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Regulation of Actin Filament Dynamics by Actin Depolymerizing Factor/Cofilin and Actin-Interacting Protein 1: New Blades for Twisted Filaments†

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ABSTRACT: Actin depolymerizing factor (ADF)/cofilin enhances turnover of actin filaments by severing and depolymerizing filaments. A number of proteins functionally interact with ADF/cofilin to modulate the dynamics of actin filaments. Actin-interacting protein 1 (AIP1) has emerged as a conserved WD-repeat protein that specifically enhances ADF/cofilin-induced actin dynamics. Interaction of AIP1 with actin was originally characterized by a yeast two-hybrid system. However, biochemical studies revealed its unique activity on ADF/cofilin-bound actin filaments. AIP1 alone has negligible effects on actin filament dynamics, whereas in the presence of ADF/cofilin, AIP1 enhances filament fragmentation by capping ends of severed filaments. Studies in model organisms demonstrated that AIP1 genetically interacts with ADF/cofilin and participates in several actin-dependent cellular events. The crystal structure of AIP1 revealed its unique structure with two seven-bladed β-propeller domains. Thus, AIP1 is a new class of actin regulatory proteins that selectively enhances ADF/cofilin-dependent actin filament dynamics.

1. Regulation of Actin Filament Dynamics by Actin Depolymerizing Factor/Cofilin

Actin is one of the major cytoskeletal proteins in eukaryotes. Actin polymerizes into a filamentous form and serves as a core of the microfilament system. Dynamic assembly and disassembly of the actin cytoskeleton are required for a number of biological processes, such as cell division, cell motility, endocytosis, and morphogenesis. Actin by itself undergoes polymerization and depolymerization under physiological conditions. However, the rate of filament turnover by actin itself is much slower than that of actin filaments *in vivo*. Therefore, a number of actin regulatory proteins regulate nucleation, depolymerization, and fragmentation when rapid reorganization of the actin filaments is needed (*1*−*4*).

ADF/cofilin[1] is one of the essential factors for enhancing actin filament turnover (*5*−*8*). ADF/cofilin weakly severs actin filaments without capping ends (*9*−*16*), thereby increasing the number of free filament ends where polymerization and depolymerization occur (Figure 1). It also enhances the rate of monomer dissociation from the pointed ends (*17*, *18*) (Figure 1). Most ADF/cofilin proteins exhibit stronger depolymerizing activity at pHs above 7.5 than at lower pHs (*11*, *12*, *19*). This pH dependence is due to an increase in the critical concentration for the actin−ADF/cofilin complex at high pHs (*20*, *21*). ADF/cofilin binds preferentially to ADP-bound actin (*22*) and inhibits its

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[1] Abbreviations: AIP1, actin-interacting protein 1; ADF/cofilin, actin depolymerizing factor/cofilin; CAP, cyclase-associated protein.

