The Structure of Aip1p, a WD Repeat Protein That Regulates Cofilin-mediated Actin Depolymerization*

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Actin-interacting protein 1 (Aip1p) is a 67-kDa WD repeat protein known to regulate the depolymerization of actin filaments by cofilin and is conserved in organisms ranging from yeast to mammals. The crystal structure of Aip1p from Saccharomyces cerevisiae was determined to a 2.3-Å resolution and a final crystallographic R-factor of 0.204. The structure reveals that the overall fold is formed by two connected seven-bladed β-propellers and has important implications for the structure of Aip1 from other organisms and WD repeat-containing proteins in general. These results were unexpected because a maximum of 10 WD repeats had been reported in the literature for this protein using sequence data. The surfaces of the β-propellers formed by the D-A and B-C loops are positioned adjacent to one another, giving Aip1p a shape that resembles an open “clamshell.” The mapping of conserved residues to the structure of Aip1 reveals dense patches of conserved residues on the surface of one β-propeller and at the interface of the two β-propellers. These two patches of conserved residues suggest a potential binding site for F-actin on Aip1p and that the orientation of the β-propellers with respect to one another plays a role in binding an actin-cofilin complex. In addition, the conserved interface between the domains is mediated by a number of interactions that appear to impart rigidity between the two domains of Aip1p and may make a large substrate-induced conformational change difficult.

The actin cytoskeleton plays a key role in cell motility, cell morphology, cytokinesis, and the organization of organelles within the cytosol. The organization of actin filaments within the cytoskeleton is tightly regulated by a number of actin-binding proteins. Among these is cofilin, an essential protein conserved in all of the eukaryotes. Cofilin and other members of the actin-depolymerizing factor/cofilin family of proteins are known to aid the rearrangement of the cytoskeleton and increase actin filament turnover by binding and severing F-actin (1–3). The actin-severing activity of cofilin is known to be regulated in three ways. It is negatively regulated by phosphorylation at Ser-3 (4, 5) and inhibited by phosphoinositides (6), and its activity is strongly pH-dependent (7, 8). Mammalian cofilin preferentially binds to F-actin without severing at pH < 7.3 and preferentially severs F-actin at more alkaline pH (7–9). Recent studies have also shown that yeast cofilin interacts with actin-interacting protein 1 (Aip1p) (10) and that the two proteins cooperate in the depolymerization of actin filaments (10–12).

Aip1p has also been shown to play an important role in the regulation of the actin cytoskeleton (13, 14) and is thought to be conserved in all of the eukaryotes. Previous studies have shown that Aip1p is involved in endocytosis and cytokinesis and that motility in Dicystostelium (13) is required for the normal localization of cofilin to cortical actin patches in Saccharomyces cerevisiae (10) and is essential for the organized assembly of muscle actin filaments in Caenorhabditis elegans (14). It has also been shown that in the absence of cofilin, Aip1p has little ability to depolymerize or bind to F-actin (12, 15). Additionally, a number of alanine-scanning mutations in yeast actin that should hinder the binding of cofilin (16) also prevent the interaction of actin with Aip1p (17), suggesting that the actual target of Aip1p in vivo is a binary complex of F-actin and cofilin. Initial studies on S. cerevisiae Aip1p suggested that the addition of substoichiometric amounts of Aip1p to F-actin in the presence of equimolar amounts of cofilin significantly improves the rate and extent of F-actin depolymerization in vitro (10, 11). More recent studies on Xenopus laevis Aip1p have indicated that Aip1p enhances the fragmentation of actin filaments by capping their barbed ends in the presence of cofilin (15), thus preventing the elongation of cofilin-severed actin filaments. These studies have indicated a likely binding site for Aip1p on F-actin (15); however, the specific nature of its interactions with cofilin and F-actin are still unknown.

