Aip1p Interacts with Cofilin to Disassemble Actin Filaments

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Abstract. A ctin interacting protein 1 (Aip1) is a conserved component of the actin cytoskeleton first identified in a two-hybrid screen against yeast actin. Here, we report that Aip1p also interacts with the ubiquitous actin depolymerizing factor cofilin. A two-hybrid–based approach using cofilin and actin mutants identified residues necessary for the interaction of actin, cofilin, and Aip1p in an apparent ternary complex. Deletion of the AIP1 gene is lethal in combination with cofilin mutants or act1-159, an actin mutation that slows the rate of actin filament disassembly in vivo. Aip1p localizes to cortical actin patches in yeast cells, and this localization is disrupted by specific actin and cofilin mutations. Further, Aip1p is required to restrict cofilin localization to cortical patches. Finally, biochemical analyses show that Aip1p causes net depolymerization of actin filaments only in the presence of cofilin and that cofilin enhances binding of Aip1p to actin filaments. We conclude that Aip1p is a cofilin-associated protein that enhances the filament disassembly activity of cofilin and restricts cofilin localization to cortical actin patches.

Key words: Aip1 • cofilin • actin • cytoskeleton • Saccharomyces cerevisiae

The actin cytoskeleton plays diverse roles in the cell, mediating such processes as endocytosis, exocytosis, cell motility, cell polarity, and cytokinesis in a spatially and temporally controlled manner. Each of these processes requires the regulation of specific dynamic properties and spatial organization of actin filaments by a subset of a large collection of actin-binding proteins. To determine how actin and associated proteins function together to control morphogenetic events, it will be important to determine how actin-associated proteins are sorted to different structures of the cytoskeleton, and how the combined actions of different actin-associated proteins affect cytoskeleton dynamics in these different structures.

Saccharomyces cerevisiae presents excellent opportunities for the study of combined effects of actin-associated proteins on actin assembly and organization both in vivo and in vitro. The actin cytoskeleton of S. cerevisiae is organized into polarized cortical patches and cytoplasmic bundles of actin filaments aligned along the mother–daughter cell axis (A dams and Pringle, 1984; A mberg, 1998). Little is known about how actin-binding proteins are sorted between different compartments of the cytoskeleton, or whether they themselves are responsible for forming these specialized actin networks.

We previously described a two-hybrid approach for characterizing interactions of actin-binding proteins with yeast actin (A mberg et al., 1995a). We used this system to identify yeast actin–associated proteins and examined their ability to interact with 35 clustered-charged-to-alanine mutants of actin. Those mutations that disrupt the binding of a particular ligand can identify regions of the actin surface important for a given interaction and can delineate an interaction footprint when displayed on the structure of actin. A ctin interacting protein 1 (Aip1p),† identified in our two-hybrid analysis using actin as bait, had a very distinct interaction footprint on actin subdomains III and IV. We report here that in addition to interacting with actin, Aip1p also associates with the small actin-binding protein cofilin.

Members of the cofilin/actin depolymerizing factor family are conserved actin monomer and filament binding proteins that induce actin filament disassembly (for review see M oon and D rubin, 1995). Yeast cofilin is 40% identical in sequence to mammalian cofilin/actin depolymerizing factor; the gene is essential in yeast and the gene product localizes to cortical actin patches (M oon et al. 1993). Recently, two advances have led to a greater understanding of cofilin function in yeast: (1) A synaptic set of cofilin mu-
tants was constructed by alanine scanning mutagenesis (Lappalainen and Rubin, 1997) and (2) the structure of yeast coflin was determined (Fedorov et al. 1997).

In this report, we have used this large set of genetic and structural tools in conjunction with classical biochemical and cell biological analyses to gain insight into the function of the interactions between Aip1p, coflin, and actin. We found that Aip1p mediates the restriction of coflin to cortical actin patches and that purified Aip1p has dramatic effects on coflin’s activity in vitro. Our results suggest that these two proteins interact in vivo to regulate actin dynamics.

**Materials and Methods**

**Yeast Strains, Media, and Genetic Methods**

Yeast strains are listed in Table I. FY23 and FY86 were provided by Fred Winston (Harvard Medical School, Boston, MA). Y187 and Y190 were provided by Steve Elledge (Baylor College of Medicine, Houston, TX). DDY319, DDY321, DDY760, and DDY496 were constructed as described (Holtzman et al., 1993, 1994; Moon et al., 1993). Standard methods were employed for growth, sporulation, and tetrad dissection of yeast (Rose et al., 1989). Yeast transformations were performed by electroporation (Becker and Garenne, 1991) or by lithium acetate (Rose et al., 1989). The medium for two-hybrid analysis was synthetic medium plus dextrose supplemented with adenine to 10 μg/ml and 3,5-amino-triazole (3-A-T) (Sigma Chemical Co.) at 25, 50, or 100 mM.

**Plasmid Construction and DNA Manipulations**

Plasmid pRB2247 was constructed by isolating a 1.4-kb product of a BglII partial digest of plasmid pRB2248 and cloning this fragment into the BamHI site of plasmid pGEX-SX-3 (Pharmacia Biotech, Inc.) such that the AIP1 gene was in frame with the glutathione-S-transferase (GST) reading frame. Plasmid pRB2251 was constructed by subcloning a 2.2-kb ClaI fragment from AIP1 genomic clone pRB2249 into YCp50 such that AIP1 and bla transcription is divergent. The deletion allele of AIP1 was constructed by double fusion PCR and has been described elsewhere (Amberg et al., 1995b).