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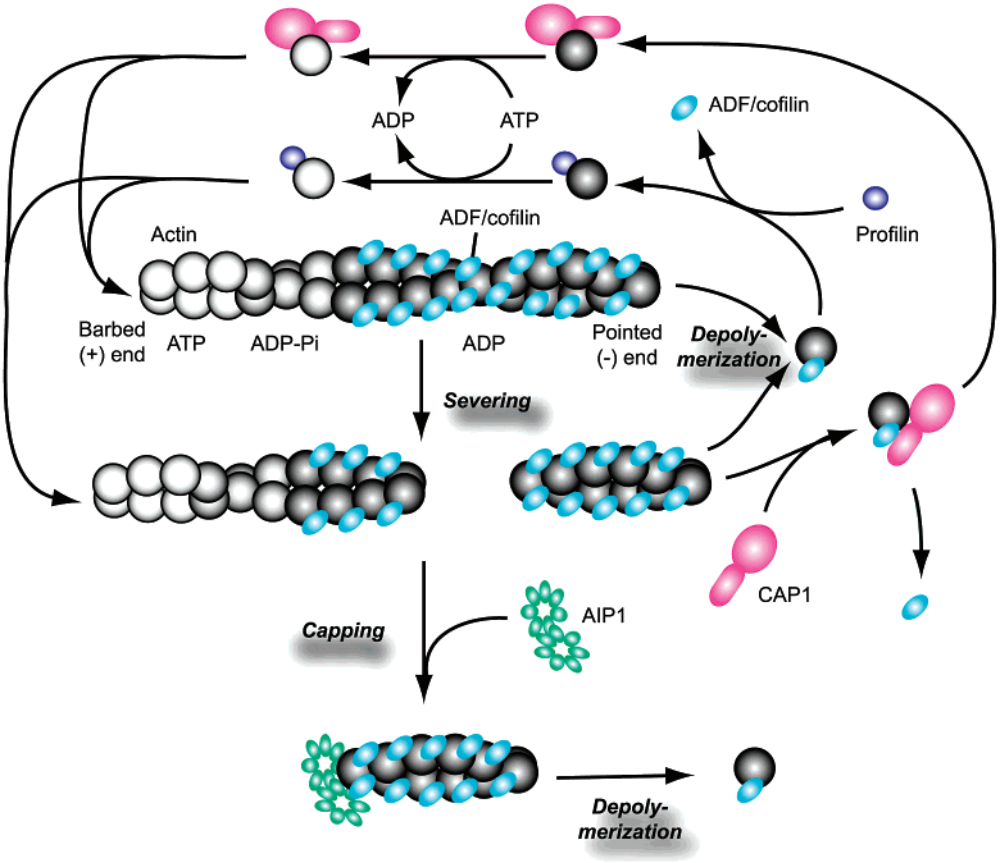

FIGURE 1: Current model of the regulation of actin dynamics by ADF/cofilin, CAP1, profilin, and AIP1. ADF/cofilin preferentially binds to ADP-actin, enhances depolymerization from the pointed ends, and severs filaments. Although ADF/cofilin inhibits exchange of actin-bound nucleotide, profilin and CAP1 enhance exchange of actin-bound ADP with ATP and promote barbed end elongation. AIP1 specifically caps ends of ADF/cofilin-bound filaments and enhances fragmentation.

nucleotide exchange (*23*). Electron microscopic observations revealed that filament binding by ADF/cofilin changes the twist of the actin filaments (*24*) or stabilizes a preexisting tilted conformation of actin subunits (*25*), and weakens lateral contacts in the filaments (*26*). An ADF/cofilin-induced conformational change in actin subunits was also observed in solution (*27*, *28*).

Structures of ADF/cofilin proteins have a core of four or five β-sheets, which is surrounded by four or five helices (*29*−*32*) (Figure 2). This structural fold is very similar to that of the individual segments of the gelsolin family of actin-severing/capping proteins (*33*). The presence of two actin-binding sites, the G/F-site and the F-site (Figure 2), has been demonstrated by various methods, including site-directed mutagenesis, cross-linking, peptide competition, electron microscopy, and synchrotron footprinting. The G/F-site that includes the long helix α3 and the N-terminus (Figure 2) is responsible for both G- and F-actin binding and sufficient for depolymerizing activity (*34*−*39*). The F-site, including the C-terminus and the loop (F-loop) at the opposite side of the G/F-site (Figure 2), is required in addition to the G/F-site for F-actin binding and severing activity (*37*, *40*−*42*). However, in the absence of the structure of the actin−ADF/cofilin complex at atomic resolution, the structural basis of severing and depolymerization by ADF/cofilin is not fully understood.

The ADF/cofilin proteins have been demonstrated to be involved in many cellular events in which rapid turnover or reorganization of actin filaments is required. In migrating

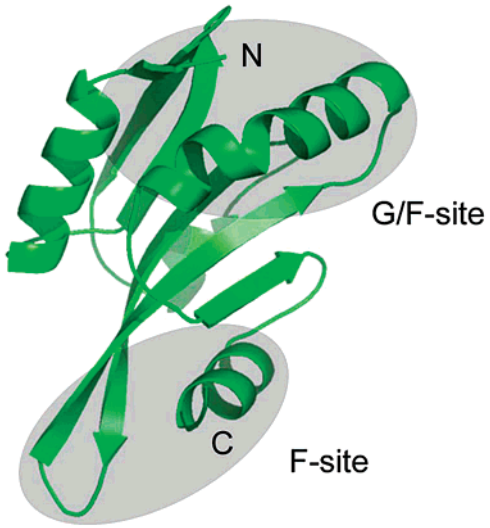

FIGURE 2: Structure of yeast cofilin (Protein Data Bank entry 1COF). The G/F-actin-binding site (G/F-site) and the F-actin-binding site (F-site) are represented by shaded regions. This figure was made with PyMOL (DeLano Scientific LLC, San Carlos, CA) and Adobe Photoshop 6.0.