Aip1p is a member of the WD repeat family of proteins and has been predicted to contain as many as 10 WD repeats (14). These repeats, also known as WD40 repeats, are homologous sequences of ~40 amino acids frequently bracketed by the amino acid pairs Gly-His on the amino end of the repeat and Trp-Asp at the carboxyl end and are almost exclusive to eukaryotes (18). Analyses of well characterized genomes such as S. cerevisiae have shown that approximately 1% codes for WD repeat-containing proteins. Domains constructed from WD repeats are utilized by many proteins in the reversible binding and regulation of specific protein targets in an extremely diverse set of cellular systems (18). Structural characterization of a number of WD repeat proteins has revealed that they form multi-bladed β-propeller structures (19–22) with nearly all of

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‡ The abbreviations used are: Aip1p, actin-interacting protein; SeMet-Aip1p, selenomethionine-labeled Aip1p; APAF1, apoptosis protease-activating factor 1.
them containing seven blades. Each of the blades in the β-propeller is formed by a small anti-parallel β-sheet consisting of four β-strands, labeled A, B, C, and D, beginning with the strand closest to the center of the propeller. A single WD sequence repeat encompasses the D strand of one blade and the A, B, and C strands of the following blade. Each repeat in sequence therefore does not coincide with a single structural repeat.

Aip1p has been predicted to contain more WD repeats than any other structurally characterized protein bringing into question whether its WD repeats form one very large β-propeller or multiple smaller β-propeller domains. This general issue has also been raised for other proteins with larger numbers of WD repeats. The nature of the interactions of Aip1p with actin and cofilin, has also been raised for other proteins with larger numbers of WD repeats.

### EXPERIMENTAL PROCEDURES

**Cloning, Protein Expression, and Purification**—The AIP1 gene was PCR-amplified from *S. cerevisiae* genomic DNA using the 5'-CTAATGGGCACTGATCATGTTCTTGAAGG-3' and the 3'-primer 5'-ACTGCCCAGGGTCGAGGACAACATTCCACTCT-3'. The resulting fragment was inserted into the NcoI and Smal sites of the plasmid pTYB4 (New England Biolabs) to yield the final bacterial expression vector. The sequencing of the plasmid indicated conservative HS30R mutation, presumably from the PCR. This plasmid was used to transform the Escherichia coli expression strain ER2566. Recombinant Aip1p was produced in fusion with plasmid-derived intein and chitin-binding domains. The wild-type protein was expressed in LB medium. Aip1p was produced in fusion with plasmid-derived intein and chitin-binding domain fusion protein were passed over a column of chitin beads, which was washed with at least 20 column volumes of Buffer A (20 mM Tris, pH 8.0, 500 mM NaCl, 0.1 mM EDTA) plus 0.1% Triton X-100 followed by 20 column volumes of Buffer B. Aip1p-intein fusion protein was then cleaved on the chitin column by incubation for 12 h in Buffer A supplemented with 50 mM β-mercaptoethanol. Aip1p was eluted in Buffer A, dialyzed into 20 mM Tris, pH 8.0, and further purified by anion-exchange chromatography on a quaternized polyethyleneimine HQ column using a BioCad Sprint fast protein liquid chromatography. Aip1p was eluted with a 0–1 M NaCl gradient, eluting at a salt concentration of ~450 mM. The purified protein was then exchanged into 20 mM HEPES, pH 7.4, with 20 mM NaCl and concentrated to 15 mg/ml using Millipore Ultrafree spin concentrators. Dynamic light-scattering experiments using a Protein Solutions Dynapro 99 instrument on purified protein at 0.2 mg/ml concentration indicated that Aip1p exists as a monodisperse monomer with a molecular mass of 61.3 kDa reasonably close to the predicted molecular mass of 67 kDa.

**Crystallization and Data Collection**—Purified Aip1p and SeMet Aip1p were crystallized at room temperature by the hanging drop vapor diffusion method. Drops containing 1 μL of protein solution at a concentration of 15 mg/ml were mixed with 1 μL of the precipitant solution (100 mM HEPES, pH 7.5, 200 mM NaCl, 13% w/v polyethylene glycol 4000) and suspended on siliconized glass coverslips over a 1-ml reservoir of the precipitant solution. Crystals used in data collection were transferred to a cryoprotectant solution consisting of 90% w/v precipitant solution and 10% w/v ethylene glycol, picked up in rayon loops, and flash-cooled in a stream of liquid nitrogen vapor at 110 K. Native crystals of Aip1p belonged to the space group *P*2₁2₁2₁ with unit cell dimensions of a = 69.1 Å, b = 154.4 Å, and c = 92.1 Å. Crystals of selenomethionine-containing Aip1p belonged to the space group *P*2₁ with unit cell dimensions of a = 60.8 Å, b = 154.5 Å, c = 89.0 Å, and β = 90.65°. Multiple wavelength anomalous dispersion data were collected on SeMet Aip1p crystals at beamline 9-2 at the Stanford Synchrotron Radiation Laboratory (SSRL) at 110 K using an ADSC CCD detector (Table I). Data were collected on native crystals at beamline 9-1 at SSRL at 110 K using a Mar 345 imaging plate detector. All of the