Plasmids encoding fusions of the GAL4 DNA binding domain (DBD) to SNF1 (pSE1112), the GAL4 DBD to lamin (pA51-lamin), and the GAL4 activation domain (AD) to SNF4 (pSE1111) were provided by Steve Elledge. The construction of the plasmids carrying fusions of the actin-alanine scan alleles to the Gal4 DBD, a fusion of the Gal4 DBD to ACT1 (pRB1516 also known as pBAD7), a fusion of the Gal4 AD to ACT1 (pAIP70), and a fusion of the Gal4 AD to AIP1 (pBH2248) previ-

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Table I. Saccharomyces cerevisiae Strains

| Name | Genotype | Source |
|------|----------|--------|
| FY23 | a ura3-52 leu2Δ1 trplΔ63 | Winston et al., 1995 |
| FY86 | a ura3-52 leu2Δ1 his3Δ200 | Winston et al., 1995 |
| DDY319 | a/a ura3-52 ura3-52 leu2Δ1/S/Leu2Δ1 TRPL1/1 TRP1 HIS3/his3Δ200 | This work |
| Y187 | a gal4 gal80 his3 trpl Δ901 ade-2-101 ura-3-52 leu-2-3,112 GAL–lacZ | Bai and Elledge, 1996 |
| Y190 | a gal4 gal80 his3 trpl Δ901 ade-2-101 ura-3-52 leu-2-3,112 URA3::GAL–lacZLYS2::GAL–HIS3cyh’ | Bai and Elledge, 1996 |
| DAY30 | a ura3-52 leu2Δ1 trplΔ63 aip1Δ1::URA3 | This work |
| DAY52 | a ura3-52 leu2Δ1 trplΔ63 his3Δ200 aip1Δ1::TRP1 | This work |
| DAY53 | a ura3-52 leu2Δ1 trplΔ63 his3Δ200 aip1Δ1::TRP1 | This work |
| DBY6527 | a ura3-52 leu2Δ1 trplΔ63 his3Δ200 aip1Δ1::URA3 | Amberg et al., 1995b |
| DBY6529 | a ura3-52 leu2Δ1 trplΔ63 his3Δ200 aip1Δ1::LEU2 | Amberg et al., 1995b |
| DBY6531 | a ura3-52 leu2Δ1 trplΔ63 his3Δ200 aip1Δ1::TRP1 | Amberg et al., 1995b |
| DBY130 | a ura3-52 leu2-3,112 his3Δ200 lys2-801 | |
| DDDY496 | a leu2-3,112 ura3-52 slaa-Δ1::URA3 | |
| DDDY319 | a sac6-Δ1::LEU2 his3Δ200 leu2-3,112 lys2-801 ura3-52 | |
| DDDY321 | a abp1-Δ1::LEU2 his3Δ200 leu2-3,112 ura3-52 | |
| DDDY760 | a slaa-Δ1::LEU2 leu2-3,112 ura3-52 ade2-1 ade3 | |
| TDS143 | a ura3-52 leu2-3,112 his3Δ200 tub2-210 act1-111::HIS3 ade4 | Wertzman et al., 1992 |
| TDS150 | a ura3-52 leu2-3,112 his3Δ200 tub2-210 act1-119::HIS3 ade4 | Wertzman et al., 1992 |
| TDS156 | a ura3-52 leu2-3,112 his3Δ200 tub2-210 act1-125::HIS3 ade4 | Wertzman et al., 1992 |
| TDS363 | a ura3-52 leu2-3,112 his3Δ200 tub2-210 act1-133::HIS3 ade2-101 | Wertzman et al., 1992 |
| TDS167 | a ura3-52 leu2-3,112 his3Δ200 tub2-210 ACT1::HIS3 ade4 | Wertzman et al., 1992 |
| DDDY355 | a ura3-52 leu2-3,112 his3Δ200 tub2-210 act1-122::HIS3 ade4 ade2-101 cry1r | |
| DDDY582 | a ura3-1 leu2-3,112 trpl-1 his3Δ11,15 ade2-101 cap2-Δ1::HIS3 | |
| DDDY1252 | a ura3-52 his3Δ200 lys2-801 COF1::LEU2 | |
| DDDY1253 | a ura3-52 his3Δ200 lys2-801 cof1-4 | |
| DDDY1254 | a ura3-52 his3Δ200 lys2-801 cof1-5 | |
| DDDY1255 | a ura3-52 his3Δ200 lys2-801 cof1-6 | |
| DDDY1256 | a ura3-52 his3Δ200 lys2-801 cof1-7 | |
| DDDY1257 | a ura3-52 his3Δ200 lys2-801 cof1-10 | |
| DDDY1258 | a ura3-52 his3Δ200 lys2-801 cof1-8 | |
| DDDY1259 | a ura3-52 his3Δ200 lys2-801 cof1-11 | |
| DDDY1260 | a ura3-52 his3Δ200 lys2-801 cof1-12 | |
| DDDY1261 | a ura3-52 his3Δ200 lys2-801 cof1-13 | |
| DDDY1262 | a ura3-52 his3Δ200 lys2-801 cof1-15 | |
| DDDY1263 | a ura3-52 his3Δ200 lys2-801 cof1-18 | |
| DDDY1264 | a ura3-52 his3Δ200 lys2-801 cof1-19 | |
| DDDY1265 | a ura3-52 his3Δ200 lys2-801 cof1-21 | |
| DDDY1266 | a ura3-52 his3Δ200 lys2-801 cof1-22 | |
| DDDY1435 | a ura3-52 leu2-3,112 his3Δ200 ade2-101 top1Δ::URA3 | Goode et al., 1998 |
| DDDY1492 | a ura3-52 leu2-3,112 his3Δ200 tab2-101 act1-159::HIS3 | Belmont et al., 1998 |
ousely were described elsewhere (Amberg et al., 1995a). The plasmid encoding a fusion of the GAL4 DBD to AIP1 (pDA b19) was constructed by removing the AIP1 open reading frame from pBAD24B as a BglII partial digest and cloning it into the BamHI site of plasmid pBR315 (Amberg et al., 1995a) (a Cen version of pSI CHY H2) so that the AIP1 open reading frame is in frame with that of the GAL4 DBD. The construct encoding a fusion of the GAL4 DBD to A BP1 (pDA b20) was constructed by excising the ABP1 open reading frame from plasmid pBR119 (Drubin et al., 1988) as a 1.9 kb XhoI-EcoR1 fragment, blunting the EcoR1 site with T4 DNA polymerase and cloning into plasmid pACTII (gift of Steve Elledge) that had been cut with XhoI and SalI in which the SalI site had been made blunt with T4 DNA polymerase. The resulting construct expresses all but the first 11 amino acids of AIP1 fused to GAL4p.