cells, ADF/cofilin is localized to the lamellipodia (*43*, *44*) where it severs filaments and increases the number of exposed ends (*45*−*47*) or enhances turnover of the Arp2/3-mediated dendritic network of actin filaments (*48*). Over-expression of ADF/cofilin enhances cell motility (*49*, *50*), whereas genetic ablation of ADF/cofilin impairs cell motility

in vivo (*51*). Recently, Dawe *et al*. demonstrated that ADF/cofilin controls the polarity of cell migration (*52*). In addition, ADF/cofilin is required for cytokinesis (*53*−*55*), endocytosis (*56*), and myofibril assembly (*57*) and is implicated in many diseases (reviewed in refs *58* and *59*). As ADF/cofilin is involved in many fundamental cellular activities, mutations in ADF/cofilin genes cause lethality in yeast (*60*, *61*), the fruit fly *Drosophila melanogaster* (*53*), and the nematode *Caenorhabditis elegans* (*62*).

Multicellular organisms have multiple ADF/cofilin isoforms that have redundant or nonredundant functions (reviewed in refs *6* and *59*). Mammals have three ADF/cofilin isoforms [ADF, cofilin-1 (non-muscle-type cofilin), and cofilin-2 (muscle-type cofilin) (*63*−*66*)], while green plants have more than 10 isoforms (reviewed in refs *8* and *67*). These isoforms are often expressed in different tissues and show quantitative differences in actin depolymerizing activity (*21*, *66*, *68*, *69*). In *C. elegans*, UNC-60A, the nonmuscle isoform, has strong depolymerizing activity and is required for early embryonic development, while UNC-60B, the muscle isoform, has weaker activity and is required specifically for myofibril assembly (*55*). Isoform-specific functions of ADF/cofilin in other organisms are yet to be examined.

2. Regulators of ADF/Cofilin

ADF/cofilin interacts with a number of cellular factors that influence actin cytoskeletal dynamics. Phosphorylation of ADF/cofilin at a conserved serine residue near the N-terminus inhibits its actin binding activity (*70*, *71*). The N-terminus of ADF/cofilin is predicted to be an actin-binding site (*72*). Introduction of an acidic residue in the N-terminal region of ADF/cofilin greatly reduces its affinity for actin (*20*, *40*, *73*). In vertebrates, LIM-kinases and TES kinases are specific for ADF/cofilin and mediate various signals for remodeling of the actin cytoskeleton (reviewed in refs *74* and *75*). On the other hand, dephosphorylation of ADF/cofilin is induced by many stimuli, but their signaling components are poorly understood (reviewed in ref *76*). Effects of pharmacological phosphatase inhibitors on dephosphorylation of ADF/cofilin are not consistent among different cell types (*77*−*80*). Recently, a specific ADF/cofilin phosphatase, Slingshot, has been identified (*81*). In addition, 14-3-3ζ binds to phosphorylated cofilin and prevents dephosphorylation by a phosphatase (*82*). However, how these regulators participate in the previously reported pathways for ADF/cofilin dephosphorylation is not known.

Phosphoinositides, such as phosphatidylinositol 4,5-bisphosphate (PIP2), bind to ADF/cofilin and compete with actin (*83*). A PIP2 binding site on ADF/cofilin is mapped to long helix α3 (*84*−*86*) and several other positively charged residues (*86*) which overlap with the actin-binding surface. At high concentrations, PIP2 inhibits the interaction between ADF/cofilin and actin *in vitro*. The activity of chicken cofilin to reorganize the actin cytoskeleton in microinjected cultured cells is significantly inhibited by its preincubation with PIP2 (*87*). However, *in vivo*, the role of PIP2 in the regulation of ADF/cofilin has not been established.

