Activation of the Arp2/3 Complex by the Actin Filament Binding Protein Abp1p

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Abstract. The actin-related protein (Arp) 2/3 complex plays a central role in assembly of actin networks. Because distinct actin-based structures mediate diverse processes, many proteins are likely to make spatially and temporally regulated interactions with the Arp2/3 complex. We have isolated a new activator, Abp1p, which associates tightly with the yeast Arp2/3 complex. Abp1p contains two acidic sequences (DDW) similar to those found in SCAR/WASp proteins. We demonstrate that mutation of these sequences abolishes Arp2/3 complex activation in vitro. Genetic studies indicate that this activity is important for Abp1p functions in vivo. In contrast to SCAR/WASp proteins, Abp1p binds specifically to actin filaments, not monomers. Actin filament binding is mediated by the ADF/cofilin homology (ADF-H) domain of Abp1p and is required for Arp2/3 complex activation in vitro. We demonstrate that Abp1p recruits Arp2/3 complex to the sides of filaments, suggesting a novel mechanism of activation. Studies in yeast and mammalian cells indicate that Abp1p is involved functionally in endocytosis. Based on these results, we speculate that Abp1p may link Arp2/3-mediated actin assembly to a specific step in endocytosis.

Key words: actin • yeast • Arp2/3 complex • Abp1 • endocytosis

Introduction
Rapid actin assembly and turnover are required for diverse cellular processes including endocytosis, cell motility, and morphogenesis. For this reason, there has been strong interest in learning how actin assembly is regulated in cells. Recently, the actin-related protein (Arp) 2/3 complex has emerged as a central regulator of actin dynamics and organization (reviewed in Machesky and Gould, 1999). This protein complex has a mass of ~200 kD and is composed of seven highly conserved subunits: p40, p35, p21, p19, p15, and the actin related proteins, Arp2 and Arp3. So far, two activities have been identified for purified Arp2/3 complex in vitro: nucleation of actin filament assembly and actin filament branching (Mullins et al., 1998; Welch et al., 1998).

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Abbreviations used in this paper: AAP, actin-associated protein; Arp, actin-related protein; HA, hemagglutinin.

Alone, the Arp2/3 complex nucleates actin assembly weakly. However, in the presence of activating proteins, actin nucleation increases dramatically. To date, three activators of the Arp2/3 complex have been identified. They are the Listeria ActA protein (Welch et al., 1998), the ubiquitous cellular SCAR/WASp proteins (Machesky et al., 1999; Rohatgi et al., 1999; Winter et al., 1999; Yarar et al., 1999), and fission yeast type I myosin (Lee et al., 2000). For each, the minimal domain requirements for activation in vitro have been defined. SCAR/WASp activation requires an acidic motif and an adjacent actin monomer binding domain (reviewed in Higgs and Pollard, 1999; Mullins, 2000). Activation by ActA requires three regions: an acidic sequence, an adjacent actin monomer binding domain, and an additional sequence with weak similarity to cofilin (Skoble et al., 2000). The acidic motif associates directly with the Arp2/3 complex and, in current models, the actin monomer binding domain is thought to present an actin subunit to Arp2 and/or Arp3, promoting the formation of a filament seed for rapid polymerization (see, for example, Skoble et al., 2000). Although fission yeast myosin I activates the Arp2/3 complex, its mechanism of activation is less well understood (Lee et al., 2000). Budding yeast myosin I also
may be involved in activating Arp2/3 complex, as suggested by its physical association with the Arp2/3 complex and genetic interactions with LAS17/EEE1, the yeast gene that encodes a homologue of SCAR/WASp (Evangelista et al., 2000; Lechler et al., 2000). However, it has not yet been shown to activate Arp2/3-nucleated actin assembly in vitro.

Here, we identify a new activator of the Arp2/3 complex, the actin binding protein Abp1p. We demonstrate that actin monomer binding activity is not a strict requirement for Arp2/3 complex activation, since Abp1p lacks this property. Instead, our data support an alternative model for activation in which Abp1p recruits the Arp2/3 complex to the sides of actin filaments to promote filament nucleation.