The constructs encoding fusions of the cofilin mutants to the GAL4 A D (used for the two-hybrid studies) were constructed by PCR into plasmid pACTII. The cofilin mutant and wild-type alleles were amplified off plasmids (Lappalainen et al., 1997) using primers D A o C OF F1 (5'-cgccggcat ggaaagaaatgtcgatctagc-3') and D A o C OF F2 (5'-cgccgacatcgatctatcccaggaagcgcgc-3') and vent polymerase (New England Biolabs Inc.). Subcloning of cof1-4 required the use of the special primer D A o C OF F3 (5'-cgccgacatcgatctatcccaggaagcgcgc-3') in the place of primer D A o C OF F1. The PCR products were cut with NcoI and EcoRI and cloned into similarly cut pACTII. Each construct was cloned and tested in duplicates generated from separate PCR reactions. When possible, constructs were confirmed by a BrdvI digest. Plasmid pACTII-COF1 was made by PCR amplification of the COFI open reading frame with oligonucleotides PL76.2 (5'-cgccgacatcgatctatcccaggaagcgcgc-3') and PL76.2 (5'-cgccgacatcgatctatcccaggaagcgcgc-3') for the COF1 product and with NcoI-BamHI, and subcloning of this insert in frame similarly digested pACTII.

To express AIP1 as a GST fusion protein in yeast, primers A R P1 (5'-gcggcggccgtccatcattgtatcattg-3') and A R P3 (5'-cgccgacatcgatctatcccaggaagcgcgc-3') for the AP1 product were used to amplify the AIP1 open reading frame from genomic DNA. This PCR product was cleaved with BamHI and E agl and cloned into similarly cut pEG202. The subsequent construct was transformed into yeast strain Y190 and Y187 derivatives together onto yeast extract/peptone/dextrose medium–plates, incubating at 30°C for 24 h, selecting the mated cells on SC-TRP, -LEU. The selected diploids, carrying both constructs encoding fusions to the Gal4 AD, were replica plated onto SC media lacking Leu (SC-LEU) for AD fusions. Mating was carried out by replica plating the Y190 and Y187 derivatives onto SC media lacking Trp (SC-TRP) or D BD fusions and synthetic complete medium lacking Leu (SC-LEU) for A IP1 fusions. Mating was carried out by replica plating the Y190 and Y187 derivatives onto synthetic complete medium–plates, incubating at 30°C for 24 h, selecting the mated cells on SC-TRP, -LEU. The selected diploids, carrying both B D and A D fusion constructs, were replica plated to media containing 25, 50, and 100 mM 3-A T (Sigma Chemical Co.) and incubated at 25°C.

Protein Purification and Antibody Production

AIP1-GST fusion protein was produced for antibody production by transforming bacterial strain UT5600 (ΔompT-ΔfepA) leu protE ProT (provided by S. Gottesman, National Cancer Institute, Bethesda, MD) carrying plasmid pBR2247 by standard methods (Smith and Johnson, 1988; GST Gene Fusion System manual, Pharmacia Biotech, Inc.). The antibodies were raised in three New Zealand white rabbits by injection of 100 μg of GST-AIP1 in 1 ml of adjuvant (R1I1 ImmunoChem Research Inc.), three times at 2-week intervals. 2 wk after the last boost, the animals were exsanguinated. Anti-AIP1 antibodies were affinity-purified on columns to which GST-AIP1 was covalently coupled (Sigma Chemical Co.). The column was washed five times with PBS and incubated with thrombin (5 U/ml; Sigma Chemical Co.) overnight at room temperature to cleave the AIP1 from the GST. The column was washed with 8 ml 50 mM HEPES, pH 7.2, 50 mM KCl, and the flow-through was concentrated to 2 ml and loaded onto a 1-ml mono-Q anion exchange column (Pharmacia Biotech Inc.). A linear salt gradient from 100 to 400 mM KCl was applied to the column and peak fractions containing AIP1 were concentrated to 15 μM, frozen in liquid N2, and stored at −80°C.

Two-Hybrid Analyses

In all cases, two-hybrid analyses were performed by mating strain Y190 carrying constructs encoding fusions to the Gal4 A D, to strain Y187 carrying constructs encoding fusions to the Gal4 A D. Transformants were lined, spotted, or spread as lawns on selective medium, synthetic complete medium lacking Trp (SC-TRP) or D BD fusions and synthetic complete medium lacking Leu (SC-LEU) for A IP1 fusions. All constructs were confirmed by replica plating the Y190 and Y187 derivatives onto SC media lacking Trp (SC-TRP) or D BD fusions and synthetic complete medium–plates, incubating at 30°C for 24 h, selecting the mated cells on SC-TRP, -LEU. The selected diploids, carrying both B D and A D fusion constructs, were replica plated to media containing 25, 50, and 100 mM 3-A T (Sigma Chemical Co.) and incubated at 25°C.

Microscopy

Immunofluorescence was performed by standard protocols using a methanol/acetone fixation (Pringle et al., 1991). Afinity-purified anti-AIP1 antibody was used at a dilution of 1:100. Afinity-purified rabbit anti-cofilin was used at 1:100. Guinea pig antianticin antibody was used at 1:2,000. For AIP1 localization, FITC-conjugated goat anti-rabbit IgG (Organon Teknika Corp.) was used at 1:1,000 and rhodamine-conjugated goat anti-guinea pig (Organon Teknika Corp.) was used at 1:1,000. For AIP1 localization in wild-type and act1-111 strains, rhodamine-conjugated goat anti-rabbit IgG (Cappel; I CN Biochemicals) was used at 1:1,000 and rhodamine-conjugated goat anti-guinea IgG (Cappel; I CN Biochemicals) was used at 1:1,000.

Actin Filament Sedimentation Assay

To test the cofilin dependence of AIP1 binding to F-actin, variable concentrations of AIP1 and cofilin, 3.75 mM actin was polymerized at room temperature in F-buffer (5 mM Tris, pH 7.5, 0.7 mM A TP, 0.2 mM D TT, 0.2 mM CaC2), and concentrated to 2 ml in Centriprep 10 devices (A micron Inc.). The actin was polymerized by adding initiation salts to a final concentration of 100 mM KCl, 2 mM MgC2, and incubating for 2 h at room temperature. Residual actin-binding proteins were stripped from F-actin at this point by slow adding KCl to 0.6 M and further incubating for 30 min. The polymerized actin was pelleted at 80,000 rpm for 30 min at room temperature in a TLA 100.3 rotor (Beck man Instruments, Inc.). The pellet was resuspended in G buffer to a final concentration of 30 μM and dialyzed against three changes of 2 liters of buffer before being frozen in liquid N2, and stored at −70°C. Co filin was used as a GST-fusion protein from E. cherichia coli and subsequently cleaved from GST by thrombin digestion as described previously (Lappalainen et al., 1997).