Tropomyosin competes with ADF/cofilin for F-actin binding and inhibits depolymerization (*9*, *88*−*92*). In yeasts, cofilin is restricted to cortical actin patches (*61*), while tropomyosin is localized to actin cables (*93*). However, in tropomyosin mutant cells, cofilin is localized to a cable-like structure (*94*). In *C. elegans*, tropomyosin is important for stabilization of actin filaments because tropomyosin protects them from ADF/cofilin (*92*). In migrating cells, ADF/cofilin is enriched in the lamellipodia near the leading edge (*43*, *44*), whereas tropomyosin is depleted from the leading edge and localized to proximal regions of the lamellipodia and the stress fibers (*95*). Thus, tropomyosin spatially segregates stable filaments from dynamic filaments to establish compartments of functionally distinct cytoskeleton in a cell (reviewed in ref *96*). However, vertebrates have more than 40 tropomyosin isoforms, and one of the nonmuscle isoform is colocalized with ADF/cofilin to actin filaments in the lamellipodia (*97*), suggesting that not all tropomyosins compete with ADF/cofilin for F-actin binding.

In addition to these regulators of ADF/cofilin, several actin-binding proteins have recently been shown to collaborate with ADF/cofilin to promote actin filament dynamics (Figure 1). Profilin binds to G-actin, enhances exchange of actin-bound ADP with ATP, and promotes filament growth from the barbed ends. However, when the barbed ends are capped, profilin sequesters actin monomers (reviewed in ref *98*). Profilin competes with ADF/cofilin for G-actin binding (*14*, *99*) and enhances exchange of actin-bound nucleotide in the presence of ADF/cofilin, thereby increasing the rate of actin turnover synergistically with ADF/cofilin (*100*) (Figure 1). Cyclase-associated protein (CAP) sequesters actin monomers by binding to G-actin through its C-terminal region (reviewed in ref *101*) that contains a WH2 (WASP-homology 2) domain (*102*). Recently, Moriyama and Yahara (*103*) demonstrated that the N-terminal domain of CAP binds to the actin−cofilin complex and accelerates actin depolymerization from the pointed ends (Figure 1). Furthermore, the C-terminal domain of CAP enhances exchange of actin-bound ADP with ATP and promotes barbed end elongation, thus enhancing rapid subunit turnover (Figure 1).

Actin-interacting protein 1 (AIP1) is a unique protein that enhances filament severing only in the presence of ADF/cofilin. The function of AIP1 is discussed in the following sections in detail.

3. Enhancement of ADF/Cofilin-Dependent Actin Filament Disassembly by AIP1

AIP1 was originally identified in yeast as one of actin-interacting proteins from a two-hybrid screen (*104*). Subsequently, AIP1 was rediscovered as a protein that binds to ADF/cofilin by affinity chromatography (*105*) or a yeast two-hybrid system (*106*) and as a high-copy number suppressor of a mutant cofilin allele in yeast (*107*). In yeasts, the *AIP1* gene is not essential for viability, whereas a deletion of *AIP1* is synthetic lethal with mutant cofilin alleles (*106*, *107*). In *C. elegans*, AIP1 is encoded by the *unc-78* gene and shows genetic interaction with the *unc-60B* ADF/cofilin gene (*108*). This genetic evidence strongly suggests that AIP1 and ADF/cofilin functionally interact *in vivo*.

Biochemical studies have revealed that AIP1 collaborates with ADF/cofilin to disassemble actin filaments (*105*, *106*, *109*). AIP1 alone has negligible effects on the dynamics of actin filaments. However, in the presence of ADF/cofilin, it induces extensive disassembly of actin filaments. Electron microscopy showed that AIP1 and ADF/cofilin increased a

