Activation of the yeast Arp2/3 complex by Bee1p, a WASP-family protein

Dirk Winter*, Terry Lechler* and Rong Li

The Arp2/3 complex is a highly conserved cytoskeletal component that has been implicated in the nucleation of actin filament assembly [1]. Purified Arp2/3 complex has a low intrinsic actin nucleation activity, leading to the hypothesis that an unidentified cellular activator is required for the function of this complex [2,3]. We showed previously that mutations in the Arp2/3 complex and in Bee1p/Las17p, a member of the Wiskott–Aldrich syndrome protein (WASP) family, lead to a loss of cortical actin structures (patches) in yeast [4,5]. Bee1p has also been identified as an essential nucleation factor in the reconstitution of actin patches *in vitro* [6]. Recently, it was reported that WASP-like proteins might interact directly with the Arp2/3 complex through a conserved carboxy-terminal domain [7]. Here, we have shown that Bee1p and the Arp2/3 complex co-immunoprecipitate when expressed at endogenous levels, and that this interaction requires both the Arc15p and Arc19p subunits of the Arp2/3 complex. Furthermore, the carboxy-terminal domain of Bee1p greatly stimulated the nucleation activity of purified Arp2/3 complex *in vitro*, suggesting a direct role for WASP-family proteins in the activation of the Arp2/3 complex. Interestingly, deletion of the carboxy-terminal domain of Bee1p neither abolished the localization of the Arp2/3 complex, as had been suggested, nor resulted in a severe defect in cortical actin assembly. These results indicate that the function of Bee1p is not mediated entirely through its interaction with the Arp2/3 complex, and that factors redundant with Bee1p might exist to activate the nucleation activity of the Arp2/3 complex.

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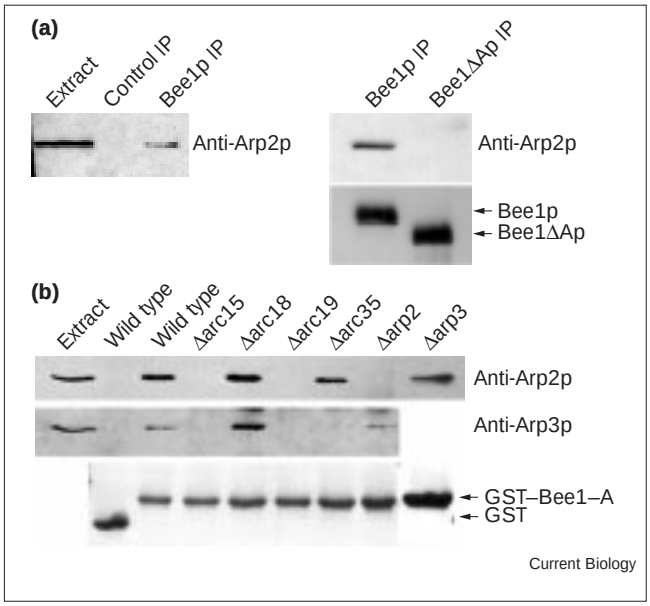
Figure 1



Physical interaction of the Arp2/3 complex with Bee1p. (a) Extracts were prepared from a wild-type strain (control), a strain expressing Bee1p tagged at its carboxyl terminus with the IgG-binding domain of protein A, and a strain expressing Bee1p lacking the carboxy-terminal acidic domain (Bee1ΔAp) tagged with protein A. After binding to IgG beads and washing with buffer, bound proteins were eluted with 0.5 M acetic acid, pH 3.6. These immunoprecipitated (IP) proteins and a pre-IP extract were subjected to SDS–PAGE, transferred to nitrocellulose and blotted with rabbit anti-Arp2p antibody [11]. Equal amounts of Bee1p and Bee1ΔAp were immunoprecipitated, as shown by western blot analysis. (b) Arc15p and Arc19p are required for Arp2/3 complex interaction with Bee1p. Extracts at 10 mg/ml were prepared from strains carrying null mutations in Arp2/3 complex subunits: *Δarc15*, *Δarc18*, *Δarc19*, *Δarc35*, *Δarp2* and *Δarp3*. These extracts were added to glutathione–agarose beads bound to either GST as a control, or GST–Bee1–A (GST fused to the carboxy-terminal 54 amino acids of Bee1p). Bound proteins were analyzed by western blotting using antibodies to the Arp2p and Arp3p subunits of the Arp2/3 complex ([11] and data not shown). Equal amounts of GST and GST–Bee1–A were present in each sample as indicated by protein staining.