Abp1p was the first actin-associated protein identified in yeast (Drubin et al., 1988). Since then, it has been shown that Abp1p and drebrins are homologous and form a family of proteins conserved from yeast to humans (Lappalainen et al., 1998). These proteins contain multiple domains with different functions: an ADF/cofilin homology domain (ADF-H), a helical region, a proline-rich domain, and an SH3 domain. In yeast, Abp1p localizes to cortical actin patches, and its overexpression causes severe defects in cellular actin organization (Drubin et al., 1988). An abp1 null mutant is viable, but is synthetically lethal in combination with mutations in genes encoding other actin-associated proteins: sac6, slal, and sla2 (Holtzman et al., 1993). In mammalian cells, Abp1p localizes to the perinuclear region, but upon activation of the GTPase Rac, Abp1p translocates to the leading edge of cells in a pattern that overlaps precisely with Arp2/3 complex localization (Kessels et al., 2000).

A variety of studies in yeast and mammalian cells have linked Abp1p to endocytosis functionally (mammalian studies reviewed in Qualmann et al., 2000). In yeast, Abp1p and the coiled coil region of Sla2p perform a redundant function required for endocytosis (Wesp et al., 1997). ABP1 is also required for proper localization in cells of the endocytosis-regulating kinases Arc1p and Prk1p (Cope et al., 1999). The proline-rich region of Abp1p binds directly to the SH3 domain of Rvs167p/amphiphysin, another factor required for endocytosis (Munn et al., 1995; Lila and Drubin, 1997). However, until now, Abp1p was thought to function simply as a physical bridge linking Arp2/3 complex to actin filaments, 0.5 M KCl. This material was dounced 10–20× (tight-fitting pestle) on ice and centrifuged at 4°C for 20 min in a TL100.3 rotor at 80,000 rpm. The supernatant contained the AAP mixture. The pellet contained actin, which was further purified on a MonoQ 5/5 column (Amershams Pharmacia Biotech), followed by a Superdex 75 gel filtration column (Amershams Pharmacia Biotech), and was stored in G buffer as described previously (Goode et al., 1999).

Arp2/3 complex was purified from AAP by two different strategies (significance described in Results). In the two-step method, the salt concentration of AAP was lowered by diluting sevenfold in HEKG buffer (HEK + 5% glycerol). The AAP was then fractionated by FPLC on a monoQ 5/5 column, eluting with a 25-ml linear KCl gradient (0.1–0.6 M) and collecting 0.5-ml fractions. Arp2/3-containing fractions were identified by the distinctive broad band pattern on SDS-PAGE gels and by immunoblotting with anti–Arp2p antibodies (Moreau et al., 1996). Peak fractions were pooled, desalted in HEKG, and concentrated to 0.3 ml using Microcon10 devices (Amicon). Arp2/3 complex was then fractionated on a Superose 12 gel filtration column (Amershams Pharmacia Biotech), equilibrated in HEKG, collecting 0.5-ml fractions. Arp2/3 complex eluted in a peak ~12 ml after loading, consistent with a mass of 220–250 kD. Peak Arp2/3 fractions were pooled, concentrated as above to 5 μM, and stored in aliquots at ~8°C. In the one-step purification, the AAP was desalted and concentrated in a Microcon10 device, and then fractionated by gel filtration as above. By this method, Arp2/3 complex eluted from the column ~11 ml after loading. To quantitate the stoichiometry of the Abp1p–Arp2p3 complex association, Abp1p and Arc1p bands were quantified by densitometry and the stoichiometry was found to be an ~1:1 molar ratio.

To purify native yeast Abp1p, the AAP-containing fractions from the mono Q column were identified by immunoblotting with anti–Abp1p antibodies (Drubin et al., 1988). Peak fractions were pooled, concentrated, and fractionated by gel filtration chromatography as above. Abp1p eluted from the Superose 12 column ~14 ml after loading and was concentrated to 20 μM and stored as above.