population of short filaments (*105*, *109*), suggesting that filament disassembly is due to enhanced filament fragmentation. Okada *et al*. (*110*) demonstrated that *Xenopus* AIP1 binds to filament ends and inhibits elongation from the barbed ends without affecting ADF/cofilin's severing activity. Therefore, enhanced filament fragmentation by AIP1 is caused by its barbed end capping activity that prevents elongation and reannealing of the severed filaments (Figure 1). AIP1 appears to recognize the ends of ADF/cofilin-bound filaments rather than ADP-actin subunits at the ends of ADF/cofilin-severed filaments, because AIP1 does not prevent reannealing of mechanically fragmented ADP-actin in the absence of ADF/cofilin (*110*). *C. elegans* AIP1 (UNC-78) weakly enhances monomer dissociation from filaments, suggesting that AIP1 has activity to enhance ADF/cofilin-mediated filament turnover (*111*). In addition, UNC-78/AIP1 does not enhance depolymerization by mutants of ADF/cofilin which binds to G-actin, but not to F-actin, suggesting filament binding by ADF/cofilin is required for AIP1 to interact with filaments (*111*). UNC-78/AIP1 shows preferential interaction with a specific ADF/cofilin isoform (*111*). Enhancement of filament disassembly by UNC-78/AIP1 is very efficient in the presence of UNC-60B, a muscle-specific *C. elegans* isoform of ADF/cofilin (*57*) but not remarkable in the presence of UNC-60A, a ubiquitously expressed isoform that has strong depolymerizing activity on its own (*55*, *69*). Thus, in multicellular organisms, AIP1 and ADF/cofilin may interact in an isoform-specific manner.

The AIP1-binding site on actin is mapped to subdomains 3 and 4 by the yeast two-hybrid system (*104*). The cofilin-binding site on G-actin is localized to subdomains 1 and 3 by molecular dynamics simulation and biochemical competition with gelsolin segment-1 that binds to the same region of G-actin (*72*). In addition, electron cryomicroscopy revealed that cofilin interacts with F-actin at two sites: subdomains 1 and 3 of the upper and subdomains 2 and 1 of the lower actin subunits (*24*, *41*). Biochemical analysis also showed two cofilin-binding sites on F-actin (*112*, *113*). Thus, the AIP1-binding site in subdomain 3 overlaps with the cofilin-binding site, while that in subdomain 4 is unique for AIP1 binding. Nevertheless, since mapping of the AIP1-binding site on actin was determined as yeast two-hybrid interactions, endogenously expressed cofilin or other proteins could mediate the interactions. AIP1 cosediments with F-actin with low affinity (estimated dissociation constant of 15 μM) (*109*), and cosedimentation is enhanced in the presence of cofilin (*105*, *106*). However, in these studies, cosedimentation of AIP1 with F-actin did not reach saturation, and in the presence of cofilin, a large amount of F-actin is disassembled. Therefore, the stoichiometry and affinity of binding between AIP1 and actin remain unclear.

4. Structure of AIP1

Orthologs of AIP1 have been identified in a wide range of eukaryotic species. They have 597−615 amino acids that are 20−95% identical. AIP1s have been predicted to contain up to 10 WD repeats that match the consensus sequence (*114*). WD repeats are found in a wide range of proteins, and they are believed to fold into a β-propeller structure in which each repeat corresponds to a single blade containing a four-stranded antiparallel β-sheet (*114*). Recently determined crystal structures of yeast AIP1 (*137*) and *C. elegans*

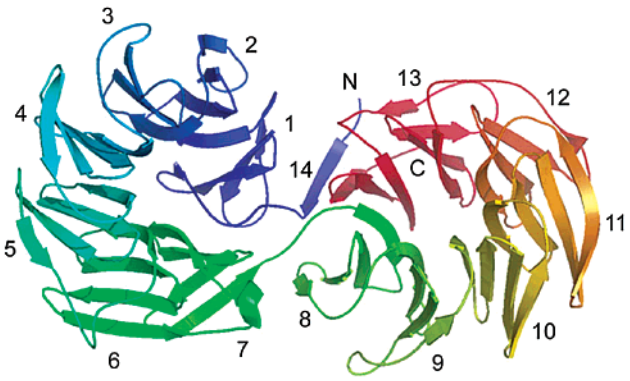

FIGURE 3: Structure of *C. elegans* AIP1 (UNC-78) (Protein Data Bank entry 1NR0; the coordinates will be released upon publication of the structure by S. Vorobiev, K. Mohri, S. Ono, and S. Almo). The chain is depicted as gradual changes in color from the N-terminus (blue) to the C-terminus (red). Blades 1−14 are denoted. This figure was made with PyMOL (DeLano Scientific LLC) and Adobe Photoshop 6.0.