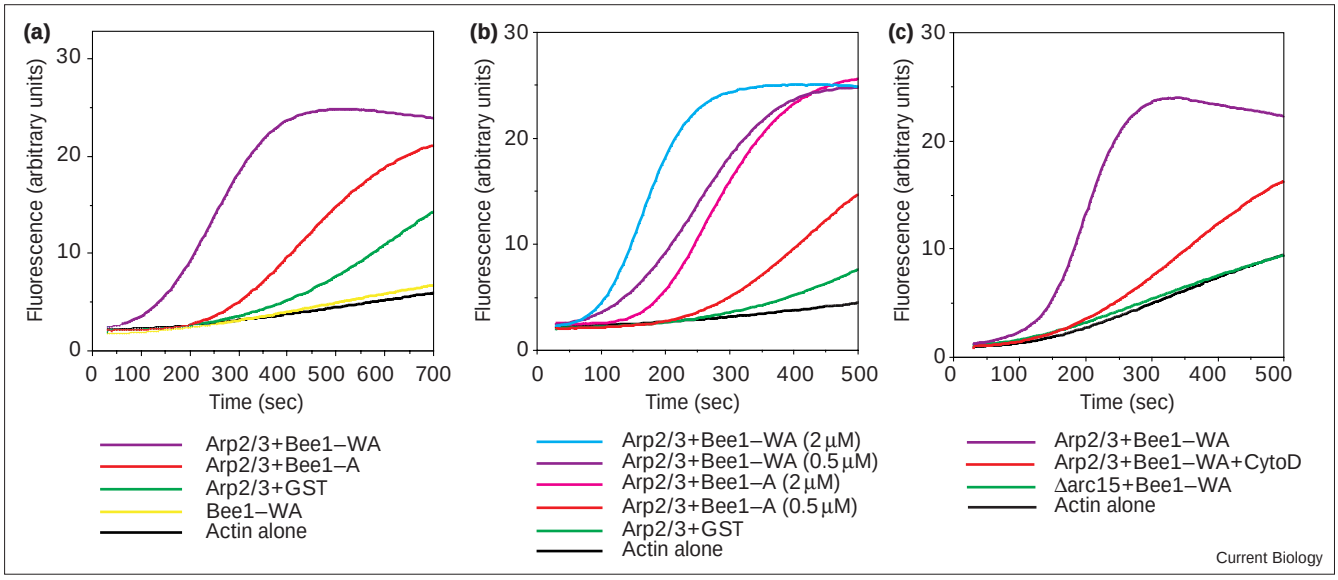
Results and discussion

Yeast actin patches are dynamic structures that provide a model for studying the control of actin polymerization at specific sites at the membrane cortex. Previous studies have identified two actin patch components crucial for the assembly of these structures: Bee1p, a yeast WASP-family protein, and the complex containing actin-related proteins 2 and 3 (Arp2/3), a multi-subunit protein complex that is conserved in eukaryotic cells. Disruption of the gene encoding Bee1p or a temperature-sensitive mutation in the Arp3p gene results in loss of the cortically associated actin patches [4,5]. These results suggest that Bee1p and the Arp2/3 complex function together in the generation of actin patches.