**Materials and Methods**

**Purification of Yeast Arp2/3 Complex and Abp1p**

Using HEK buffer (20 mM Hepes, pH 7.5, 1 mM EDTA, 50 mM KCl) supplemented with 10% glycerol and with 1 mM PMSF plus an aqueous cocktail of protease inhibitors, a high-speed supernatant was generated from lysed yeast cells (DDY1519), as described previously (Goode et al., 1999). Actin assembly stimulated by addition of 5 mM MgCl2 was carried out for 1 h on ice. To isolate assembled actin, the reactions were centrifuged at 4°C for 1 h in an SA600 rotor (Sorvall) at 17,000 rpm. Pellets were washed once in 6 ml HEKGM buffer (HEK supplemented with 10% glycerol and 5 mM MgCl2), and repelleted by centrifugation (80,000 rpm) for 20 min at 4°C in a TLA100.3 rotor (Beckman Coulter). The actin pellet was rinsed once in HEKGM buffer, and actin-associated proteins (AAPs) were isolated by resuspending the pellet in 3 ml HEKGM buffer + 0.5 M KCl. 1.5 ml of HEKGM buffer (HEK + 5% glycerol) and 1 ml of 3 M NaCl were added to 10 ml of AAP mixture. The AAP was then fractionated by centrifugation at 4°C for 1 h. The beads were then washed five times in HEKG in a TLA100.3 rotor. The AAP was then fractionated by centrifugation at 4°C for 1 h. The beads were then washed five times in HEKG, once in HEKG, + 0.4 M KCl and once in HEKG. Samples of the Arp2/3 complex eluted from the column ~11 ml after loading. To quantitate the stoichiometry of the Arp2p3 complex association, Abp1p and Arc1p bands were quantified by densitometry and the stoichiometry was found to be an ~1:1 molar ratio. To purify native yeast Abp1p, the AAP-containing fractions from the mono Q column were identified by immunoblotting with anti–Abp1p antibodies (Drubin et al., 1988). Peak fractions were pooled, concentrated, and fractionated by gel filtration chromatography as above. Abp1p eluted from the Superose 12 column ~14 ml after loading and was concentrated to 20 μM and stored as above.

**Abp1p–Arp2p3 Binding Assays**

Anti–HA.11 monoclonal antibodies (BAbCO) were covalently attached to CL48 protein A beads (Amershams Pharmacia Biotech) using Dimethyl pimelimidate (DMPP; Pierce Chemical Co.). A high-speed supernatant (HSS) was generated as above from yeast (YMW15) expressing a functional 3′/hemagglutinin (HA)-tagged Arp2p3 as the only source of Arp2p3 (Moreau et al., 1996). 1.5 ml of HSS/10 μl antibody beads was mixed in a rotator at 4°C for 1 h. The beads were then washed five times in HEKG, once in HEKG, + 0.4 M KCl and once in HEKG. Samples of the Arp2p3-loaded beads were boiled in SDS sample buffer without reducing agent, and fractionated on SDS-PAGE gels that were subsequently stained with Coomassie blue. beads contained 5–10 μM Arp2p3 complex. 1 μM Abp1p was added to 10 μl of Arp2p3-loaded beads or control beads (no Arp2p3) in a 100-μl reaction in HEKG (final concentration Arp2p3 = 0.5–1 μM). Reactions were mixed on a rotator for 20 min at 25°C. Beads were washed three times with 100 μl HEKG and analyzed by SDS-PAGE as above.

**Actin Filament Cosedimentation**

For copelling assays, 1 μM Abp1p was added to variable concentrations of assembled purified yeast actin (Goode et al., 1999), incubated for 20 min at 25°C, and centrifuged for 30 min in a TL100 rotor at 90,000 rpm. Pellets and supernatants were fractionated on 12% SDS-PAGE gels that were subsequently stained with Coomassie blue. Band intensities were quantitated by densitometry. To measure the ability of Abp1p to recruit Arp2p3 complex to actin filaments, 0.5 μM Abp1p and 1 μM F-actin were mixed with variable concentrations of Arp2p3 complex (0, 15, 30, 60, 125, and 250 nM). The reactions were incubated for 15 min at 25°C, and then centrifuged for 15 min at 90,000 rpm in a TL100 rotor. Pellets and supernatants were fractionated on 12% SDS-PAGE gels, blotted, and probed with Arp2 antibodies. Densitometry was performed to determine the level of cosedimentation of Arp2p3 complex with actin filaments.

**Actin Assembly Assays**

To measure actin assembly kinetics, light scattering and pyrene fluorescence assays were performed as described previously (Goode et al., 1999). 60 μl of 2.5-μM yeast actin (or 2.5-μM yeast actin + 0.5-μM pyrene-labeled yeast actin) were mixed rapidly with 3.5 μl of initiation buffer and 6.5 μl HEKG, Arp2p3, and or Abp1p, and then immediately transferred to a cu-
vete in a fluorometer. Polymer mass levels were determined by sedimentation 1 h after initiation of assembly (Goode et al., 1999).

**Purification and Analyses of Abp1p Mutants**

Abp1p mutants were generated by PCR in pEG(KT) (Mitchell et al., 1993) or in pDD187 (Lila and Drubin, 1997), and sequenced. GST fusion proteins were purified from *Saccharomyces cerevisiae* strain DDY1810 essentially as described (Rodal et al., 1999). Mutants of pDD187 were tested for genetic interactions in DDY995, DDY996, and DDY997 as described previously (Lila and Drubin, 1997).