AIP1 (UNC-78) (S. Vorobiev, K. Mohri, S. Ono, and S. C. Almo, manuscript in preparation) revealed that AIP1 indeed folds into a β-propeller, but surprisingly that it has two seven-bladed β-propeller domains totaling 14 blades in a single molecule (Figure 3). The two domains are connected with a hinge and slightly twisted. AIP1 is the first protein to be characterized as a β-propeller protein with two propeller domains in a single molecule. Other WD repeat proteins, such as the β-subunit of trimeric G-protein (*115*−*117*), the p40 subunit of the Arp2/3 complex (*118*), and the CDC4 ubiquitin ligase (*119*), have seven or eight repeats (blades) in a single propeller. The β-propeller fold is also present in other nonhomologous proteins, but only four to eight blades have been found to date in a single molecule (*120*). Interestingly, from sequence analysis, a protein with 16 WD repeats is predicted (*114*). Therefore, the structure of AIP1 suggests that other WD repeat proteins with more than nine repeats could have multiple β-propeller domains.

Functional residues of AIP1 have not yet been identified. In addition, no sequence motif for actin binding has been proposed for AIP1. Therefore, the structure of AIP1 will be very important in the study of its function and molecular interactions with actin and ADF/cofilin. Four mutations in the *C. elegans unc-78* gene that cause disorganization of the myofibril structure have been reported (*108*). However, recombinant UNC-78 proteins with these mutations become insoluble when they are expressed in *Escherichia coli* (K. Mohri and S. Ono, unpublished observations), suggesting that the structure, rather than the functional residues, is altered by the mutations. Structure-based mutagenesis analysis of surface residues should be a straightforward approach to identifying functional sites on AIP1.

5. Cell Biological Function of AIP1

5.1. Yeasts. As briefly described above, the budding yeast *Saccharomyces cerevisiae* AIP1 gene, *AIP1*, is nonessential and genetically interacts with the cofilin gene. However, the function of AIP1 is not clear because deletion of the *AIP1* gene does not cause detectable growth or morphological defects (*106*, *107*). AIP1 is localized to cortical actin patches where cofilin is also localized (*61*). Its localization to cortical patches is dependent on actin and cofilin as AIP1 becomes

cytosolic in several actin or cofilin mutant backgrounds that do not disrupt the integrity of the patches (*106*). On the other hand, in the absence of AIP1, cofilin is found in actin cables where cofilin is not normally found (*106*, *107*), suggesting that AIP1 has a role in restricting the localization of cofilin to cortical patches. Deletion of *AIP1* is synthetic lethal with the V159N mutation of the *ACT1* actin gene, which decreases rates of actin filament turnover (*94*), suggesting that AIP1 supports enhancement of actin turnover. It has not yet been demonstrated whether AIP1 has a role in endocytosis in which cofilin is required (*56*). An AIP1 sequence has been identified in the fission yeast *Schizosaccharomyces pombe*, but functional study on this gene is not reported.

5.2. Slime Molds. In *Physarum polycephalum*, an AIP1 ortholog, p66, was identified as a heat shock protein (*121*). Upon heat shock, the *Physarum* amoeboid cells reorganize the actin cytoskeleton and undergo transformation into dormant microcysts. p66 is strongly upregulated by heat shock and becomes associated with cytoplasmic actin rods (*122*). Partially purified p66 coprecipitates with F-actin (*122*), but highly purified p66 does not (*121*), suggesting the presence of a factor(s), possibly cofilin, that mediates interaction of p66 with F-actin. These reports suggest that *Physarum* p66 is involved in actin reorganization during microcyst formation. However, its mechanism and the role of cofilin in this process have not been reported.