To determine whether Bee1p and the Arp2/3 complex interact physically, extracts were prepared from a yeast strain expressing Bee1p tagged at the carboxyl terminus with protein A under the control of the *BEE1* promoter. The tagged Bee1p complements the *BEE1* null mutation (data not shown). After IgG beads were incubated with the extract and washed with buffer, the bound proteins were analyzed by immunoblotting for the presence of Arp2p. This Arp2/3 complex subunit was detected in the immunoprecipitate of the Bee1–protein A extract but not in that of an extract from the untagged control strain (Figure 1a), or from a strain carrying a carboxy-terminal truncation of Bee1p (Bee1ΔAp) lacking the acidic domain, which has been shown to mediate the interaction of the WASP-like protein Scar1 with the Arp2/3 complex [7]. This shows that Bee1p and the Arp2/3 complex interact physically *in vivo*, and that the acidic domain is necessary for this association. The interaction is not stable, however, as Bee1p and the Arp2/3 complex did not co-fractionate on a gel filtration column (data not shown). To test whether the carboxy-terminal acidic region (Bee1–A) is sufficient for binding, Bee1–A was expressed as a glutathione-S-transferase (GST) fusion protein in *Escherichia coli* and coupled to glutathione beads (Figure 1b). These beads, but not the control GST beads, were able to pull down Arp2p and Arp3p from the wild-type yeast extract (Figure 1b). The GST–Bee1–A beads were also able to pull down Arp2p and Arp3p when mixed with purified Arp2/3 complex, indicating that the interaction is direct (see Supplementary materials published with this article on the internet).

To determine which subunit(s) of the Arp2/3 complex are required for the interaction with Bee1p, beads to which Bee1–A was coupled were incubated with extracts prepared from strains bearing gene disruptions affecting each of the Arp2/3 complex subunits (data not shown). Although it was previously reported that gene disruption of Arp2p or Arp3p genes is lethal, we have been able to obtain viable *Δarp2* and *Δarp3* strains by using the right strain background (S288c) and optimal growth conditions (data not shown). As shown in Figure 1b, there was no detectable interaction of Bee1–A with Arp2p or Arp3p in either the *Δarc15* or the *Δarc19* cell extracts, suggesting that Arc15p and Arc19p are required for the interaction of

Figure 2



Bee1p activates the nucleation activity of the Arp2/3 complex. **(a)** Stimulation of the nucleation activity of Arp2/3 complex by Bee1–WA and Bee1–A fragments: black, actin alone; yellow, 0.5 μM Bee1–WA; green, 0.25 μM Arp2/3 complex plus 0.5 μM GST; red, 0.25 μM Arp2/3 complex, 0.5 μM Bee1–A; purple, 0.25 μM Arp2/3 complex, 0.5 μM Bee1–WA. **(b)** Comparison of Bee1–WA and Bee1–A effects on the Arp2/3 complex actin nucleation activity: black, actin alone; green, 0.25 μM Arp2/3 complex plus 0.5 μM GST; red, 0.25 μM Arp2/3 complex plus 0.5 μM Bee1–A; magenta, 0.25 μM Arp2/3 complex plus 2 μM Bee1–A; purple, 0.25 μM Arp2/3 complex plus 0.5 μM Bee1–WA; cyan, 0.25 μM Arp2/3 complex plus 2 μM Bee1–WA. **(c)** Bee1–WA-stimulated Arp2/3 complex nucleation activity is inhibited by cytochalasin D and a partial complex missing Arp2p and Arc15p does not stimulate actin assembly. Black, actin alone; green, 0.25 μM Arp2p- and Arc15p-deficient complex plus 1 μM Bee1–WA; red, 0.25 μM Arp2/3 complex plus 1 μM Bee1–WA, 200 nM cytochalasin D (cytoD); purple, 0.25 μM Arp2/3 complex plus 1 μM Bee1–WA. All pyrene assays were performed in UBA buffer (50 μM Hepes pH 7.5, 100 μM KCl, 3 μM $MgCl_2$, 1 μM EGTA, 0.5 μM ATP) at 1.5 μM total actin, consisting of a 1:1 mix of yeast actin and 30% pyrene-labeled rabbit muscle actin. Yeast Arp2/3 complex and Arp2p/Arc15p-deficient complex were purified from wild-type and *Δarc15* cells expressing His_6–Myc_5-tagged Arp3p by Q-sepharose, ammonium sulfate precipitation and Ni^{2+} affinity steps (data not shown). GST–Bee1–WA is the carboxy-terminal 125 amino acids of Bee1p fused to the carboxyl terminus of GST.

