Molecular Dissection of *ARP1* Regions Required for Nuclear Migration and Cell Wall Integrity Checkpoint Functions in *Saccharomyces cerevisiae*

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ABSTRACT. The dynactin complex is one of the components required for the regulation of the cell wall integrity checkpoint, which ensures the completion of cell wall remodeling before mitosis. The core of the dynactin complex is a backbone filament composed of monomers of an actin-related protein, Arp1, which is also involved in nuclear migration. To examine the molecular basis for the dual functions of the dynactin core subunit Arp1p in yeast, we constructed 32 mutated *arp1* alleles. We assessed the effects of the mutations on cell wall integrity checkpoint and nuclear migration functions and identified four categories of mutants: 1) those showing no change from the wild type; 2) those resulting in a defective cell wall integrity checkpoint but normal nuclear migration; 3) those with a normal cell wall integrity checkpoint but defective nuclear migration; and 4) those defective in both the cell wall integrity checkpoint and nuclear migration functions. Our results show a separation of the two functions in the molecular structure of Arp1p and indicate that a local surface region of Arp1p is important in maintaining the cell wall integrity checkpoint function.

Key words: cell wall integrity checkpoint/dynactin complex/*ARP1*/alanine scanning/nuclear migration/1,3- β -glucan synthase

Introduction

Cell cycle progression is regulated by several checkpoint mechanisms that monitor cell cycle landmarks. When a cell cycle event is perturbed, progression of the cell cycle to subsequent events is halted until the completion of the former event. Well-known checkpoint targets are DNA damage, DNA replication (Weinert *et al.*, 1994), kinetochore attachment to the mitotic spindle (Rudner and Murray, 1996), and bud morphogenesis (Lew and Reed, 1995).

We recently discovered a new cell cycle checkpoint, termed the cell wall integrity checkpoint (Suzuki *et al.*, 2004). In response to a defect in cell wall synthesis, cells of the yeast *Saccharomyces cerevisiae* arrest cell growth at the small bud before entry into mitosis. The cell wall of *S. cerevisiae* consists predominantly of 1,3- β -glucan (Cid *et al.*, 1995). Mutants defective in 1,3- β -glucan synthesis cannot form the medium or large bud, and these mutants

arrest the cell cycle progression before nuclear division without forming a bipolar spindle (Suzuki *et al.*, 2004). This observation indicates that the perturbation of 1,3- β -glucan synthesis triggers the cell wall integrity checkpoint, leading to cell cycle arrest before the separation of the spindle pole body (SPB). An analysis of the mutant defective in the cell wall integrity checkpoint, *wac1* (wall checkpoint defective), revealed that this mutant accumulates cells with a bipolar spindle after 1,3- β -glucan synthesis has stopped (Suzuki *et al.*, 2004). *WAC1* is identical to *ARP1*, and the *wac1* product possesses a single amino acid replacement (P268L). These findings suggest that Arp1p (Wac1p) is required to arrest the cell cycle at G2 phase when cell wall synthesis is perturbed, but little is known about how Arp1p actually functions in the cell wall integrity checkpoint.

Biochemical and microscopic studies have revealed that Arp1p is the core subunit of the dynactin complex. Nine or 10 Arp1p monomers form the actin-like short filament backbone of the dynactin complex, as well as multiple species of subunits assemble along this filament (Eckley *et al.*, 1999; Schafer *et al.*, 1994). The dynactin complex of the budding yeast contains at least three species of polypeptides: Arp1p, Nip100p, and Jnm1p (Geiser *et al.*, 1997; Kahana *et al.*, 1998; McMillan and Tatchell, 1994). In addition to these proteins, comprehensive two-hybrid screening