AIP1 was purified from the yeast strain D D Y 130 (carrying the plasmid pAR3) as a GST fusion protein under the control of the GAL promoter. 4 liters of cells were grown at 30°C in synthetic medium with 2% dextrose and without uracil or leucine to an OD of 1.0 at 600 nm, harvested by centrifugation, and resuspended in 4 liters of synthetic medium with 2% glycerol and without uracil or leucine. A filter overnight incubation at 30°C to derepress the galactose promoter, cells were again harvested and resuspended in 4 liters of rich medium with 2% galactose. The cultures were induced for 8 h at 30°C before cells were once again harvested, washed twice with 100 ml water, resuspended in 10 ml water, frozen as 50-μl pellets in liquid N2, and stored at −80°C. Y east pellets were lysed by freezing in liquid N2 in a Waring blender and thawed in PBS to a final concentration of 1×, with 1 mM PMSF and 0.5 μg/ml each of antipain, leu pep tin, pepstatin A, chymostatin, and aprotin. The lysate was cleared first by spinning at 17,000 g in a D upont G SA rotor. The supernatant from this spin was cleared by spinning at 30,000 rpm for 50 min in a Beckman 70Ti rotor. This high speed supernatant was dialyzed overnight against PBS and passed twice over a column with a 4 ml bed of glutathione agarose beads (Sigma Chemical Co.). The column was washed five times with PBS and incubated with thrombin (5 U/ml; Sigma Chemical Co.) overnight at room temperature to cleave the AIP1 from the GST. The column was washed with 8 ml 50 mM HEPES, pH 7.2, 50 mM KCl, and the flow-through was concentrated to 2 ml and loaded onto a 1-ml mono-Q anion exchange column (Pharmacia Biotech Inc.). A linear salt gradient from 100 to 400 mM KCl was applied to the column and peak fractions containing AIP1 were concentrated to 15 μM, frozen in liquid N2, and stored at −80°C.
A ip1p from plasmid pAR20 was incubated for 20 min with the co-filin–F-actin and pelleted for 20 min at 90,000 rpm in a TLA100 rotor (Beckman Instruments). Equal amounts of supernatants and pellets were fractionated on 13% SDS-PAGE gels and visualized by autoradiography. Results were quantified on a PhosphorImager using ImageQuant software (STORM 860; Molecular Dynamics, Inc.) and on an IS2000 densitometer using AlphaImager software.

### Molecular Modeling

Images of actin and coflin molecular models were generated on a Silicon Graphics Indigo™ workstation running Sybil software (Tripos Inc.). Coordinates for actin (file 1ATN) and yeast coflin (file 1CFY) were retrieved from the Brookhaven database.

### Results

#### Identification and Sequence of Aip1p

Aip1p was first identified as a 67-kD yeast protein that interacts with actin in the two-hybrid system (Amberg et al., 1995a; GenBank accession number P46680) and is the first discovered member of a family of conserved proteins (Fig. 1).

**Figure 1.** Sequence alignment of Aip1p and its homologues. Identities are boxed. Sequences were aligned with ClustalW 1.7 and analyzed in Seqvu 1.1 (Garvan Research Institute). Identity is assigned if 4 of 6 residues at a position are identical. SwissProt accession numbers for the given sequences are the following: S. cerevisiae (P46680), S. pombe (O14301), P. polychaetum (P90587), D. discoideum (P54686), C. elegans (Q11176), and Homo sapiens (GenBank AF020056).
1). Homologues of Aip1p have been identified in Schizosaccharomyces pombe, Phusarum polycephalum (Matsumoto et al., 1998), Dictyostelium discoideum, Caenorhabditis elegans (where there are two homologues), Mus musculus, and humans. Additionally, members of the Aip1p family show weak homology to proteins that contain reiterated motifs called WD repeats. These repeats were first identified in β subunits of trimeric G-proteins (Fong et al., 1986) and have since been found in proteins with highly diverse functions. Members of the Aip1p family contain from four to eight WD repeats.

**Two-Hybrid Interactions between Aip1p and Cofilin**

To identify additional Aip1p ligands we used a two-hybrid construct of Aip1p fused to the GAL4 DBD (plasmid pDA b189) to screen a large set of yeast actin interacting proteins and cell polarity proteins fused to the GAL4 activation domain. Included in this set were clones encoding AIP1, AIP2, AIP3, OYE2, SRV2, PFY1, COF1, BNR1, LAS17, MNN10, ABP1, RVS167, BEM1, FUS1, and SAC6. As can be seen in Fig. 2, we found that in addition to actin, Aip1p also interacted with Cof1p (yeast cofilin). These interactions are specific since no activation was observed between Aip1p and the transcription factor Snf4p or between Aip1p and the actin cortical patch protein Abp1p (data not shown). Furthermore, the Aip1p–actin and Aip1p–cofilin two-hybrid interactions are reciprocal. An apparent cofilin–cofilin interaction also was detected but is likely the result of bridging through actin. The focus of this study is the functional significance of the Aip1p–cofilin interaction.

**The Aip1p and Cofilin Binding Footprints on Actin**

To obtain a structural framework for understanding the functions of the Aip1p–actin and cofilin–actin interactions, we used actin mutations (Wertman et al., 1992) in conjunction with the two-hybrid system to identify likely sites of interaction for these proteins on actin. This approach was described previously for the identification of the binding footprint of Aip1p on actin (Amberg et al., 1995a) and was repeated here to identify the binding footprint for cofilin on actin (Fig. 3, A and B and Table I).

Yeast strain Y 187, carrying a fusion of the Gal4p AD to cofilin, was mated to strain Y 190 containing plasmids encoding fusions of 35 actin mutants to the Gal4p DBD (Amberg et al., 1995a). Diploids were replica plated on 3-A T medium to assess activation of the His3p two-hybrid reporter (Fig. 3 A). Failure to grow on this medium indicates that the actin mutant contained in that strain is defective for the cofilin–actin interaction. This result suggests that the mutation may lie in or near the cofilin binding site on actin. We discovered that eight actin mutants failed to interact with cofilin (Fig. 3 A). Of these eight, five have thus far failed to interact with any actin-binding protein tested (act1-107, act1-130, act1-127, act1-128, and act1-108) and probably encode either unfolded or unstable proteins. Therefore, nothing can be concluded from these mutants. However, the remaining three mutants (act1-103, act1-106, and act1-126) display specific effects on the cofilin–actin interaction (Fig. 3 A).