Intracellular localization of *Dictyostelium* AIP1 (DAIP1) is very similar to that of cofilin (*123*, *124*), and it is enriched in dynamic actin-rich structures such as leading edges of motile cells, phagocytic cups, and macropinosomes, but not in a cleavage furrow (*125*). DAIP1 is also colocalized with cofilin to hyperosmotic stress-induced cortical actin bundles and dimethyl sulfoxide-induced intranuclear actin rods (*109*). These suggest that DAIP1 closely collaborates with cofilin in reorganization of the actin cytoskeleton. A null mutation of DAIP1 causes reduced rates of growth, cytokinesis, fluid-phase uptake, phagocytosis, and cell motility (*125*). However, unlike in yeasts and *C. elegans* (see below), the localization of cofilin is not changed in DAIP1-null cells (*125*). Overexpression of DAIP1 enhances fluid-phase uptake (*125*) and partially impairs cytokinesis (*109*). These cellular events require dynamic regulation of actin filaments, suggesting that DAIP1 is important in enhancing the filament dynamics by disassembling filaments. Nonetheless, the phenotypes of DAIP1-null cells are not severe, suggesting that DAIP1 has a supporting role in actin dynamics mediated by cofilin and/or other actin regulatory proteins.

5.3. C. elegans. Two AIP1 genes are predicted in the *C. elegans* genome sequence. One of them (C04F6.4) has been identified as the *unc-78* gene (*108*). The *unc-78* locus was originally defined from mutant screens for muscle-affecting uncoordinated worms (*126*, *127*). An *unc-78*-null mutation causes severe disorganization of actin filaments and formation of actin aggregates in body wall muscle, resulting in a defect in muscle contractile activity (*108*) (Figure 4). Point mutations in the WD repeats of the UNC-78 protein also cause similar, but weaker, phenotypes as compared to the null mutant. *unc-78* mutations enhance the motility defects of the *unc-60B* ADF/cofilin mutants, which also exhibit disorganized actin filaments in body wall muscle (*57*). In addition, UNC-60B (ADF/cofilin) is mislocalized to actin aggregates in *unc-78* mutants (*108*), while it is normally localized in the diffuse cytoplasm and to a part of myofibrils (*57*, *92*). UNC-78 is expressed in body wall muscle and other muscle tissues and localized to thin filaments (*111*). Thus, UNC-78/AIP1 functionally interacts with UNC-60B to organize actin filaments in muscle cells. Interestingly, UNC-78 is colocalized with actin in *unc-60B*-null mutant muscle cells, suggesting that there is an unknown mechanism that mediates interaction between UNC-78 and actin. Functional analysis of the second AIP1 isoform (K08F9.2) is not reported except that large-scale RNA interference projects yielded no detectable phenotype for this gene (*128*, *129*).

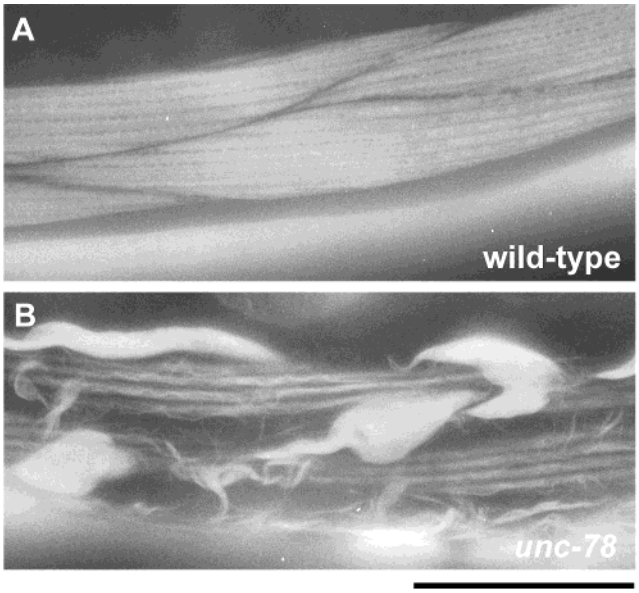

FIGURE 4: Disorganization of actin filaments by a null mutation of the *C. elegans* AIP1 (*unc-78*) gene. Wild-type (A) or *unc-78*-null (B) worms were stained with tetramethylrhodamine phalloidin to visualize filamentous actin, and parts of the body wall muscle are shown. Striated organization of actin filaments in the wild type (A) is significantly disturbed in the *unc-78* mutant with formation of extensive aggregates (B). The bar is 20 μm.