**Results**

**Identification of a New Arp2/3 Complex-associated Activator**

*Saccharomyces cerevisiae* Arp2/3 complex was purified by a new procedure we developed to study actin assembly in yeast extracts (see Materials and Methods), a method that will be described in greater detail elsewhere (Goode, B.L., A.A. Rodal, G. Barnes, and D.G. Drubin, manuscript in preparation). In this procedure, actin assembly was stimulated in yeast lysates after clarification by ultracentrifugation. The actin structures that formed in the extracts contained >90% of the cellular actin, >80% of the cellular Arp2/3 complex, and many other known yeast actin-associated proteins (determined by quantitative immunoblotting). These actin structures were isolated by low-speed centrifugation, and the actin-associated proteins were stripped from the filaments with high salt (0.5 M KCl). The Arp2/3 complex is an abundant component of the salt-stripped AAP mixture and was purified away from the other proteins using two additional steps, ion exchange and gel filtration chromatography (Fig. 1, a and b). The isolated complex is composed of seven polypeptides of the expected molecular weights: 15, 19, 21, 35, 40, 43, and 53 kD (Fig. 1 b, d). The complex includes the 40-kD subunit that has been identified in preparations from other organisms, but was reported previously to not copurify with the yeast Arp2/3 complex (Winter et al., 1997). We refer to this as the “core” Arp2/3 complex.

Core Arp2/3 complex modestly accelerated actin assembly (Fig. 2, △). It was activated by a carboxyl-terminal fragment of Las17p/WASP (see below), consistent with recent reports (Machesky and Insall, 1998; Winter et al., 1999), and by the ActA protein from *Listeria monocytogenes* (Welch et al., 1998) (Fig. 2, ○).

We next investigated the possibility that ion exchange chromatography had removed factors normally associated with the Arp2/3 complex. Therefore, we purified the Arp2/3 complex from AAP by a one-step approach using gel filtration chromatography. This revealed a doublet protein band with an apparent molecular weight of 85 kD that cofractionated with the Arp2/3 complex in a 0.94:1 molar ratio to other subunits in the complex (Fig. 1 c, *). We refer to this material as “intact”Arp2/3 complex. Intact Arp2/3 complex eluted earlier from the gel filtration column than the core Arp2/3 complex, consistent with an increase in mass from the associated factor. Comparison of the nucleation activities of the intact and core Arp2/3 preparations revealed a striking difference: intact Arp2/3 complex nucleated actin assembly with rapid kinetics (Fig. 2, ●), similar to ActA-activated Arp2/3 complex.

**Abp1p Associates Directly with the Arp2/3 Complex and Activates Actin Assembly**

We identified by immunoblotting the associated doublet in the intact Arp2/3 complex as Abp1p (Fig. 1 d), one of the first actin binding proteins found in yeast (Drubin et

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**Figure 1.** Purification of yeast Arp2/3 complex and identification of tightly associated Abp1p. (a) Coomassie-stained gel showing purification steps for isolation of core Arp2/3 complex: high speed supernatant (S), actin pellet (P), anion exchange column peak (Q), and gel filtration peak (GF). Coomassie-stained gel of the gel filtration column fractions for isolation of core Arp2/3 complex (b) and intact Arp2/3 complex (c). (*) The associated Abp1p doublet. (d) Immunoblot of gel filtration column fractions from the intact Arp2/3 preparation probed with Abp1p antibodies.
Figure 2. Abp1p activates Arp2/3-mediated actin nucleation in the pyrene assay. Graph shows assembly of 2 μM yeast actin and 0.5 μM pyrene-labeled rabbit muscle actin, with 25 nM core Arp2/3 complex, 25 nM intact Arp2/3 complex, 50 nM ActA, and 70 nM GST-Abp1p. (▲) Actin alone; (□) GST-Abp1p; (△) core Arp2/3; (×) core Arp2/3 + GST-Abp1p; (○) core Arp2/3 + ActA; (●) intact Arp2/3.

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Abp1p Requires Its Two Acidic Motifs and ADF-H Domain for Arp2/3 Complex Activation In Vitro

Abp1p has multiple domains: an NH-terminal ADF-H domain, two acidic sequences with homology to those found in SCAR/WASp proteins, a proline-rich region, and an SH3 domain (Fig. 4 A). To investigate the domain requirements of Abp1p for actin filament binding and Arp2/3 activation, we purified a set of Abp1p mutants as GST fusion proteins (Fig. 4, A and B). GST was left on the fusion proteins because attempts to remove GST by thrombin digestion resulted in severe proteolysis of Abp1p.

