ARPC1/Arc40 Mediates the Interaction of the Actin-related Protein 2 and 3 Complex with Wiskott-Aldrich Syndrome Protein Family Activators*

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The actin-related protein 2 and 3 (Arp2/3) complex is a seven-subunit protein complex that nucleates actin filaments at the cell cortex. Despite extensive cross-linking, crystallography, genetic and biochemical studies, the contribution of each subunit to the activity of the complex remains largely unclear. In this study we characterized the function of the 40-kDa subunit, ARPC1/Arc40, of the yeast Arp2/3 complex. We showed that this subunit is indeed a stable component of the Arp2/3 complex, but its highly unusual electrophoretic mobility eluded detection in previous studies. Recombinant Arc40 bound the VCA domain of Wiskott-Aldrich syndrome protein family activators at a $K_d$ of 0.45 μM, close to that of the full complex with VCA (0.30 μM), and this interaction was dependent on the conserved tryptophan at the COOH terminus of VCA. Using a newly constructed Δarc40 yeast strain, we showed that loss of Arc40 severely reduced the binding affinity of the Arp2/3 complex with VCA as well as the nucleation activity of the complex, suggesting that Arc40 contains an important contact site of the Arp2/3 complex with VCA. The Δarc40 cells exhibited reduced growth rate, loss of actin patches, and accumulation of cables like actin aggregates, phenotypes typical of other subunit nulls, suggesting that Arc40 functions exclusively within the Arp2/3 complex.

The Arp2/3 complex is a highly conserved actin regulator that nucleates branched actin network associated with regions of the plasma membrane (1, 2). This complex contains seven subunits, including two actin-related proteins Arp2 and Arp3 and five novel polypeptides named ARPC1–5 (3–5). Purified Arp2/3 complex is inactive but stimulates rapid actin polymerization in the presence of activator proteins, the most potent type being the VCA domain-containing Wiskott-Aldrich syndrome protein (WASP) family members (1, 2). This complex contains seven subunits, Arp2, Arp3, and ARPC1, are in close contact with the VCA domain and the two reconstructions clearly did not show any difference as large as a missing 40-kDa subunit (12). Another unexpected result on Arc40 came from genetic analysis of yeast mutants bearing deletions of each of the Arp2/3 subunits (10). Unlike the other Arp2/3 subunit nulls, a previous effort to recover viable Arc40 null cells was unsuccessful, raising the possibility that the function of Arc40 may extend beyond that of the Arp2/3 complex.

ARPC1 has been a particularly enigmatic subunit. Whereas this subunit is present in the Arp2/3 complex from various organisms, Arc40, the budding yeast ARPC1, was apparently absent from most preparations of the purified yeast complex (5). Since the yeast complex preparations demonstrate a VCA-dependent actin nucleation activity comparable to that of the bovine Arp2/3 complex, the lack of ARPC1 implied that this subunit may not be required for the activity of the Arp2/3 complex. Despite the apparent lack of ARPC1/Arc40 in the purified yeast complex, it was clear that Arc40 can associate with the Arp2/3 complex in yeast (10) and appeared to be present in at least some preparations of the yeast complex (11). Moreover electron cryomicroscopy reconstruction showed that the yeast complex was nearly identical to the bovine complex, and the two reconstructions clearly did not show any difference as large as a missing 40-kDa subunit (12). Another unexpected result on Arc40 came from genetic analysis of yeast mutants bearing deletions of each of the Arp2/3 subunits (10). Unlike the other Arp2/3 subunit nulls, a previous effort to recover viable Arc40 null cells was unsuccessful, raising the possibility that the function of Arc40 may extend beyond that of the Arp2/3 complex.

In this study, we reinvestigated the involvement of Arc40 in the function of the Arp2/3 complex through biochemical and genetic analysis. We present evidence that ARPC1/Arc40 is indeed a stable component of the Arp2/3 complex and mediates a major interaction between the complex and WASP family activators.

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The abbreviations used are: Arp2/3, actin-related proteins 2 and 3; WASP, Wiskott-Aldrich syndrome protein; GST, glutathione S-transferase; HA, hemagglutinin; Ni-NTA, nickel-nitrilotriacetic acid; TAP, tandem affinity purification.

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EXPERIMENTAL PROCEDURES

Media and Genetic Manipulations—Yeast cell culture and genetic techniques were carried out by methods described in Ref. 13. YPD contained 2% glucose, 1% yeast extract, and 2% Bactopeptone (Difco Laboratories).