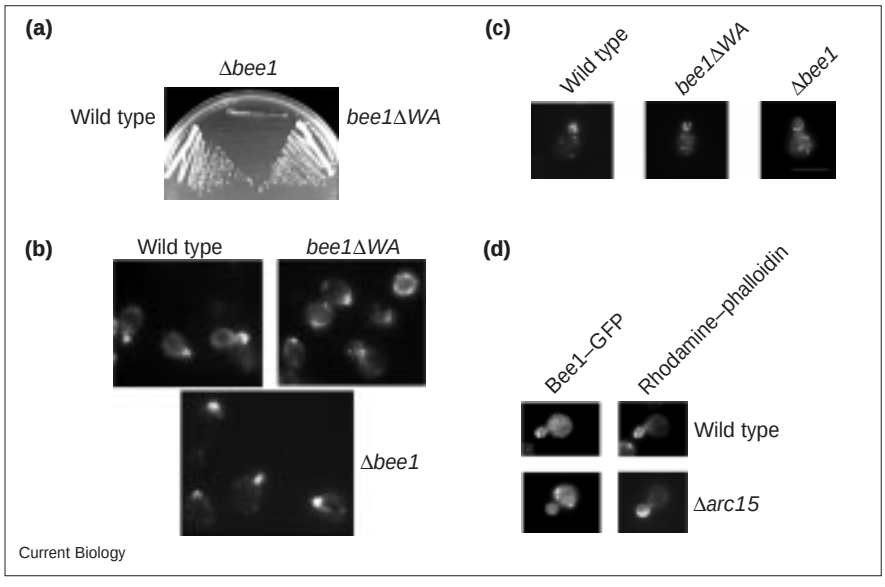
the Arp2/3 complex with Bee1–A. Yeast Arc19p is homologous to human Arc21, the subunit shown, by yeast two-hybrid analysis, to interact with WASP-family members [7]. A partial Arp2/3 complex that lacks Arc15p and Arp2p can be purified from the *Δarc15* extract (data not shown). Because this complex contains Arc19p, this suggests that Arc19p is not sufficient for the interaction with the carboxy-terminus of Bee1p. Arc18p is not required for the interaction with Bee1–A, as both Arp2p and Arp3p can be precipitated with the beads from the *Δarc18* extract (Figure 1b). In the *Δarc35* extract, only Arp2p (and not Arp3p) was found associated with Bee1p. Because *Δarc35* disrupts the Arp2/3 complex (data not shown), it is possible that Arp2p associates with Bee1p through complex formation with a small subunit, most likely Arc15p. In an extract prepared from *Δarp2* cells, Arp3p still bound to Bee1–A, and in *Δarp3* extracts, Arp2p still associated with Bee1–A. This indicates that Arp2p and Arp3p are not required for the interaction of the Arp2/3 complex with Bee1p. Taken together, the above results suggest that the Arc15p and Arc19p subunits mediate the interaction with Bee1–A. Because genetic and crosslinking data suggest that Arc15p is a more peripheral subunit than Arc19p (data not shown and [8]), Arc15p is more likely to physically contact Bee1–A. Alternatively, Bee1p might contact the surfaces of both proteins.