Deletion of the amino-terminal ADF-H domain disrupted both actin-filament binding (Fig. 4 C) and Arp2/3 activation (D), while deletion of the SH3 domain of Abp1p caused no change in either activity (not shown). The ADF-H domain alone had limited solubility, and therefore we were unable to test its activity in F-actin copelleting assays. It also showed no activity in the pyrene actin assembly assay (data not shown), possibly because it is not folded correctly. Triple substitutions (DDW/AAA) within either acidic motif of Abp1p disrupted Arp2/3 activation, but not completely (Fig. 4 E). These mutations had no effect on actin filament binding (Fig. 4 C). A double mutant targeting both acidic motifs caused a stronger negative effect on Arp2/3 activation than each single mutant alone. Like the single mutants, this double mutant had no effect on actin-filament binding (Fig. 4, C and E).

Genetic Interactions Support an In Vivo Role for Arp2/3 Activation by Abp1p

To test the in vivo relevance of the Abp1p-Arp2/3 complex activity, we introduced the double acidic sequence mutant (A-N*4C*) into genetic backgrounds in which the abp1 Δ mutant has a phenotype: sla1-7, sla2-5, and sac6-102. Although no synthetic effects were seen in combination with sla1-7 or sla2-5, the ABP1 double acidic mutant reduced the permissive temperature of growth in the sac6-102 background, indicating that Arp2/3 complex activation by Abp1p is important in vivo (Fig. 5). However, the effects were not as strong as a complete abp1 deletion (see Discussion).

Discussion

We have identified a new activator of the Arp2/3 complex, the conserved actin binding protein Abp1p. This protein has been linked functionally to endocytosis by a number of studies in yeast and mammalian cells (reviewed in Qualmann et al., 2000; also see below). Our data therefore suggest that Abp1p links Arp2/3 complex-mediated actin assembly to a step in endocytosis. They also provide important insights into the mechanism(s) by which activation of the Arp2/3 complex can be achieved.

A New Mechanism for Arp2/3 Complex Activation: Recruitment to the Sides of Actin Filaments

The most well-characterized activators of the Arp2/3 complex are ActA (Welch et al., 1998) and SCAR/WASp (Ma-
chesky et al., 1999; Rohatgi et al., 1999; Winter et al., 1999; Yarar et al., 1999). These factors require both an acidic motif and an actin monomer binding domain for activation (reviewed in Higgs and Pollard, 1999). Similar to ActA and SCAR/WASp proteins, Abp1p requires acidic sequences for activating the Arp2/3 complex. However, a key difference is that ActA and SCAR/WASp require a single acidic sequence for activation, whereas Abp1p requires two acidic sequences for full activation. A possible explanation for this requirement is discussed below. Another fundamental difference between Abp1p and ActA or SCAR/WASp is that activation of the Arp2/3 complex by Abp1p requires an actin filament binding domain instead of an actin monomer binding domain. Importantly, this demonstrates that actin monomer binding is not a strict requirement for activation. Fungal type I myosin (Lee et al., 2000) may work by a similar mechanism. However, it has not yet been established whether the carboxyl terminus of this myosin I, which activates the Arp2/3 complex in vitro, binds to actin monomers and/or actin filaments. Actin filament binding of Abp1p is mediated by its ADF-H domain, consistent with functional analyses of mouse Abp1 (Kessels et al., 2000). Our data support the hypothesis that the ADF-H module has been adapted for different interactions with actin (Lappalainen et al., 1998). Twinfilins use these modules to bind specifically to actin.

Figure 3. Abp1p binds directly to actin filaments and to the Arp2/3 complex. (A) Coo massie-stained gel of purified native yeast Abp1p. (B) Coo massie-stained gel of HA-Arp2/3 complex isolated with HA antibody-coupled beads (1). 0.5 μM purified Abp1p cosediments with HA-Arp2 beads (3) but not with beads alone (2). (C) 0.15 μM Abp1p binds tightly to actin filaments (3 and 0.75 μM) in cosedimentation assays. (D) Abp1p binds to actin filaments with a 1:1 molar saturation stoichiometry. (E) Abp1p (0.5 μM) strongly enhances the association of Arp2/3 complex (0.5 μM) with actin filaments (2 μM) by cosedimentation assay. (●) The seven subunits of the core Arp2/3 complex.
monomers (Goode et al., 1998), Abp1 uses it to bind actin filaments (Kessels et al., 2000; and this study), and ADF/cofilins use it to bind to actin monomers and filaments (reviewed in Lappalainen et al., 1998). A careful structural and functional comparison of ADF-H domains in twinfilin, Abp1p, and cofilin/ADF would help reveal how functional diversity is achieved in this module.