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has isolated three Arp1p binding proteins: Arp10p, Jsn1p, and Yjr008wp (Ito *et al.*, 2001; Uetz *et al.*, 2000). Genetic studies have shown that the dynactin complex is required for the nuclear migration process after anaphase. The deletion of the *ARP1* gene causes the defect in dynein-mediated nuclear migration, and cells with two nuclei in the mother cell are frequently observed (Muhua *et al.*, 1994). The dynactin complex in *S. cerevisiae* is proposed to associate with the cytoplasmic dynein motor, resulting in the pulling force to promote nuclear migration (Hildebrandt and Hoyt, 2000). In addition, components of the dynactin complex, namely Arp1p, Nip100p, and Jnm1p, are required for the cell wall integrity checkpoint. Surprisingly, the dynactin-related proteins Dyn1p, Pac11p, Num1p, Pac1p, and Bik1p are not required for the checkpoint (Suzuki *et al.*, 2004). Thus Arp1p functions in the cell wall integrity checkpoint as well as in nuclear migration, although the relationship between these two functions remains unclear.

In this study, we used a genetic approach to determine whether the cell wall integrity checkpoint and nuclear migration functions of Arp1p are based on independent molecular functions. We constructed 32 mutated *arp1* alleles with alanine mutations at the clusters of charged amino acids that are predicted to be on the surface of the Arp1p ultrastructure. We then examined the effects of the mutations on the cell wall integrity checkpoint and nuclear migration functions of Arp1p in yeast cells.

Materials and Methods

Strains and plasmids

Standard molecular biological techniques for yeast and bacteria were used (Sambrook *et al.*, 1989; Sherman *et al.*, 1986).

The yeast strains used in this study are listed in Table I. All strains are derivatives of YPH499 and YPH500 (Sikorski and Hieter, 1989). The *FKS1* or *fks1-1154* mutant strains used in this study harbored an *FKS1* or *fks1-1154* temperature-sensitive allele integrated into the $\Delta fks1 \Delta fks2$ background, in order to avoid the effect of Fks2p that has overlapping functions with Fks1p. The *FKS1 wac1* (YOC2857) and *fks1-1154 wac1* (YOC2858) strains have the *wac1* mutation (*arp1*^{P268L}) in *ARP1* of the *FKS1* (YOC1001) and *fks1-1154* (YOC1087) strains, respectively.

The genomic *ARP1* was replaced with the gene *Cg-LEU2* (*Candida glabrata* *LEU2* gene compatible with *Saccharomyces cerevisiae* *LEU2*) in strains *FKS1* and *fks1-1154* to generate the *FKS1 Δ arp1* (YOC2888) and *fks1-1154 Δ arp1* (YOC2889) strains, respectively.

The *arp1-x::URA3* strains (YOC3032-3099) were constructed by replacing the *Δ arp1::LEU2* locus of the *FKS1 Δ arp1* (YOC2888) and *fks1-1154 Δ arp1* (YOC2889) strains with the *HindIII-XbaI* fragment containing *arp1-x::URA3* prepared from the pBS-arp1-x plasmid. In the *wac1::URA3* strains, the genomic *Δ arp1::LEU2* region was replaced with a *HindIII-DraI* fragment, because *wac1*

was digested with *XbaI*. The integrants were selected on SD-uracil plates, and the correct integrations were verified by PCR.

The YOC4106-4112 were constructed by transforming the plasmid pYO2763, centromeric vector containing *KAR9* and *URA3*, into *FKS1* (YOC1001), *FKS1 wac1* (YOC2857), *FKS1 Δ arp1::KanMX* (YOC4113), *FKS arp1-31* (YOC3035), *FKS1 arp1-236* (YOC3052), *FKS1 arp1-266* (YOC3056) and *FKS1 arp1-374* (YOC3065) strains (*URA3* marker of genomic YOC3035, 3052, 3056 and 3065 were deleted). The genomic *KAR9* gene of the resultant strains was then replaced with the *Cg-LEU2* marker.

The plasmids used in this study are listed in Table II. The plasmid pRS315-ARP1 (pYO2521) contains the 2.1-kb *HindIII-XbaI* fragment, including the entire ORF of *ARP1*, inserted into pRS315. For pBS-ARP1-URA3 (pYO2522), the 2.1-kb *HindIII-XbaI* fragment, including the entire ORF of *ARP1*, was prepared from pRS315-ARP1 and was inserted into pBluescript II SK(+). The yeast *URA3* fragment amplified from pRS316 by PCR was digested with *SmaI* and was inserted at the blunt-ended *EcoT22I* site located 200 bp upstream from the *XbaI* site.