Purified Arp2/3 complex has a low intrinsic actin nucleation activity, which led to the hypothesis that it requires other cellular factors for activation. Because analyses of mutants had suggested that Bee1p and the Arp2/3 complex function synergistically in actin patch formation [4,5], we tested whether carboxy-terminal fragments of Bee1p could stimulate the nucleation activity of the Arp2/3 complex using the pyrene actin assembly assay [9]. Consistent with the published observations, Arp2/3 complex alone did not dramatically reduce the lag (nucleation) phase (Figure 2a) [2,3]. A Bee1 fragment containing the WH2 and acidic domains (Bee1–WA) by itself had little effect on the polymerization kinetics (Figure 2a), but when it was combined with the Arp2/3 complex, the lag phase for actin polymerization was reduced. Higher Bee1–WA levels led to a greater reduction in the lag phase (Figure 2a,b). The effect on actin polymerization by Bee1–WA and the Arp2/3 complex was blocked by cytochalasin D (Figure 2c), suggesting that barbed-end polymerization was stimulated, consistent with the model suggesting that the Arp2/3 complex nucleates polymerization toward the barbed-end [10].

The acidic region (Bee1–A) without the adjacent G-actin-binding domain is less potent than Bee1–WA in stimulating actin polymerization in the presence of the Arp2/3 complex (Figure 2a). At high concentrations, Bee1–A promotes a similar rate of polymerization as Bee1–WA, but retains a significant lag period (Figure 2b). The above results might be explained if the acidic domain of Bee1p can act as an allosteric activator of the Arp2/3 complex and the actin-binding domain facilitates actin nucleation by providing actin monomers in a polymerization-favorable conformation.

Because overexpression of the acidic region of Scar1 abolishes Arp2/3 complex localization in fibroblasts [7], we investigated whether the Bee1–Arp2/3 interaction was also required for the localization of the Arp2/3 complex. A mutant strain was generated that lacks both the

Figure 3



The Arp2/3-interacting domain of Bee1p is not required for Arp2/3 complex localization or Bee1p function. (a) Wild-type, *Δbee1*, and *bee1ΔWA* (encoding amino acids 1–348 of Bee1p, lacking the WH2 and acidic domains) strains were streaked onto a YPD plate and incubated at 30°C. (b) Rhodamine–phalloidin staining of wild-type, *Δbee1*, and *bee1ΔWA* cells to visualize F-actin. (c) Immunofluorescence localization of Arp2p in wild-type, *bee1ΔWA*, and *Δbee1* cells. Rabbit anti-Arp2p antibodies and donkey anti-rabbit FITC were used to localize endogenous Arp2p. Conditions for Arp2p immunofluorescence prevented staining with rhodamine–phalloidin. (d) Wild-type and *Δarc15* cells expressing Bee1–GFP were fixed with formaldehyde and immunostained with rabbit anti-GFP antibodies followed by donkey anti-rabbit FITC to visualize Bee1p. Cells were also stained with rhodamine–phalloidin to visualize F-actin. The scale bar represents 10 μm.

actin-binding and acidic regions of Bee1p (*bee1ΔWA*). The deletion mutation was confirmed both by restriction digest analysis and by western blotting using an antibody raised against the acidic region (data not shown). Surprisingly, these cells grew nearly as well as wild-type cells at 30°C (Figure 3a). Actin organization closely resembled that of wild-type cells, although there was an increase in the number of cells with depolarized actin or aberrant actin structures (Figure 3b). Immunofluorescence staining of Arp2p in these mutant cells showed that the complex was properly localized to actin patches (Figure 3c). Arp2p was also localized to cortical patches in over 90% of cells in a *Δbee1* strain (Figure 3c), suggesting that Bee1p is not required for the cortical association of the Arp2/3 complex. Arp2p is unlikely to co-localize with actin in this strain, as fewer than 15% of these cells have actin patches. Conversely, a Bee1–GFP construct expressed in the *Δarc15* strain was found to localize to cortical patches, but these patches were not concentrated in the bud and no longer co-localized with actin as in wild-type cells (Figure 3d). Although 85% of Bee1p patches co-stain with actin in wild-type cells, only 9% of Bee1p patches contained actin in *Δarc15* cells (n = 40 cells). These results indicate that Bee1p and the Arp2/3 complex are unlikely to depend on their interaction with each other for localization to cortical patches. Significantly, neither the Bee1p patches in the *Δarc15* cells nor the Arp2p patches in the *Δbee1* strain co-localized with actin as in wild-type cells (Figure 3b–d), suggesting that Bee1p and the Arp2/3 complex depend on each other to assemble actin filaments in these cortical patches.