Antibodies—Polyclonal antibody against yeast Arp3 (YG-18) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-yeast Arp2 polyclonal antibody was raised in rabbits against an Arp2 peptide as described previously (14). The anti-yeast ARPC1/Ar40 antibody (YAR40) was raised in rabbits as described previously (10). The anti-yeast ARPC3/Ar18 antibody (YAR18) was raised in rabbits against a GST-Ar15 recombinant protein. The anti-yea-Ar15 antibody (12CA5) was a gift from Dr. Frank McKeon (Harvard Medical School).

Gene Disruption of AR40—The AR40 gene was disrupted by replacement with the TRP1 gene. The AR40 gene was amplified from genomic DNA and cloned into pSK+ to generate pD9W. A 685-bp XcmI-XbaI fragment (corresponding to amino acids 67–295 of Arc40) was replaced with a 900-bp Smal-Nhel fragment containing the TRP1 gene. The Δarc40 strain was generated by transforming the diploid strain RLY141 with the Xhol-NotI Δarc40:TRP1 fragment. Gene disruption was verified by PCR amplification and restriction enzyme analysis (data not shown), and haploid Δarc40 cells were obtained by sporulation and tetrad dissection.

Preparation of Yeast Extracts—Yeast extracts used for binding studies, gel filtration analysis, and pyrene-actin assays were prepared essentially as described previously (15). Unless specified otherwise, extracts were prepared in 1× UBA buffer (50 mM K-Hepes, pH 7.5, 100 mM KCl, 3 mM MgCl2, 1 mM EGTA, 0.2 mM ATP, and 1 mM dithiothreitol). For the pyrene-actin assay, yeast extracts were fractionated by an additional ammonium sulfate precipitation (55%). The precipitate was resuspended and dialyzed overnight against 1× UBA buffer and clarified by centrifugation at 100,000 g. Gel filtration analysis, and pyrene-actin assays were prepared in 1× UBA buffer with 10% pyrene-labeled G-actin before polymerization by a brief incubation in excess of beads, yeast Myo5-CA (400 ng of protein/H11003/H9262), GST (350 ng of protein/H11003/H9262), yeast Myo5-CA (400 ng of protein/H11003/H9262), or GST-Bee1-VCA (320 ng of protein/H11003/H9262).

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The Budding Yeast Arp2/3 Complex Always Contains ARPC1/Ar40—Previously we used two different methods to purify the Arp2/3 complex from budding yeast, one involving Ni-NTA affinity and conventional columns and the other using an affinity column to which the CA domain was covalently coupled (5, 15). CA domain is the VCA region lacking the G-actin-binding V motif. To determine whether the lack of ARPC1/Ar40 was due to the purification procedures, we developed a purification of the complex using the TAP tag, which was faster and gentler (see “Experimental Procedures”). However, like the CA affinity-purified complex, the complexes purified using the TAP tag still lacked Ar40 as visualized by SDS-PAGE and Coomassie Blue staining (Fig. 1, A and B). As a positive control, bovine Arp2/3 complex purified using the CA affinity column contained the 40-kDa ARPC1 (Fig. 1A). We noticed, however, that the yeast complexes purified by both methods had a smear at the top of the gel (>200 kDa) (Fig. 1, A and B, large black arrows) and occasionally an additional band around 85 kDa. To identify the proteins present in the smear, the region of the gel (boxed) was excised and subjected to mass spectrometry analysis. The peptide sequences obtained for the band exclusively matched the sequence of Ar40 and cover 36% of the open reading frame (Fig. 1C, bold). Immuno blot analysis using a polyclonal anti-yeast Ar40 antibody (10) further confirmed the identity of the high molecular weight smear and the occasional 85-kDa bands to be Ar40 and also revealed Ar40 species at monomer size (see Fig. 2, B and C). This result suggests that the yeast Ar40 copurifies with the complex but exhibits abnormally high and heterogeneous molecular weights.