### Table I

| Data collection<sup>a</sup> | SeMet Aip1p | SeMet Aip1p | SeMet Aip1p | Native Aip1p |
|-----------------------------|-------------|-------------|-------------|--------------|
| λ<sub>1</sub>               | 0.979       | 0.918       | 0.9793      | 0.9700       |
| Resolution range (Å)        | 25.0–2.3    | 25.0–2.3    | 25.0–2.3    | 25.0–2.5     |
| Unique observations         | 51,350      | 51,163      | 56,061      | 22,190       |
| Total observations          | 192,792     | 246,522     | 329,569     | 192,575      |
| Completeness (%)            | 90.9 (93.1) | 90.5 (92.9) | 99.9 (99.0) | 93.5 (83.5)  |
| R<sub>w</sub>                | 0.069 (0.230) | 0.067 (0.247) | 0.071 (0.255) | 0.063 (0.235) |
| I/σ(I)                      | 13.2 (2.7)  | 12.1 (2.9)  | 12.6 (4.3)  | 14.6         |
| % reflections I > 3σ        | 74.3 (49.2) | 69.8 (37.9) | 83.0 (54.3) | 78.8 (44.4)  |

### Table II

| Phasing                       | SeMet Aip1p | Native Aip1p |
|-------------------------------|-------------|--------------|
| Number of Se Sites            | 12          |              |
| Figure of Merit               | 0.46 (25–2.7 Å) |              |

<sup>a</sup> Values in parentheses are for the highest resolution shells: 2.34–2.30 Å for SeMet Aip1p λ<sub>1</sub>, λ<sub>2</sub>, λ<sub>3</sub>, and 2.54–2.50 Å for the native data.

<sup>b</sup> R<sub>merge</sub> = Σ|I<sub>meas</sub>–I<sub>avg</sub>|/ΣI<sub>meas</sub> where the summation is over all reflections.

<sup>c</sup> R-factor = Σ|F<sub>calcd</sub> – F<sub>obs</sub>|/ΣF<sub>calcd</sub>. Seven percent of the reflections were reserved for calculation of R<sub>merge</sub>. 

...pyroanalyzer and lowering the temperature to 15 °C. Cells were harvested by centrifugation 12 h after induction and lysed using a microfluidizer at 15,000 p.s.i. Lysate was clarified by centrifuging at 39,000 g for 30 min. The resulting cell extracts containing either wild-type or SeMet Aip1p in chitin-binding domain fusion protein were passed over a column of chitin beads, which was washed with at least 20 column volumes of Buffer A (20 mM Tris, pH 8.0, 500 mM NaCl, 0.1 mM EDTA) plus 0.1% Triton X-100 followed by 20 column volumes of Buffer B. The Aip1p-intein fusion protein was then cleaved on the chitin column by incubation for 12 h in Buffer A supplemented with 50 mM β-mercaptoethanol. Aip1p was eluted in Buffer A, dialyzed into 20 mM Tris, pH 8.0, and further purified by anion-exchange chromatography on a quaternized polyethyleneimine HQ column using a BioCad Sprint fast protein liquid chromatography. Aip1p was eluted with a 0–1 M NaCl gradient, eluting at a salt concentration of ~450 mM. The purified protein was then exchanged into 20 mM HEPES, pH 7.4, with 20 mM NaCl and concentrated to 15 mg/ml using Millipore Ultrafree spin concentrators. Dynamic light-scattering experiments using a Protein Solutions Dynapro 99 instrument on purified protein at 0.2 mg/ml concentration indicated that Aip1p exists as a monodisperse monomer with a molecular mass of 61.3 kDa reasonably close to the predicted molecular mass of 67 kDa.
data were processed using the programs Denzo and Scalepack (24).