The results presented in this paper show that the carboxy-terminal WA domain of Bee1p interacts directly with the Arp2/3 complex, and that this interaction can stimulate actin nucleation. Activating the Arp2/3 complex nucleation activity might be a conserved function of WASP-family proteins, given that the characteristic Arp2/3-complex-interacting carboxy-terminal region is conserved among them. This finding supports our previous hypothesis that Bee1p and the Arp2/3 complex act synergistically in the assembly of cortical actin filaments [4]. It is perplexing, however, that deletion of the Arp2/3-interacting domain of Bee1p, as opposed to deletion of the entire *BEE1* gene, causes little cellular defect. This suggests that Bee1p does not function solely through its interaction with the Arp2/3 complex. The lack of phenotype associated with *bee1ΔWA* also suggests that other, as yet unidentified, cellular factors act redundantly with Bee1p to activate the Arp2/3 complex.

Supplementary material

A supplementary figure showing a direct interaction between the acidic domain of Bee1p and the Arp2/3 complex is published with this paper on the internet.

Acknowledgements

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Supplementary material

Activation of the yeast Arp2/3 complex by Bee1p, a WASP-family protein

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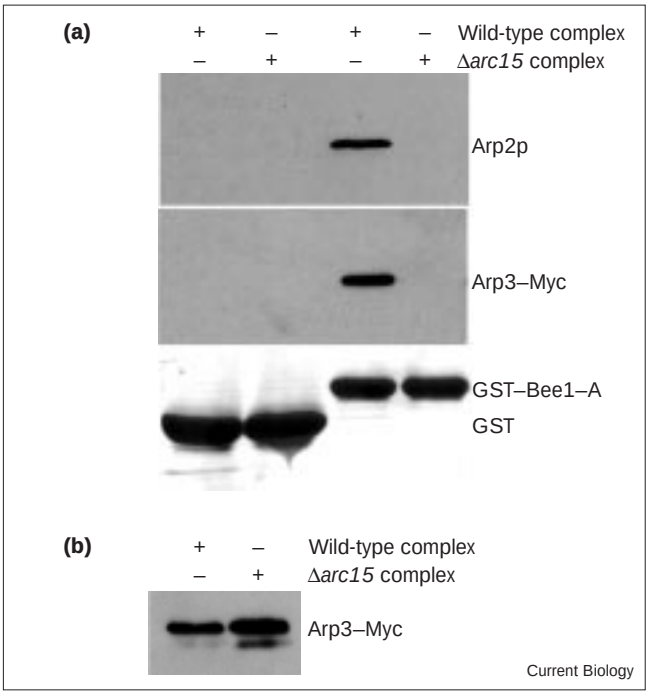
Figure S1



The Arp2/3 complex binds directly to the carboxy-terminal acidic domain of Bee1p. (a) GST–Bee1–A and GST (control) were bound to glutathione–agarose beads, then mixed with 50 nM Arp2/3 complex purified from wild-type or *Δarc15* strains. Bound proteins were analyzed by western blotting using anti-Arp2p and anti-myc (Arp3–Myc) antibodies. (b) Equivalent amounts of Arp2/3 complex purified from wild-type and *Δarc15* strains.