How, then, does actin filament binding by Abp1p contribute to Arp2/3 complex activation? It has been shown that activity of the Arp2/3 complex increases markedly upon association with actin filaments (Machesky et al., 1999). However, the Arp2/3 complex has a relatively weak binding affinity for actin filaments \((K_d = 2 \mu M;\) Mullins et al., 1998; and this study). We demonstrated that Abp1p has a strong actin filament binding affinity \((K_d < 0.1 \mu M)\) and recruits Arp2/3 complex to filaments. This effect was not due to an increase in the number of pointed ends caused by an Abp1p severing activity. Even high concentrations of Abp1p did not increase the rate of actin filament disassembly or assembly (not shown), characteristics

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**Figure 4.** Actin filament binding and Arp2/3 complex activation by Abp1p mutants. (A) Schematic diagram of GST-Abp1p fusion proteins and alignment of acidic sequences. (B) Coomassie-stained gel of purified fusion proteins. (C) The ADF-H domain of Abp1p is required for actin filament binding, but its acidic sequences and the SH3 domain are not. Cosedimentation assays were performed using 2 μM F-actin and 250 nM GSTAbp1p mutants. (D and E) The ADF-H domain and both acidic sequences in Abp1p are required for activation of the Arp2/3 complex. Pyrene assay for actin assembly using 3 μM actin (2.85 μM yeast actin, 0.15 μM rabbit skeletal muscle pyrene-labeled actin), 10 nM yeast Arp2/3 complex, 400 nM Las17/WASp WA domain, and 70 nM Abp1p mutants.
of filament-severing proteins. Based on these data, we propose that Abp1p may activate Arp2/3 complex-mediated actin nucleation by recruiting Arp2/3 complex to filament sides.

This model also provides an explanation for why Abp1p requires two acidic sequences for full activation of the Arp2/3 complex. It has been suggested that ActA and SCAR/WASp proteins may stimulate Arp2/3 complex by a “hit and run” mechanism (Higgs and Pollard, 1999), which would require only transient interactions with the Arp2/3 complex. This is consistent with their relatively weak binding affinities to activators for Arp2/3 complex (e.g., WASp has a $K_d \approx 1 \mu M$; Higgs et al., 1999). In contrast, Abp1p binds to the Arp2/3 complex with much higher affinity, remaining tightly and stoichiometrically associated through gel filtration (Fig. 1) at 0.3 $\mu M$ each of Abp1p and Arp2/3 complex. This observation indicates that Abp1p binds to Arp2/3 complex with a dissociation constant well below 0.3 $\mu M$. Abp1p also displaces efficiently the “WA” fragment of human SCAR protein from Arp2/3 complex in binding competition experiments (B.L. Goode, unpublished data). Therefore, Abp1p may require two acidic sequences to anchor the Arp2/3 complex effectively to the sides of actin filaments, which in turn promotes Arp2/3 complex-mediated nucleation of actin assembly.

The Cellular Functions of Abp1p

Until now, the only activities found for Abp1p were actin filament binding (through its ADF-H domain) and interactions with other proteins (e.g., Rvs167p/amphiphysin and Srv2p/CAP) through its carboxyl-terminal SH3 domain and proline-rich region. These binding interactions led to the hypothesis that Abp1p might serve as an adapter, linking actin filaments to endocytic machinery (Lila and Drubin, 1997). Our demonstration here that Abp1p stimulates Arp2/3 complex-mediated actin nucleation suggests that Abp1p may link activated actin polymerization to an endocytic function. Evidence that Arp2/3 activation by Abp1p is important in vivo comes from the observation that mutation of the two acidic sequences in Abp1p enhanced the severity of the phenotype resulting from mutation of the actin filament cross-linking protein, Sac6p/fimbrin. Further genetic investigations will be required to elucidate the specific in vivo roles of Arp2/3 activation by Abp1p, and to determine whether this activity is important for Abp1p’s endocytic function.

Note Added in Proof. After our manuscript was submitted, two publications appeared that demonstrate activation of the Arp2/3 complex by the actin filament binding protein cortactin. This work shows that cortactin requires its actin filament binding domain for activation, and thus its mechanism for activation may be similar to that of Abp1p (Uruno et al., 2001; Weaver et al., 2001).

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