The three mutations that specifically disrupt the cofilin–actin interaction are located in a small region of subdomain III on actin. Interestingly, these three mutations form one-half of the Aip1p binding footprint (Amberg et al., 1995a) that includes act1-109, act1-111, and act1-112, as well as act1-103, act1-106, and act1-126 (Fig. 3 B). These data are consistent with the model that Aip1p binding to actin is facilitated by cofilin.

**The Aip1p and Actin Binding Footprints on Cofilin**

A large set of mutant alleles of cofilin has been constructed (Lappalainen et al., 1997) and the yeast cofilin structure has been determined (Federov et al., 1997). This presented us with the opportunity to use our two-hybrid methodology to identify surfaces on cofilin required for its interactions with Aip1p and actin. Toward this end, we cloned the cofilin mutants into the two-hybrid activation domain vector pACTII (gift of S. Elledge) and scored the interactions with Aip1p and actin. Toward this end, we cloned the cofilin mutants into the two-hybrid activation domain vector pACTII (gift of S. Elledge) and scored the ability of these mutants to interact with Aip1p and actin. Four cofilin mutants failed to interact with both actin and Aip1p: cof1-9, cof1-16, cof1-17, and cof1-20. Two cofilin mutants specifically failed to interact with actin: cof1-6 and cof1-14. Three mutants, cof1-4, cof1-13, and cof1-22, failed to interact with Aip1p but interacted well with actin.

We displayed the two-hybrid data on the molecular model of cofilin (Fig. 3, C and D). In agreement with the in vitro binding data of cofilin mutants to actin (Lappalainen et al., 1997), the two-hybrid data identified a ridge that is involved in the actin interaction (Fig. 3, red and purple in B and C), on the edge of the disc-shaped cofilin protein. A subset of the mutations that disrupted the cofilin–actin interaction constitute part of the Aip1p footprint on cofilin (shown in purple in Fig. 3, C and D). A different set of mutations specifically affected cofilin interactions with Aip1p and not actin (shown in blue). These data are consistent with the model that Aip1p binding to actin is facilitated by cofilin.

![Figure 2](image-url)
Two-hybrid analysis was performed between 36 actin alleles fused to the GAL4 DBD and cofilin fused to the GAL4 AD. Activation of the HIS3 two-hybrid reporter was assessed on medium containing 50 mM 3-AT at 25°C (A). A model of the actin monomer is shown in B. In red are the amino acids altered by mutations that disrupt both the actin–cofilin and actin–Aip1p two-hybrid interactions. In blue are the amino acids altered by mutations that disrupt only the actin–Aip1p interaction. Marked with green are those mutations that had no effect on either interaction. The allele numbers of disruptive mutations are indicated. Displayed in C and D are two views, 180° apart, of yeast cofilin. Mutations that disrupted both the cofilin–actin and the cofilin–Aip1p two-hybrid interactions are indicated in purple. Mutations that disrupted the cofilin–actin interaction alone are indicated in red. Mutations that disrupted the cofilin–Aip1p interaction alone are indicated in blue, and mutations that had no effect on either the cofilin–actin or cofilin–Aip1p interactions are shown in green.
with the model that Aip1p binding to coflin is facilitated by actin because, according to this model, disruption of the coflin–actin interaction would be predicted to also disrupt the Aip1p–cofilin interaction. Overall, the foot-printing data suggest that there is a ternary complex between Aip1p, coflin, and actin, and that the members of this complex make distinct contacts with each other.

**Synthetic Interactions with aip1Δ Mutants**

The actin cytoskeleton consists of a large number of interacting components. Often the deletion of a gene encoding one of these components does not in itself cause a readily detectable phenotype. However, combinations of mutations can produce informative synthetic phenotypes that suggest a shared or parallel function for the proteins involved (A dams et al., 1993). Deletion of the AIP1 gene had no effect on cell growth on a variety of media at a variety of temperatures (data not shown). Therefore, we investigated its genetic interactions with mutations in genes that encode other actin-binding proteins.

First we crossed the aip1Δ strain to the collection of clustered-charged-to-alanine mutants of COF1 (cof1-4, cof1-5, cof1-6, cof1-7, cof1-10, cof1-11, cof1-12, cof1-13, cof1-15, cof1-18, cof1-19, cof1-21, and cof1-22). The results of these crosses are shown in Table II. The aip1Δ mutation is synthetically lethal with cof1-5 and cof1-22, which are both temperature sensitive for growth on their own and show defects in actin turnover rates in vivo at the permissive temperature (L appalainen and Drubin, 1997). In addition, the aip1Δ mutant is synthetically lethal with cof1-4, which has no growth phenotype of its own but has actin organization defects as visualized by rhodamine-phalloidin staining (L appalainen et al., 1997). The aip1Δ mutant is also synthetically temperature sensitive at 37°C with cof1-6, which has no actin organization or growth phenotype on its own (L appalainen et al., 1997). We examined actin and coflin localization in the aip1Δ cof1-6 double mutant. At the permissive temperature, the double mutant grows slowly and has actin clumps in the mother cell. These clumps stain with rhodamine-phalloidin, which binds specifically to F-actin and not G-actin. At the restrictive temperature, actin is depolarized, actin clumps are apparent, and unbudded cells accumulate. Coflin colocalizes with the actin structures at both the permissive and restrictive temperatures (data not shown).

We also determined if the aip1Δ mutation displayed synthetic lethality with any of the viable actin–alanine scan alleles of actin (act1-1, -101, 102, 104, 105, 108, 111, 113, 115, 116, 117, 119, 120, 121, 122, 123, 124, 125, 129, 132, 133, 136) and act1-159, which decreases rates of actin filament turnover in vivo and in vitro (B elmont et al., 1998). When a yeast strain carrying the act1-159 mutation was crossed to the aip1Δ strain, double mutant spores failed to grow at 25°C. We also observed subtle synthetic growth defects in double mutants containing the aip1Δ allele and three other actin alleles: act1-133, act1-119, and act1-125 (data not shown). These three mutations are not located near the Aip1p interaction interface on actin (Fig. 3), suggesting that the weak synthetic interactions are not a function of compromised Aip1p–actin interactions but of cumulative defects in cytoskeletal function.
Finally, we determined if the aip1Δ allele was synthetically lethal with deletion alleles of six other components of the yeast cortical cytoskeleton: ABP1, SLA1, SLA2, TWF1, CAP2, and SAC6. No clear synthetic lethality was found. However, the sac6Δ, cap2Δ-1, and sla1Δ mutations had slight synthetic growth defects in combination with the aip1Δ allele (data not shown).