For pBS-wac1-URA3 (pYO2523), a fragment including the *wac1* allele was amplified from the *FKS1 wac1* genome by PCR, digested with *HindIII* and *DraI*, and used to replace the *HindIII-DraI* region of pBS-ARP1-URA3. Positive clones were selected based on *XbaI* digestion, because an additional *XbaI* site was generated by the *wac1* mutation.

To construct pBS-arp1-x-URA3 (pYO2524-pYO2555), the *NruI-SphI* fragment of pBS-ARP1-URA3 (or, in the case of *arp1-294* to *-374*, the *SphI-EcoT22I* fragment) was replaced with *EGFP*. Next, the *EGFP* fragment inserted in pBS-ARP1-URA3 was replaced with the *NruI-SphI* fragment (or, in the case of *arp1-294-arp1-374*, the *SphI-EcoT22I* fragment), which included a part of the ORF of *arp1-x* containing an alanine mutation, prepared from mutated pRS315-ARP1. Positive clones were selected based on plasmid digestion with *HindIII* and *Bsp1407I*.

Site-directed mutagenesis

A QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) was used to make point mutations. We used 32 synthetic oligonucleotides to change selected charged amino acids in yeast *ARP1* to alanine. The designed mutations were placed in the middle of each oligonucleotide, with 15–20 bases of correct sequence in each flanking region. Using pRS315-ARP1, which included intact *ARP1* as a template, the mutagenic oligonucleotide primers complementary to opposite strands of the plasmid were extended by PCR. Following the PCR, the products were treated with *DpnI* (target sequence: 5'-Gm⁶ATC-3'), which is specific for methylated and hemimethylated DNA, to digest the parental DNA template and to select for the mutation-containing synthesized DNA. Template plasmid isolated from *Escherichia coli* is methylated and therefore susceptible to *DpnI* digestion. The nicked plasmids incorporating the desired mutation were then transformed into *E. coli* XL-1-Blue, and the mutations were confirmed by DNA sequencing.