High Molecular Weight Species of Ar40 Are Formed during SDS-PAGE—Since most of the Ar40 associated with purified yeast Arp2/3 complex was seen as high molecular weight aggregates on SDS-PAGE, we performed gel filtration fractionation to determine whether these aggregates were present in the native complex. If the large aggregates were formed before gel electrophoresis, the Arp2/3 complex should elute faster than expected of a 220-kDa globular complex and exhibit size heterogeneity, but if Ar40 associates with the complex as a monomer, it should cofractionate with the other subunits with
expected elution rate. A Superose 12 gel filtration column with calibrated separation range from 15 to 669 kDa was used. As shown in Fig. 2, A and B, the large aggregates cofractionated with the complex in the expected fractions (peak at fraction 26). Immunoblot analysis confirmed that the large aggregates in these fractions were indeed Arc40. This result suggests that Arc40 associates with the Arp2/3 complex normally but forms large aggregates during SDS-PAGE. Attempts to prevent or solubilize these aggregates were unsuccessful. Serendipitously we analyzed by SDS-PAGE purified Arp2/3 complex that was solubilize these aggregates were unsuccessful. Serendipitously we analyzed by SDS-PAGE purified Arp2/3 complex that was isolated on a CA affinity column. The yeast complex was calibrated separation range from 15 to 669 kDa was used. As expected elution rate. A Superose 12 gel filtration column with calibrated separation range from 15 to 669 kDa was used. As shown in Fig. 2, A and B, the large aggregates cofractionated with the complex in the expected fractions (peak at fraction 26). Immunoblot analysis confirmed that the large aggregates in these fractions were indeed Arc40. This result suggests that Arc40 associates with the Arp2/3 complex normally but forms large aggregates during SDS-PAGE. Attempts to prevent or solubilize these aggregates were unsuccessful. Serendipitously we analyzed by SDS-PAGE purified Arp2/3 complex that was bound to CA-coated beads. The beads were directly boiled in the sample buffer before gel loading. We found that binding to CA strongly reduced Arc40 aggregates and increased the proportion of monomeric Arc40 in the gel (Fig. 2C, arrow).

Arc40 Directly Binds VCA—The above result led us to suspect that Arc40 directly interacts with VCA, and this interaction somehow prevents Arc40 aggregation in the gel. To test this hypothesis, recombinant baculovirus-expressed His<sub>6</sub>-HA-tagged Arc40 was used to test the interaction with GST-VCA from Bee1 and N-WASP and CA from yeast Myo5. Purified VCA and CA fragments were cross-linked to Affi-Gel beads and tested for interaction with purified Arc40 by bead pull-down assays (see “Experimental Procedures”). Both VCA fragments demonstrated similar levels of binding to recombinant Arc40, while CA from yeast Myo5 also showed strong binding, whereas the GST control did not bind (Fig. 3A). For the following analysis, we used the GST-VCA and GST-VCA<sup>W503A</sup> peptides from N-WASP because the mutant and wild-type N-WASP-VCA peptides were characterized previously for binding to the Arp2/3 complex (26). Mutations affecting the conserved tryptophan in the A region were previously shown to disrupt the interaction of VCA with the Arp2/3 complex (8, 26). As shown in Fig. 3A, GST-N-WASP-VCA bound Arc40 in a dose-dependent manner, and GST-N-WASP-VCA<sup>W503A</sup> mutant had significantly reduced affinity to Arc40. Quantification of the amount of Arc40 bound to GST-N-WASP-VCA and GST-N-WASP-VCA<sup>W503A</sup> at 1.5 μM Arc40 showed that binding to the mutant was decreased by 21-fold compared with the wild type, suggesting that Arc40 binds directly to VCA and this interaction specifically requires the conserved tryptophan in the A region. The K<sub>d</sub> values measured for Arc40-VCA and Arp2/3-VCA interactions were 0.45 ± 0.16 and 0.30 ± 0.12 μM, respectively (Fig. 3B). The similarity in binding affinities of these interactions suggests that Arc40 is a major contributor of Arp2/3 complex binding to VCA. This conclusion was further supported by analysis of the Arc40 null complex (see below).