5.4. Vertebrates. In *Xenopus* embryos, AIP1 (XAIP1) is localized to the cell cortex, diffuse cytoplasm, and nuclei (*105*). During the first cleavage, XAIP1 is accumulated in the cytoplasmic side of the progressing cleavage furrow (*105*) where *Xenopus* ADF/cofilin (XAC) is also enriched (*54*) and active actin polymerization occurs (*130*). Microinjection of blastomeres with purified XAIP1 causes arrest of cleavage and later development by disrupting cortical accumulation of actin and XAC (*105*), suggesting that XAIP1 function together with XAC to regulate actin organization *in vivo*.

In chickens, an AIP1 ortholog, WDR1, was identified as a gene that is upregulated in the auditory epithelium of the inner ear upon noise damage (*131*). WDR1 is expressed in normal auditory epithelium and colocalized with ADF/cofilin and actin, but noise damage transiently induces overexpression of WDR1 in the supporting cells that are important for the structural integrity of the epithelium (*132*). This epithelium has the ability to regenerate the damaged tissues and restore hearing capability, suggesting that WDR1 is involved in cytoskeletal reorganization during regeneration. cDNAs for human and mouse WDR1 were cloned (*131*), but no functional analysis of these proteins has been reported.

5.5. Plants. In the green plant *Arabidopsis thaliana*, two AIP1 genes are identified (*133*). Interestingly, AtAIP1-1 is

specifically expressed in reproductive tissues, whereas AtAIP1-2 is in both reproductive and vegetative tissues. An antibody against AtAIP1-1 is cross-reactive with a lily protein that is localized to actin bundles in pollen grains and diffuse cytoplasm in pollen tubes (*133*). This pattern is very similar to that of LIADF1, a pollen-specific ADF/cofilin in lily, suggesting collaboration of AIP1 and ADF/cofilin in actin reorganization in pollens.

6. Conclusion and Future Directions

Functional studies in different model systems agree that ADF/cofilin and AIP1 collaborate to enhance actin filament disassembly and participate in cytoskeletal reorganization. ADF/cofilin has been studied in a variety of systems, but studies on AIP1 are still limited. Therefore, it is not known whether AIP1 is generally involved in a number of cellular events that are regulated by ADF/cofilin. For example, ADF/cofilin promotes filament severing during lamellipodial extension and enhances cell motility. AIP1 is also localized at the leading edge of motile *Dictyostelium* amoeboid cells, and AIP1-null cells show impaired cell motility (*125*). However, how AIP1 regulates cell motility is not known. For efficient protrusion of lamellipodia, capping protein is recruited to the leading edge, blocks most barbed ends, and contributes to maintaining high concentrations of monomeric actin (*134*, *135*). AIP1 may function as a barbed end capping protein specific for ADF/cofilin-severed filaments. Therefore, when AIP1 is absent, inefficient capping will allow excessive filament elongation and consumption of actin monomers, thus impairing efficient protrusion of lamellipodia. ADF/cofilin and capping protein are also identified as essential components of an *in vitro* model of actin-based motility (*136*). Therefore, it would be interesting to test whether AIP1 can substitute for capping protein in this system.

Recent advances in the structure and function of the AIP1 proteins should greatly help further the understanding of the mechanism of cytoskeletal regulation by ADF/cofilin and AIP1. First of all, the structure should help in identifying functional surface residues on AIP1, which will be essential in modeling the structure of the complex of actin, ADF/cofilin, and AIP1. However, binding of AIP1 to actin or ADF/cofilin has been demonstrated only by immunoelectron microscopy or crude systems (yeast two-hybrid interactions and affinity chromatography of cell lysates). Therefore, the interaction of AIP1 with actin and ADF/cofilin is not fully understood. Evidence of binding of AIP1 to the side of actin filaments is presented (*110*), but its functional significance is not known. Also, AIP1 enhances actin depolymerization in the presence of ADF/cofilin, but it is not known whether AIP1 affects the off rate of actin subunits at the pointed ends. In conclusion, AIP1 is a conserved helper for ADF/cofilin in enhancing filament fragmentation and promoting reorganization of the filaments. Even though AIP1 is not a central player in actin filament dynamics, it may be required for fine-tuning of the ADF/cofilin-mediated actin dynamics in the highly complex *in vivo* cytoskeletal systems.

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