Aip1p Localizes to Cortical Actin Patches

As a further test of the importance of the Aip1p–actin and Aip1p–cofilin interactions in vivo, we sought to determine if Aip1p colocalizes with actin and cofilin. Toward this end, we generated antibodies to a GST-Aip1p fusion protein. These antibodies specifically recognize a 67-kD protein on Western blots of wild-type (FY 23) yeast extract (Fig. 4 A, inset, lane 1) that is of the expected size based on the primary sequence of AIP1. This band is absent from AIP1 deletion strain DAY 53 (Fig. 4 A, inset, lane 2), but is restored when a low copy number vector carrying a 2.2-kb AIP1 genomic fragment (pRB 2251) is introduced into the deletion strain (Fig. 4 A, inset, lane 3).

Wild-type strain FY 23×86 was stained with the anti-Aip1p antibodies (Fig. 4 B) and guinea pig antiactin antibodies (Fig. 4 A). Aip1p localized only to cortical actin patches and was not detected along the actin cables. This localization pattern is identical to that observed for cofilin (Moon, 1993), but simultaneous colocalization of Aip1p and cofilin was not possible, since both antibodies were raised in rabbits. Since both Aip1p and cofilin appear to be found in all actin cortical patches recognizable by anti-actin antibodies, we presume that Aip1p and cofilin colocalize. Aip1p also localized diffusely throughout the cytoplasm and this cytoplasmic staining is not seen in the aip1Δ strain (data not shown).
The Aip1p–Actin Interaction Is Required for Aip1p Localization to Cortical Patches

To assess the importance and relevance of the Aip1p–actin two-hybrid interaction, we localized Aip1p in a strain bearing actin mutations that disrupt the Aip1p–actin two-hybrid interaction. Fig. 5 A shows Aip1p localization in the act1-111 strain TD 5143. Aip1p was not detected in cortical patches in the mutant strain and there is an apparent increase in the cytosolic Aip1p signal. The failure of Aip1p to localize in this strain is not due to a loss in the ability of cofilin to bind to actin (Fig. 5 B). Similarly, Aip1p localization to cortical patches in an act1-112 strain is severely compromised (Fig. 5 C), whereas cofilin localization to patches in this strain is not affected (Fig. 5 D). Aip1p was well localized to cortical patches in act1-119, act1-132, act1-124, and act1-125 strains (data not shown) indicating that Aip1p mislocalization is not caused by generalized defects in the actin cytoskeleton. These results suggest that Aip1p must bind to F-actin for stable association with cortical actin patches and is consistent with the two-hybrid data suggesting that Aip1p contacts actin in the vicinity of the act1-111 and act1-112 mutations.

Aip1p Localization in Cofilin Mutants

To test the importance of the Aip1p–cofilin interaction on Aip1p localization, we examined Aip1p localization in viable cofilin mutants (Lappalainen et al., 1997). Both Aip1p and cofilin were localized by indirect immunofluorescence in strains bearing 14 different cof1 alleles: cof1-4, cof1-5, cof1-6, cof1-7, cof1-8, cof1-10, cof1-11, cof1-12, cof1-13, cof1-15, cof1-18, cof1-19, cof1-21, cof1-22, and a wild-type congenic strain (Table II). The cells were grown at 25°C, a permissive temperature for all the strains, before fixation. Aip1p localized to patches in all of the cofilin mutants except the strain carrying the cof1-19 allele. Fig. 5 E shows Aip1p localization in cof1-19 strain D DY 1264. Aip1p was seen with the act1-111 strain, Aip1p is lost completely from the cortical patches in cof1-19 cells and there is an apparent increase in the cytoplasmic pool of Aip1p. Double labeling of this strain with antiactin and anticofilin antibodies showed that Cof1-19p is associated with cortical actin patches (Fig. 6).

The cof1-19 strain, like the aip1Δ strain, is viable and has a wild-type growth phenotype. We have examined the actin cytoskeleton in these strains and found no obvious defects. However, both strains do appear to have slightly aberrant cortical actin patches: they appear by rhodamine-phalloidin staining to be slightly larger or perhaps to contain more F-actin (data not shown). In addition, the cof1-19 cells have slightly depolarized actin patches and misoriented actin cables.

Aip1p is Required for Normal Cofilin Localization

To examine the role of Aip1p in cofilin localization, we immunolocalized cofilin in the aip1Δ strain. Surprisingly, cofilin localized not only to cortical patches but also to actin cables (Fig. 6 D). We confirmed colocalization of cofilin with actin cables by double staining with the guinea pig antiactin antibody, as shown in Fig. 6 C. This result suggests that Aip1p is required to restrict cofilin to cortical actin patches in the yeast actin cytoskeleton.

We asked if exclusive localization of cofilin to cortical actin patches depends on localization of Aip1p to these patches. To address this question, we examined cofilin localization in the cof1-19 mutant strain D DY 1264, in which Aip1p is localized in the cytoplasm. As can be seen in Fig. 6 F, Cof1-19p is localized to both the patches and the cables. Fig. 6 E shows the same cells stained with the antiactin antibody confirming association of Cof1-19p with the actin cables. Though act1-111 and act1-112 mutants also fail to localize Aip1p to actin patches (Fig. 5), we were unable to confirm that cofilin also localizes to actin cables in these strains because their actin cytoskeletons are more generally disrupted and cables are undetectable by antibody staining (data not shown).

We next asked if the localization of other proteins normally localized to the cortical actin patches was affected in the aip1Δ strain. We found that immunolocalization of Aip1p, fimbrin/Sac6p, and coronin/Crn1p was unaffected by the absence of Aip1p (data not shown). This indicates that the role of Aip1p in cofilin localization is specific and not reflective of gross structural defects in the cortical patches.

Aip1p Increases the Extent of Cofilin-induced Depolymerization

To evaluate directly the functional and physical interaction between cofilin, actin, and Aip1p, we investigated the effects that these proteins might have together in vitro. Aip1p was expressed in yeast as a GST fusion protein under the control of the GAL promoter. GST-Aip1p was affinity-purified from extracts on glutathione-agarose beads, cleaved from GST by thrombin digestion, and purified by cation exchange chromatography. This protein has two additional amino acids NH2-terminal to the primary sequence of Aip1p. No contaminants are apparent in the preparation on overloaded (5 μg) Coomassie stained gels (data not shown).