The Generation and Phenotypic Analysis of Δarc40 Cells—To directly test whether Arc40 is required for the activity of the Arp2/3 complex in vivo and in vitro, it would be useful to have a viable Δarc40 yeast strain. In previous work we found that Arc40 was the only Arp2/3 subunit that was essential for cell viability. With the newly annotated yeast genome data base, we realized that the original AR4C0 disruption construct affected an adjacent small reading frame encoding an essential protein, Dad3 (27). Thus, we generated a new disruption construct that deletes amino acids 67–295 of Arc40 (see “Experimental Procedures”). The new Δarc40/ARC40 heterozygous diploid strain was sporulated and dissected on a YPD plate. The Δarc40 spores were viable but grew very slowly compared with wild-type colonies (Fig. 4A), and only 30% (from 60 tetrad rads) of Δarc40 spores were able to form colonies. The mutant cells exhibit highly aberrant morphology and size heterogene-
ity (data not shown). Rhodamine-phalloidin staining of the mutant cells showed disruption of cortical actin patches and accumulation of aberrant actin cable-like structures (Fig. 4B). These phenotypes are entirely consistent with those of other Arp2/3 subunit null mutants (10, 28) as well as with the disruption of \(\text{BEE1}\) encoding the only WASP-like protein in yeast (25). Images from thin section electron microscopy revealed that \(\text{H9004/arc40}\) cells accumulated a large number (74 ± 12/section) of post-Golgi vesicles in the bud, whereas only a few (5 ± 1/section) vesicles were observed in wild-type cell sections (Fig. 4C). A similar vesicle accumulation phenotype was also observed in \(\text{bee1}\) cells (25). These results suggest that Arc40 functions exclusively in the Arp2/3 complex and further confirm that disruption of cortical actin patches affects a late step of exocytosis.

A Partial Arp2/3 Complex Is Present in \(\text{arc40}\) Cells and Has Reduced Affinity with VCA—Since we were able to recover viable \(\text{arc40}\) cells, we examined the effect of this mutation on the integrity of the Arp2/3 complex. Yeast extracts prepared from the wild-type or \(\text{arc40}\) strain were analyzed by gel filtration chromatography. Fractions were resolved by SDS-PAGE, transferred, and blotted with antibodies against Arp2, Arp3, \(\text{ARPC3/Arc18}\), and \(\text{ARPC5/Arc15}\). In wild-type extracts, these subunits were mainly present in the complex fraction and were undetectable in the monomer fractions (Fig. 5A). In \(\text{H9004/arc40}\) extracts, Arp3, Arc15, and Arc18 were still mainly present in the complex fractions, but 60% of Arp2 shifted into the monomer fractions, and 40% remained in the complex fractions (Fig. 5B). Since ARPC2/Arc35 and \(\text{ARPC4/Arc19}\) are core subunits (6), these must be present in the complex in \(\text{H9004/arc40}\) extracts for assembly of any subcomplex. Therefore, this result suggests that an Arp2/3 complex lacking only Arc40 exists in the mutant extract, although the affinity of Arp2 with the complex is reduced.

Because Arc40 alone binds to VCA with an affinity close to that between VCA and the purified Arp2/3 complex, we tested whether loss of Arc40 had any effect on the latter interaction. Equal amounts of high speed extracts from wild-type and \(\text{arc40}\) cells were incubated with VCA-coated Affi-Gel beads.
Bound and free Arp2/3 complexes were resolved by SDS-PAGE and blotted with antibodies against Arp3. Fig. 6A shows that loss of Arc40 strongly reduced the affinity of VCA with the Arp2/3 complex. Furthermore little Arp2 in Δarc40 extract was bound to VCA beads, suggesting that Arp2 itself does not bind VCA (Fig. 6B). To further rule out that the reduction in VCA affinity observed in Δarc40 extract was due to partial loss of Arp2, we also tested Δarp2 extracts in the above experiment. The lack of Arp2 had only a slight effect on the interaction between Arp2/3 complex with VCA (Fig. 6A), suggesting that Arp2 does not contribute significantly to the binding affinity of Arp2/3 complex with VCA. Previous cross-linking results also implicated Arp3 in Arp2/3 interaction with VCA (7, 8); however, the GST-VCA beads exhibited the same level of interaction with the Arp2/3 complex in Δarp3 extracts as that in wild-type extracts (Fig. 6C). These results strongly suggest that ARPC1/Arc40 mediates a major interaction between the Arp2/3 complex and VCA.

**Arc40 Is Required for VCA-stimulated Actin Nucleation—** Previous analysis of VCA from different WASP-like proteins and VCA mutants suggested that the ability of VCA to activate the Arp2/3 complex does not strictly correlate with the binding affinity between the complex and VCA (26, 29). Therefore, although Arc40 contributes significantly to the interaction with the Arp2/3 complex, it was unclear whether Arc40 is required for the nucleation activity of the Arp2/3 complex. However, because Δarc40 cells grew extremely poorly, we were not able to obtain enough cells to purify the Arc40 null complex. To circumvent this problem, we developed a strategy to assay Arp2/3 complex activity in crude yeast extracts. In this assay, resolubilized and dialyzed 55% ammonium sulfate precipitates of high speed supernatants were used in the pyrene-actin polymerization assay (see “Experimental Procedures”). In the absence of VCA, the extract exhibited no stimulation of actin polymerization, whereas in the presence of VCA, actin nucleation was strongly stimulated as evidenced by the shortened lag phase and increased rate during the polymerization phase (Fig. 7). This nucleation activity showed dose dependence on extracts and VCA as expected for Arp2/3-based actin nucleation (Fig. 7, A–D).

