ca^{2+}-independent actin binding activity.

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