Table I. YEAST STRAINS USED IN THIS STUDY

Strains	Genotype
YOC1001 (<i>FKS1</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1</i>
YOC2857 (<i>FKS1 wac1</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, wac1</i>
YOC2888 (<i>FKS1 Δarp1</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::Cg-LEU2*</i>
YOC4113 (<i>FKS1 Δarp1::KanMX</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::KanMX</i>
YOC3032 (<i>FKS1 ARP1:URA3</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::ARP1:URA3</i>
YOC3033 (<i>FKS1 wac1:URA3</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::wac1:URA3</i>
YOC3034 (<i>FKS1 arp1-2</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-2:URA3</i>
YOC3035 (<i>FKS1 arp1-31</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-31:URA3</i>
YOC3036 (<i>FKS1 arp1-35</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-35:URA3</i>
YOC3037 (<i>FKS1 arp1-46</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-46:URA3</i>
YOC3038 (<i>FKS1 arp1-67</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-67:URA3</i>
YOC3039 (<i>FKS1 arp1-73</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-73:URA3</i>
YOC3040 (<i>FKS1 arp1-79</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-79:URA3</i>
YOC3041 (<i>FKS1 arp1-84</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-84:URA3</i>
YOC3042 (<i>FKS1 arp1-108</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-108:URA3</i>
YOC3043 (<i>FKS1 arp1-122</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-122:URA3</i>
YOC3044 (<i>FKS1 arp1-133</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-133:URA3</i>
YOC3045 (<i>FKS1 arp1-162</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-162:URA3</i>
YOC3046 (<i>FKS1 arp1-185</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-185:URA3</i>
YOC3047 (<i>FKS1 arp1-192</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-192:URA3</i>
YOC3048 (<i>FKS1 arp1-202</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-202:URA3</i>
YOC3049 (<i>FKS1 arp1-214</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-214:URA3</i>
YOC3050 (<i>FKS1 arp1-222</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-222:URA3</i>
YOC3051 (<i>FKS1 arp1-233</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-233:URA3</i>
YOC3052 (<i>FKS1 arp1-236</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-236:URA3</i>
YOC3053 (<i>FKS1 arp1-251</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-251:URA3</i>
YOC3054 (<i>FKS1 arp1-256</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-256:URA3</i>
YOC3055 (<i>FKS1 arp1-263</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-263:URA3</i>
YOC3056 (<i>FKS1 arp1-266</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-266:URA3</i>
YOC3057 (<i>FKS1 arp1-282</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-282:URA3</i>
YOC3058 (<i>FKS1 arp1-294</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-294:URA3</i>
YOC3059 (<i>FKS1 arp1-300</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-300:URA3</i>
YOC3060 (<i>FKS1 arp1-317</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-317:URA3</i>
YOC3061 (<i>FKS1 arp1-326</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-326:URA3</i>
YOC3062 (<i>FKS1 arp1-336</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-336:URA3</i>
YOC3063 (<i>FKS1 arp1-344</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-344:URA3</i>
YOC3064 (<i>FKS1 arp1-368</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-368:URA3</i>
YOC3065 (<i>FKS1 arp1-374</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-374:URA3</i>
YOC1087 (<i>fks1-1154</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1</i>
YOC2858 (<i>fks1-1154 wac1</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, wac1</i>
YOC2889 (<i>fks1-1154 Δarp1</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::Cg-LEU2</i>
YOC3066 (<i>fks1-1154 ARP1:URA</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::ARP1:URA3</i>
YOC3067 (<i>fks1-1154 wac1:URA</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::wac1:URA3</i>
YOC3068 (<i>fks1-1154 arp1-2</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-2:URA3</i>
YOC3069 (<i>fks1-1154 arp1-31</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-31:URA3</i>
YOC3070 (<i>fks1-1154 arp1-35</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-35:URA3</i>
YOC3071 (<i>fks1-1154 arp1-46</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-46:URA3</i>
YOC3072 (<i>fks1-1154 arp1-67</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-67:URA3</i>
YOC3073 (<i>fks1-1154 arp1-73</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-73:URA3</i>
YOC3074 (<i>fks1-1154 arp1-79</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-79:URA3</i>
YOC3075 (<i>fks1-1154 arp1-84</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-84:URA3</i>
YOC3076 (<i>fks1-1154 arp1-108</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-108:URA3</i>
YOC3077 (<i>fks1-1154 arp1-122</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-122:URA3</i>
YOC3078 (<i>fks1-1154 arp1-133</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-133:URA3</i>

Table I. YEAST STRAINS USED IN THIS STUDY (CONTINUE)

Strains	Genotype
YOC3079 (<i>fks1-1154 arp1-162</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-162:URA3</i>
YOC3080 (<i>fks1-1154 arp1-185</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-185:URA3</i>
YOC3081 (<i>fks1-1154 arp1-192</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-192:URA3</i>
YOC3082 (<i>fks1-1154 arp1-202</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-202:URA3</i>
YOC3083 (<i>fks1-1154 arp1-214</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-214:URA3</i>
YOC3084 (<i>fks1-1154 arp1-222</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-222:URA3</i>
YOC3085 (<i>fks1-1154 arp1-233</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-233:URA3</i>
YOC3086 (<i>fks1-1154 arp1-236</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-236:URA3</i>
YOC3087 (<i>fks1-1154 arp1-251</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-251:URA3</i>
YOC3088 (<i>fks1-1154 arp1-256</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-256:URA3</i>
YOC3089 (<i>fks1-1154 arp1-263</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-263:URA3</i>
YOC3090 (<i>fks1-1154 arp1-266</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-266:URA3</i>
YOC3091 (<i>fks1-1154 arp1-282</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-282:URA3</i>
YOC3092 (<i>fks1-1154 arp1-294</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-294:URA3</i>
YOC3093 (<i>fks1-1154 arp1-300</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-300:URA3</i>
YOC3094 (<i>fks1-1154 arp1-317</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-317:URA3</i>
YOC3095 (<i>fks1-1154 arp1-326</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-326:URA3</i>
YOC3096 (<i>fks1-1154 arp1-336</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-336:URA3</i>
YOC3097 (<i>fks1-1154 arp1-344</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-344:URA3</i>
YOC3098 (<i>fks1-1154 arp1-368</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-368:URA3</i>
YOC3099 (<i>fks1-1154 arp1-374</i>)	<i>MATa fks1::HIS3, fks2::LYS2, ade3::fks1-1154:TRP1, arp1::arp1-374:URA3</i>
YOC4106	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1 kar9::Cg-LEU2 [pYO2763]</i>
YOC4107	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, wac1, kar9::Cg-LEU2 [pYO2763]</i>
YOC4108	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::KanMX, kar9::Cg-LEU2 [pYO2763]</i>
YOC4109	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-31:URA3, kar9::Cg-LEU2 [pYO2763]</i>
YOC4110	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-236:URA3, kar9::Cg-LEU2 [pYO2763]</i>
YOC4111	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-266:URA3, kar9::Cg-LEU2 [pYO2763]</i>
YOC4112	<i>MATa fks1::HIS3, fks2::LYS2, ade3::FKS1:TRP1, arp1::arp1-374:URA3, kar9::Cg-LEU2 [pYO2763]</i>