Using this assay, we compared the activity of extracts prepared from a wild-type and a Δarc40 strain. Both extracts had the same concentration of Arp2/3 complex as determined by immunoblot analysis (Fig. 7E, inset). In contrast to wild-type extracts, extracts prepared from the mutant strain exhibited no nucleation activity at a wide range of extract concentrations (Fig. 7E). To determine whether the lack of activity in the Δarc40 extract was due to lack of Arc40 as opposed to indirect effects from unhealthy Δarc40 cells, we added back baculovirus-expressed His6-HA-Arc40 to the Δarc40 extract. The actin nucleation activity was significantly restored to the Δarc40 extract in a His6-HA-Arc40 concentration-dependent manner (Fig. 7F). This result suggests that Arc40 is required for the VCA-stimulated actin nucleation activity of the Arp2/3 complex.

**DISCUSSION**

Previously it was unclear whether Arc40 was a necessary component of the active Arp2/3 complex and whether the function of Arc40 exists beyond that of the Arp2/3 complex. Here we first showed by mass spectrometry and gel filtration that Arc40 is indeed a component of purified yeast Arp2/3 complex but has a strong tendency to form high molecular weight species when analyzed by SDS-PAGE. Attempts to reduce this tendency, including addition of fresh 0.1 M dithiothreitol or 10% β-mercaptoethanol to SDS-PAGE sample buffer and low temperature (37 °C)
heat denaturation, were unsuccessful. However, treatment with 6 M urea prior to the addition of sample buffer could enrich the fraction of Arc40 that ran with expected mobility (data not shown). One possible cause for the formation of the high molecular weight species may be the amino acid composition and structural characteristics of Arc40. The budding yeast Arc40 is composed of 384 amino acids, a large portion of which are hydrophobic (grand average of hydropathicity (GRAVY) = −0.221). The crystal structure of bovine ARPC1 (30) revealed a seven-blade β-propeller composed of WD40 repeats. The top face of the β-propeller is normally in contact with the rest of the complex. It is possible that when the complex is denatured in the presence of SDS, the extensive β-sheets and high hydrophobicity led to formation of the aggregates.

In addition to confirming that ARPC1/Arc40 is a component of the Arp2/3 complex, our results also suggest that this subunit contains a major contact site between the Arp2/3 complex and VCA. Purified Arc40 bound to the activator VCA with a $K_d$ of 0.45 μM, close to that of the yeast Arp2/3 complex with VCA (0.30 μM). The latter was similar to the affinity ($K_d = 0.9$ μM) measured for the bovine Arp2/3 with human WASP-VCA by fluorescence anisotropy (26) and the affinity ($K_d = 0.76$ μM) for the bovine Arp2/3 complex with human N-WASP-VCA by pull-down assays (8). Loss of Arc40 from the Arp2/3 complex resulted in a 5–10-fold reduction of the affinity with VCA, and
FIG. 7. **Arc40 is required for the actin nucleation activity of the Arp2/3 complex in yeast extracts.** A, pyrene-actin polymerization in the presence of varying concentrations (as indicated next to the graphs) of yeast extracts. The reaction mixture contained a final concentration of 1.5 μM G-actin and 250 nM VCA. B, barbed end concentration was calculated from the data in A using the equation ([barbed ends] = (elongation rate)/([actin monomers]) - k-). The elongation rate was taken at 50% of maximum polymerization. The concentration of Arp2/3 complex present in the extract was determined by Western blot in comparison with known concentrations of purified complex.

C, pyrene-actin polymerization in the presence of varying concentrations (as indicated next to the graphs) of VCA. The reaction mixture contained a final concentration of 1.5 μM G-actin and 250 ng/μl extract (total protein). D, barbed end concentration was calculated from the data in C as described in B and plotted against VCA concentrations.