To evaluate the interaction of Aip1p with F-actin at steady state, we carried out cosedimentation assays with 2.5 μM prepolymerized yeast F-actin. For all of the assays described here, after 20 min of coincubation the reaction is at steady state as evaluated by light-scattering at 400 nm (data not shown). Although a small proportion (5–10%) of purified Aip1p sedimented in the absence of actin in these assays, this amount did not perceptibly increase upon addition of actin (data not shown). Given the two-hybrid interaction of Aip1p and cofilin, it seemed possible that an actin–Aip1p interaction might be mediated by cofilin. To test this hypothesis, we performed the cosedimentation assay in the presence of recombinant cofilin, which binds to F-actin and accelerates disassembly rates (increasing subunit turnover), but does not significantly change actin polymer levels at steady state (Fig. 7 B, lanes 1 and 2). Strikingly, in the presence of both Aip1p and cofilin, we observed a dramatic shift of actin and cofilin from the pellet to the supernatant (Fig. 7 A, lane 2). This shift might be explained by invoking a monomer sequestering model as applies for twinfilin (Goode et al., 1998), a protein that binds stoichiometrically to actin monomer, preventing nucleotide exchange and polymerization. We examined the
Figure 5. Aip1p localization to cortical patches requires actin and cofilin. Immunofluorescence was performed on strain TDS143 (act1-111) grown at 25°C (A and B), strain DDY355 grown at 37°C (act1-112; C and D), and strain DDY1264 (cof1-19) grown at 25°C (E) using affinity-purified anti-Aip1p antibody (A, C, and E), affinity-purified rabbit anticofilin antibody (B and D), and a fluorescein-conjugated goat anti-rabbit secondary antibody.
effects of stoichiometry on the Aip1p-dependent shift by varying the concentration of Aip1p (0.012–2.5 μM; Fig. 7 A, lanes 2–6) or cofilin (0.12–2.5 μM; Fig. 7 B, lanes 3–7) in cosedimentation assays with constant concentrations of F-actin (2.5 μM). Intriguingly, though the shift into the supernatant showed a linear dependence on cofilin, it did not require Aip1p at similar stoichiometry. In fact, a significant shift occurred at molar ratios of Aip1p/cofilin/actin as low as 1:50:50 (Fig. 7 A, lane 4), and can even be seen at molar ratios of Aip1p/cofilin/actin as low as 1:200:200 (Fig. 7 A, lane 6).

F-Actin Binding of Aip1p Is Facilitated by Cofilin

We used radiolabeled in vitro–translated Aip1p to evaluate binding to F-actin at a low concentration of Aip1p, which would not promote net disassembly of the filaments. The in vitro-translated Aip1p product sedimented with actin filaments. This cosedimentation was abolished by addition of excess nonradiolabeled Aip1p or by dilution of the sample, suggesting that the binding is specific (data not shown). To establish the dependence of this binding on cofilin, we pelleted increasing concentrations of actin filaments with or without stoichiometric cofilin with in vitro-translated Aip1p. Aip1p cosedimented with the F-actin in the absence of added cofilin, but addition of 1:1 cofilin increased the amount that cosedimented (Fig. 8 A). The binding of Aip1p to cofilin-F-actin is saturable with a $K_d$ of $\sim 4 \mu M$. Similar results were obtained by Western blot analysis of identical experiments with purified Aip1p (data

![Figure 6](image_url)

Figure 6. Selective localization of cofilin to cortical patches is dependent on Aip1p. Immunofluorescence was performed on wild-type strain D D Y 1252 (A and B), aip1Δ strain D A Y 30 (C and D), and on cof1-19 strain D D Y 1264 (E and F) using guinea pig antiactin antibodies and a rhodamine-conjugated goat anti–guinea pig secondary antibody (A, C, and E), and anticofilin antibodies in conjunction with an FITC-conjugated goat anti–rabbit secondary antibody (B, D, and F).
The Journal of Cell Biology, Volume 145, 1999 1263

not shown). We next examined how much added cofilin was required to get the increased binding. The amount of Aip1p cosedimenting with 7.5 μM F-actin falls off linearly with cofilin concentration (Fig. 8 B). Aip1p cosedimentation with F-actin could be increased in the presence of cofilin either because cofilin creates more binding sites for Aip1p, or because cofilin increases the affinity of Aip1p for actin. To distinguish between these possibilities, we added 15 and 45 nM purified Aip1p to 5 μM cofilin-saturated actin filaments (which is below the concentration that saturates Aip1p binding) and ran the sedimentation reaction. A threefold higher concentration of Aip1p increases the fraction of Aip1p cosedimenting with actin, suggesting that Aip1p binding sites are not saturated, and that the cofilin-dependent increase in binding reflects an increased affinity of Aip1p for F-actin in the presence of cofilin (data not shown). To determine stoichiometries of these proteins in yeast cells, we estimated the ratio of Aip1p/cofilin/actin in the cell by comparing immunoblots of whole-cell extracts and purified proteins of known concentration (data not shown). Aip1p, cofilin, and actin are each present in whole-cell extracts at a ratio of about 1:1:5 or 1:1:10.

Discussion

Aip1p–Cofilin–Actin Interactions Suggest a Ternary Complex

Aip1p was originally identified by its two-hybrid interaction with actin (Amberg et al., 1995a). Further two-hybrid analysis revealed an interaction between Aip1p and cofilin (Fig. 2) raising the possibility that actin, Aip1p, and cofilin might form a ternary complex. The existence of such a ternary complex is supported by evidence that Aip1p and cofilin are dependent on each other and on actin for their correct localization in vivo (Fig. 5). Binding experiments with purified proteins also support an Aip1p–cofilin–actin complex (Fig. 8). Binding of purified or in vitro–translated Aip1p to F-actin is concentration-dependent and increases at high ratios of cofilin/actin. Since cofilin is a contaminant in yeast actin preparations, despite efforts to deplete it (~1 μM/100 μM actin), we cannot rule out the possibility that contaminating native cofilin is responsible for the baseline binding to F-actin in the absence of added recombinant cofilin. Thus, it is possible that Aip1p binding to F-actin is strictly cofilin-dependent. We were unable to show a direct interaction between Aip1p and cofilin and/or G-actin by native gel shift or by cosedimentation with GST–Aip1p, further suggesting that Aip1p interacts with cofilin on F-actin.