**Structure Determination, Model Building, and Refinement**—The positions of 12 selenium sites were located, and multiple wavelength anomalous dispersion phases were calculated to a 2.7-Å resolution using SOLVE (25). Density-modified phases using the program RESOLVE (26) yielded interpretable electron density maps with a figure of merit of 0.61. Phases were extended to 2.5 Å using the program CNS (27). Two molecules of SeMet Aip1p were then built into the asymmetric unit using the program O (28). The molecular model was refined by iterative cycles of rigid body refinement, energy minimization, simulated torsional annealing, and individual B-factor refinement in the program CNS (27) followed by model rebuilding in program O. Non-crystallographic symmetry restraints were used in the initial rounds of refinement and released once the model of the protein molecules was completed. The $R_{free}$ value was used to monitor the progress of the refinement and was calculated using 7% of the data. Solvent molecules were added in peaks $>3\sigma$ in $F_o - F_c$ difference electron density maps and retained if their B-values remained $<60$ Å$^2$ after a full round of refinement. Two peaks $>8\sigma$ in $F_o - F_c$ difference electron density maps that were each surrounded by a tetrahedral arrangement of two histidines and two glutamate residues were modeled as zinc ions and refined with B-values similar to those of the surrounding residues. The observed metal ions were bound in pockets created by the crystal packing of the two SeMet-Aip1p molecules present in the asymmetric unit and are probably not bound to Aip1p outside of the crystal lattice. The final SeMet Aip1p molecular model was refined to a 2.3-Å resolution with $R_{free} = 0.257$ and $R_{cryst} = 0.204$. The final model of SeMet-Aip1p comprises residues 2–543 and 550–613 in one Aip1p molecule and residues 2–543 and 550–615 in the other. Superposition of the two SeMet-Aip1p molecules in the asymmetric unit reveals no significant differences with a root mean square difference of 0.31 Å over 606-Ca positions. The structure of native Aip1p was determined by molecular
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Fig. 2. Stereoview of 2Fo – F electron density map contoured at 1.4σ for residues 336–340, 350–354, and Trp-362 which confer rigidity at the β-propeller interface.

replacement in CNS using the finished model of SeMet Aip1p without solvent molecules or zinc ions as the search model. The molecular model of native Aip1p was refined to a 2.5-Å resolution in the same manner as SeMet Aip1p. Superposition of the final models of native Aip1p and molecule A of SeMet Aip1p in program O shows that the two structures are virtually identical with a root mean square difference of only 0.30 Å over 604-Cα positions. Figures were made in MOLSCRIPT (29), BOBSCRIPT (30), RASTER3D, and Grasp (31).

RESULTS

Overall Structure of Aip1p—The structure of the WD repeat protein Aip1p is formed by two connected seven-bladed β-propellers domains. These domains are both regular and do not contain any significant structural excursions. These are oriented such that the top surfaces of the propellers, those formed by the D-A and B-C loops of each blade, face toward one another (Fig. 1). The resulting overall structure of Aip1p resembles a clamshell opened at an angle of −110° with the two doughnut-shaped β-propeller domains of Aip1p twisted −15° askew relative to one another. The two β-propeller domains are similar in size, each roughly 45 Å in diameter and 25 Å high. The first β-propeller domain (propeller 1) is formed by blades 1–7 (residues 20–335), and the second β-propeller domain (propeller 2) is formed from blades 8–14 (residues 4–10 and 340–613). The only significant difference in the makeup of the two individual domains is that propeller 1 consists of a continuous string of residues, while the N-terminal 10 residues of Aip1p form the final β-strand of blade 14 in propeller 2. This structural motif, sometimes referred to as “molecular velcro,” has been observed in the structures of other WD repeat proteins and other β-propeller proteins as well (18, 32). These interactions help to keep propeller 2 tightly folded and are believed to be a major factor in the stability of the WD fold. Therefore, there are two separate segments involved in the region connecting the two domains of Aip1p. These regions are conserved and are composed of residues 10–20 and 337–341.

The Interdomain Interface—There is an extensive interface mediated by numerous contacts between the two β-propeller domains of Aip1p. This surface is formed by the β-strands of blades 1, 7, and 14, various residues from blade 8, and the loop connecting blades 7 and 8 (residues 337–341). A hydrogen-bonding network links the side chains of Ser-352 and Ser-354 on strand 8B with the imidazole ring of His-338 from the loop connecting blades 7 and 8 (Fig. 2). Additionally, the hydroxyl group of Ser-352 forms a hydrogen bond with the indole ring nitrogen of Trp-362, possibly helping to orient its hydrophobic side chain. Significant interactions are also observed in the three hydrophobic pockets formed by: (a) Leu-6, Ile-9, Pro-11, Phe-42, and Val-56; (b) Ile-10, Leu-350, Trp-362, and Ile-607; and (c) Pro-14, Leu-315, Leu-321, Phe-323, and Ile-335. Other notable interactions are hydrogen bonds between the side chain amino group of Lys-584 and the carbonyl oxygen of Gly-63; the guanidinium group of Arg-358 and the carbonyl oxygens of Asp-318 and Gly-337; and the phenolic hydroxyl group of Tyr-35, the guanidinium group of Arg-44, and the carbonyl oxygen of Pro-14. These interactions may all contribute to the formation of a relatively rigid-connecting region between the two β-propellers of Aip1p that allows very limited movement of its two domains in the absence of its binding partners.