E, various amounts of extracts from wild-type (WT) and Δarc40 strains (Δ40) were used for assaying Arp2/3-mediated actin nucleation (as indicated next to the graphs). The reaction mixture contained a final concentration of 1.5 μM G-actin and 250 nM VCA. *Inset*, the same amounts of extracts from wild-type (WT) and Δarc40 (Δ40) strain were analyzed by immunoblot analysis against Arp2 and Arp3.

F, pyrene-actin polymerization assay in the presence of Δarc40 extracts with increasing amounts of His6-HA-Arc40 (p40). The reaction mixture contained a final concentration of 1.5 μM G-actin, 250 nM VCA, and 250 ng/μl Δarc40 extract. The final concentrations of Arc40 added are indicated next to the graph. a.u., absorbance units; ext, extract.
this effect was not due to partial loss of Arp2 from the complex since Arp2 null complex had only slightly reduced binding to VCA.

A potential involvement of ARPC1 in activator interaction has been suggested by previous chemical cross-linking experiments, which showed that VCA can be cross-linked to ARPC1, Arp2, and Arp3 (7, 8). Interestingly one study concluded that the COOH-terminal conserved tryptophan of VCA is involved in binding to Arp2 because truncating the DDW motif from the VCA COOH terminus reduced cross-linking to Arp3 by 81% and to ARPC1 by 37% (8). However, our results showed that the conserved tryptophan is required for the interaction with purified ARPC1/Arc40. These results are not necessarily contradictory because cross-linking data do not quantitatively reflect affinity. This is exemplified by the findings that whereas both Arp3 and Arp2 cross-link strongly with VCA, loss of each of these subunits had little effect on the interaction of the Arp2/3 complex with VCA. If binding between the VCA tryptophan and Arc40 contributes strongly to the affinity between VCA and Arp2/3 complex, a loss of this interaction could have a large effect on low affinity contacts at adjacent sites. However, we tested whether an excess of recombinant Arc40 could inhibit the Arp2/3 activity in the extract, but we did not detect any effect. This is not too surprising since the interaction between the Arp2/3 complex and VCA is strongly enhanced upon F-actin binding (31), and hence Arc40 is not necessarily an effective inhibitor in the nucleation assay.

The recent homology modeling of Arp2/3 complex did not explore ARPC1 as a potential site for VCA binding (32). The recent homology modeling of Arp2/3 complex did not explore ARPC1 as a potential site for VCA binding (32). The recent homology modeling of Arp2/3 complex did not explore ARPC1 as a potential site for VCA binding (32). The recent homology modeling of Arp2/3 complex did not explore ARPC1 as a potential site for VCA binding (32).

Through analysis using a new ARC40 deletion construct, we showed that Arc40 not only contributes to binding the activator peptide but also is required for the actin nucleation activity of the Arp2/3 complex. The role for Arc40 in the nucleation process is probably not restricted to activator binding. Since ARPC1 can directly bind F-actin (data not shown) and VCA, this subunit is in a unique position to mediate the cooperativity between the interactions of Arp2/3 complex with F-actin and with the activator. Additionally our data suggested that Arc40 is required for tight association of Arp2 with the rest of the complex, and therefore Arc40 could also have a strong influence on the orientation and/or conformation of Arp2. A function in VCA binding and in tethering Arp2 to the complex could both explain a loss of actin nucleation in Δarc40 extract. Our result is also largely consistent with the results from reconstitution experiments using human Arp2/3 subunit expressed in insect cells (33). Partial complex was purified from a reconstitution mixture that lacked both ARPC1 and ARPC5. Interestingly stoichiometric Arp2 was present in this partial complex, suggesting that ARPC1 in the human complex is not as important for Arp2 association with the complex as it is in yeast. This partial complex also exhibited a severe defect in actin nucleation, although residual activity (5% of the wild-type) was observed. Together that and our study demonstrate a critical role for the ARPC1 subunit in Arp2/3 complex and VCA-mediated actin nucleation.

Phenotypically Δarc40 cells are indistinguishable from other subunit null cells except Δarc18, whose defects are less severe (10). This indicates that Arc40 functions entirely within the Arp2/3 complex unlike originally thought. The vesicle accumulation phenotype is intriguing. This was previously observed in Δbee1 cells, which also lack cortical actin patch components (25). The actin patches in yeast are thought to be sites of endocytosis as indicated by phenotypes of mutants affecting a large number of actin patch components (34). The exocytic defects in Δbee1 and Δarc40 mutants may suggest that endocytosis and exocytosis are directly linked in yeast or that endocytic recycling is required for continuous activity of the machinery required for the last steps of exocytosis.

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