A large set of mutations in both actin (Wertman et al., 1992) and cofilin (Lappalainen et al., 1997) was used in conjunction with the two-hybrid system to identify regions of actin and cofilin involved in the Aip1p interaction (Fig. 3). Those data that describe the cofilin–actin interface appear sound since they agree with similar studies using biochemical (Lappalainen et al., 1997), modeling (Wriggers et al., 1998), and structural (Amy McGough, personal communication) approaches. The binding footprint (as obtained by two-hybrid analysis) for Aip1p on the surface of actin (Fig. 3 B, blue and red) partially overlaps with the

Figure 7. Aip1p enhances the extent and rate of cofilin-mediated actin dynamics. 2.5 μM actin filaments were incubated with varying concentrations of cofilin and/or Aip1p. Coomassie stained gels and accompanying quantification show the dependence of the Aip1p–cofilin interaction on Aip1p concentration (A) and on cofilin concentration (B).
cofilin binding site (red). Similarly, the footprints of Aip1p and actin on cofilin overlap as four cofilin mutants were specifically defective for both Aip1p and actin binding. Though overlapping interaction interfaces are consistent with both competitive interactions and a ternary complex, we favor the latter model because it is consistent with the localization and in vitro binding data discussed above.

While most of our structural data can be incorporated into a coherent model for interaction in a ternary complex, there were several discrepancies between the biochemical and two-hybrid data. First, only by two-hybrid assay was cof1-9 defective for binding to actin. Additionally, although cof1-6 has a wild-type growth phenotype, by two-hybrid analysis it appears to be completely defective for actin binding. This interaction has not been tested biochemically. These discrepancies may be an artifact resulting from the fusion to Gal4p in the two-hybrid system. Alternatively, the cofilin-actin two-hybrid interaction might be subtly different than that observed in vitro with purified components. Note that cof1-19 is the only cofilin mutant that is defective for Aip1p localization, but it appears to interact well with both Aip1p and actin by two-hybrid analysis. This cofilin mutant may interact well with actin and Aip1p in the two-hybrid complex but have subtly altered binding properties in vivo.

**In Vivo Interactions Support a Role for Aip1p in Promoting Actin Dynamics**

Though the AIP1 deletion mutant has no obvious phenotype on its own, allele-specific synthetic lethality was observed between aip1Δ and act1-159, cof1-4, cof1-5, cof1-22, and cof1-6 (at 37°C). These results suggest that in the double mutants, a common function is compromised enough that cell viability is lost. Because act1-159, cof1-5, and cof1-22 have all been shown to decrease the rate of F-actin disassembly in vivo (Lappalainen and Drubin, 1997; Belmont and Drubin, 1998), we postulate that Aip1p also promotes actin filament turnover. This conclusion is supported by our biochemical studies of Aip1p that demonstrate that it causes cofilin-dependent actin filament disassembly (Fig. 7). Note that CoF1-22p has defects in actin binding in vitro, but that CoF1-5p does not (Lappalainen et al., 1997), suggesting that the synthetic interaction is not simply a function of compromised actin binding by cofilin. The cofilin–actin interaction has not been tested biochemically for cof1-4 or for cof1-6.

Specific sorting of cofilin to cortical patches but not cytoplasmic cables is lost in aip1Δ (Fig. 6 C) and cof1-19 (Fig. 6 E) strains. One model that could explain these results in terms of the in vitro effects of Aip1p on actin filaments assumes that two populations of actin cables, one cofilin-bound and one tropomyosin-bound, form in yeast cells. Cofilin-bound cables would undergo net depolymerization in the presence of Aip1p, as occurs for purified actin filaments in vitro. Tropomyosin, which is localized to actin cables (Liu and Bretscher, 1989), can compete for cofilin binding sites on actin (Bernstein and Bamburg, 1982). Thus, tropomyosin would stabilize a subset of cables against Aip1p–cofilin depolymerization and these would go on to be the normal cables visualized in cells. In the aip1Δ strain or in a cofilin mutant that mislocalizes Aip1p (cof1-19), Aip1p would not be able to function synergistically with cofilin to destabilize the filaments, and both cofilin-bound and tropomyosin-bound populations of filaments would be maintained. In support of this model, cofilin also localizes to rare actin cables in act1-159 tpm1Δ and act1-159 mrm2Δ double mutant strains (Belmont et al., 1998), which would be predicted to have hyperstable F-actin structures that would not be readily disassembled by cofilin–Aip1p.

The aip1Δ allele was also found to enhance the defects observed in specific actin mutants (act1-119, act1-125, and act1-133) and in null alleles of genes encoding several components of the cortical actin cytoskeleton (Sac6p, Sla1p, and Cap2p). We believe that these double mutants most likely suffer from a general, cumulative derangement of the actin cytoskeleton, a conclusion that further supports that the actin cytoskeleton is affected in the aip1Δ strain.

**The Aip1p–Cofilin–Actin ComplexPromotes Disassembly In Vitro**

We demonstrated that Aip1p causes cofilin-mediated ac-
tin filament depolymerization in vitro (Fig. 7). Interestingly, we discovered that Aip1p can induce cofilin-mediated actin filament depolymerization at stoichiometries as low as 1:50:50 Aip1p/actin/cofilin. On the other hand, cofilin must be present at a 1:1 ratio with actin for optimal Aip1p-mediated activation of depolymerization.

Though we were able to detect concentration-dependent F-actin cosedimentation of Aip1p at a low molar ratio with actin (by Western blot analysis or using in vitro-translated Aip1p), we were unable to detect cosedimentation at higher Aip1p/actin ratios. One hypothesis that explains these results is that Aip1p saturates binding at a low stoichiometry with F-actin. A second hypothesis is that high ratios of cofilin/actin are required for Aip1p binding, but that at high concentrations of Aip1p, net depolymerization prevents cosedimentation of Aip1p with actin. A model that is consistent with substoichiometric or cofilin-dependent filament binding, net filament depolymerization, and the two-hybrid footprinting data is that Aip1p enhances the weak severing activity of cofilin (Maciver et al. 1991; McGough et al., 1997).

Aip1p is the first protein aside from actin and LIM-kinase to show a physical interaction with cofilin. The fact that Aip1p is highly conserved in eukaryotes suggests that it may be a cofactor for cofilin activity in all eukaryotic cells. Though the mechanistic details of these interactions remain to be elucidated fully, our biochemical data demonstrate clearly that Aip1p stimulates cofilin-mediated actin filament disassembly and our genetic and cell biological data provides powerful evidence for the relevance of this activity in vivo.

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