Location of Conserved Residues in the Structure of Aip1p—Actin, one of the substrates of Aip1p, is highly conserved, which suggests that the residues mediating the Aip1p-actin interface will also be conserved. Amino acid homology and identity between the yeast Aip1p sequence and that of other eukaryotes is 50 and 30%, respectively. Residues in the amino acid sequence of S. cerevisiae Aip1p were considered to be strongly conserved if unchanged or replaced by a homologous residue (Arg ↔ Lys or Asp ↔ Glu) in at least four of six representative Aip1p proteins from Schizosaccharomyces pombe, Physarum polycephalum, Dictyostelium discoideum, C. elegans, X. laevis, and Homo sapiens. Dense patches of conserved residues occur in the structure of Aip1p in two areas: neighboring regions in blades 2–5 and at the interface between the two β-propellers (Fig. 3, B and D). The residues that form the C-D loops at the bottom of blades 3, 4, and 5 are highly conserved and are part of a contiguous surface of conserved residues on the side and bottom of propeller 1. At the β-propeller interface, conserved residues involved in the formation of hydrophobic patches include Ile-335 and Trp-362 from blades 7 and 8. All four of the residues involved in the hydrogen-bonding network linking blades 7 and 8 are highly conserved in other organisms as well. Additionally, two glycines at the β-propeller interface, Gly-319 in blade 7 and Gly-357 in blade 8, are highly conserved. Notably, mutation of these glycines in C. elegans Aip1p have been shown to disrupt actin filament organization in body wall muscle (14). Finally, a few conserved residues are scattered across the solvent-exposed surface on top of the two β-propellers. These residues, which include Arg-18, Phe-208, Lys-340, and Glu-408, may be very important if Aip1p binds part of an actin-cofilin complex between its two β-propeller domains.

DISCUSSION

Structure of Aip1p as a Model for Other WD Proteins—The crystal structure of Aip1p reveals the novel arrangement of two connected WD repeat β-propeller domains in an open clamshell-shaped molecule, an architecture that is almost certainly conserved among species. This arrangement of the β-propeller domains in Aip1p allows for the existence of multiple adjacent protein binding surfaces oriented at specific angles with respect to one another. In related proteins with multiple WD repeat-containing domains, it may be particularly advantageous for binding large substrates, such as protein complexes, or the simultaneous binding of multiple target proteins. Another interesting aspect of the structure is that both of the β-propellers in Aip1p contain seven blades, which supports the idea that a WD repeat structure constructed from multiple seven-bladed β-propellers is more favorable than a single β-propeller containing a large number of blades. A number of other proteins are predicted to contain more than seven WD repeats, and their structures may also be composed of multiple seven-bladed β-propellers as opposed to a single β-propeller constructed from more than eight blades. The structure of Aip1p may serve as a good model for the WD repeat regions of these proteins.

One well studied example is human apoptotic protease-activating factor 1 (APAF1), a protein that is 24% identical to homologous regions of Aip1p. APAF1 is a component in a 7-fold symmetrical, 1.4-MDa complex dubbed the apoptosome, a key complex in the activation of an initiator caspase. A substantial portion of APAF1 is predicted to be composed of as many as 12 or 13 WD repeats (33). Cryo-electron microscope images of the apoptosome, a heptameric complex containing APAF1 and cy-
tochrome c, reveal that the WD repeat domains of APAF1 form spokes radiating from the caspase-binding hub of this complex (34). Although the structure has been determined at a relatively low resolution (27 Å), the cryo-electron microscopy electron density of the domains in the spoke regions is consistent with a clamshell-shaped molecule formed by two β-propeller domains with one or two cytochrome c molecules bound between the β-propeller domains (34).

Hidden WD Repeats—Because of the diversity found in these motifs, identifying WD repeats by sequence alone appears to be difficult. Varying numbers of repeats have been reported in the Aip1p proteins, ranging from 7 in Xenopus (12) and 9 in Dicyostelium (13) and chick (35) to 10 in C. elegans (14). Based upon sequence and functional conservation among these related proteins, it seems clear that all will fold into a homologous two-domain structure.

Moreover, it is clear from the Aip1p structure that all of the “extra” blades are derived from hidden WD repeats. Structure-based sequence alignments of these repeats within Aip1p (Fig. 4) suggest plausible albeit weaker homology with the repeat consensus. Structural overlap of these repeats within the Aip1p model (Fig. 5) confirms that they are authentic with root mean square deviations for the Cα positions in the A, B, and C strands of each WD repeat relative to those in the sixth WD repeat (residues 236–280) ranging from 0.49 to 1.89 Å. These results collectively suggest that there may be many of these repeats that have yet to be identified in the sequence data.

Aip1p-Actin Interactions—Recent studies have shown that the interactions of Aip1p with F-actin strongly depend on the presence of coflin (15), implying that Aip1p binds to newly formed barbed ends of actin while coflin is still bound. However, the specific manner in which Aip1p interacts with F-actin and coflin is still unclear, as the residues on Aip1p that directly interact with F-actin have not yet been identified. The
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structures and amino acid sequences of actins are highly conserved, and it seems likely that any residues in Aip1p that directly interact with F-actin would also be conserved. A potential interaction site is the large patch of conserved residues along the circumference and bottom loops of blades 2–5 (Fig. 3). This surface of Aip1p includes the residues Arg-130, Arg-172, Lys-194, and Lys-237, which form a positively charged area that could interact strongly with residues on the surface of F-actin.

A second potential binding area for an actin-cofilin complex is the region between the two β-propellers within the Aip1p clamshell. This space between the β-propeller domains measures ~50 Å across the clamshell and maximally 15 Å deep and, by inspection, appears to be large enough to accommodate at least part of such a complex. This would implicate the top surfaces of the β-propellers of Aip1p in binding, a region commonly used by WD repeat proteins to bind partner proteins (18). The interface between the two domains of Aip1p consists of hydrophobic pockets formed partly by residues on loops connecting the two propellers and a number of hydrogen-bonding interactions that link blades 7 and 8. Due to these interactions, the hinge region connecting the two β-propellers is likely to be inflexible, preventing significant changes in the orientation of the two domains relative to one another in the absence of binding partners. Many of the residues involved in these interactions are conserved in other organisms as well, implying that any rigidity they impart on the Aip1p interface is probably also conserved.

A loss of function was observed C. elegans Aip1p mutants (14) corresponding to G319E and G357E in S. cerevisiae Aip1p. Based upon the structure of S. cerevisiae Aip1p, the G319E mutation would cause significant steric clashes between the mutated residue and either Gln-13, Asn-339, or Arg-358. At the very least, this mutation would probably result in the displacement of the B-C loop in blade 7 and the disruption of the hydrogen bonds between Asp-318 and Arg-358 at the β-propeller interface. Similarly, the G357E mutation would cause a steric clash between the mutated residue and the D-A loop connecting blades 8 and 9. This mutation would probably cause the displacement of the B-C loop of blade 8, disrupting the interfacial hydrobonding network formed by Arg-358 and possibly disrupting the interactions formed by Trp-362. These observations support the hypothesis that disruption of the β-propeller interface and displacement of the β-propellers from their proper orientation prevents Aip1p from recognizing its substrate. A lack in flexibility may actually aid Aip1p function, possibly making it more specific for particular actin-cofilin complexes, such as those found at the barbed ends of actin filaments (15).

The interaction of Aip1p with F-actin appears to be dependent upon the binding of cofilin, as most of the mutations in actin that should hinder cofilin binding also interrupt the interaction of actin with Aip1p (10). However, a double mutation has been made in actin (E253A, R254A) that disrupts interactions with Aip1p but does not interfere with cofilin-actin interactions (10). These findings suggest that Aip1p interacts with actin directly in subdomain 4 and through cofilin-mediated interactions, and that all of these interactions are crucial for proper Aip1p function (36). It seems likely that one of more of the patches of conserved residues on the surface of Aip1p are involved in these interactions, and more work on the Aip1p-cofilin-actin system is necessary to elucidate their specific na-
ture. In these efforts, the structure of Aip1p can be used as a tool in the identification of key residues that interact with F-actin and cofillin.

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