* *Cg-LEU2* indicates *Candida glabrata LEU2* gene.

Examination of the cell wall integrity checkpoint defect

Cells were cultured to a density of 10^7 cells/ml at 25°C in YPD medium, and 100 ml of cells were centrifuged and resuspended in 10 ml of aqueous medium. The cells were sonicated and loaded into an elutriator (CR20E centrifuge with R5E rotor, Hitachi, Tokyo, Japan). Centrifugation was carried out at $870 \times g$ at 4°C. The flow rate was adjusted to 10 ml/min and was shifted to 12 ml/min before collecting 200 ml of G1 cells to be used for synchronized culture. The collected cells were concentrated by centrifugation, resuspended in fresh medium, and incubated at the restrictive temperature (37°C). Cells were collected every 30 min and fixed with 3.7% formaldehyde. The fixed cells were washed three times with SP buffer (0.5 mg/ml BSA, 150 mM NaCl, 50 mM HEPES, 0.1% Tween-20, and 1 mM NaN_3 at pH 7.5) and treated with 28 $\mu\text{g}/\text{ml}$ zymolyase 100T (Seikagaku Corporation, Tokyo, Japan), for 30 min at 30°C. The cells were washed with SP buffer and incubated in SP buffer containing 0.1% Triton X-100 for 10 min at room temperature. The cells were then washed twice and suspended in an appropriate volume of SP buffer. To visualize spindles, the cells were spread on a poly-L-lysine-coated slide and stained with rat monoclonal anti-yeast-tubulin antibody (1/34 YOL;

Oxford Biotechnology, Oxford, UK) and Alexa488-conjugated goat-anti-rat antibody (Molecular Probes, Eugene, OR) as first and second antibodies, respectively. After the antibody treatment, the cells were mounted in mounting buffer (90% glycerol containing 1 mg/ml *p*-phenylenediamine) containing 200 ng/ml DAPI, overlaid with a coverslip, and sealed. The stained cells were observed and photographed with a fluorescence microscope (DMRE, Leica, Bannockburn, IL) using a 100 $\times$ objective. At least 200 cells were counted.

Examination of the nuclear migration defect

Cells were cultured to mid-log phase in YPD medium at 25°C. Synchronized G1 cells prepared by centrifugal elutriation were incubated in YPD medium at 37°C for 4 h; cells were collected every 30 min and fixed in 3.7% formaldehyde. The fixed cells were stained with DAPI and observed with a DMRE microscope (Leica). Only cells that had completed nuclear division were counted, and the percentage of cells with two nuclei in the mother cell after the first cell cycle was determined. At least 200 cells were counted.

Table II. PLASMIDS USED IN THIS STUDY

Name	Description
pYO2521 (pRS315-ARP1)	<i>LEU2, ARP1, CEN</i>
pRS316	<i>URA3, CEN</i>
pBluescript II SK(+)	Used for constructing plasmids for integration
pEGFP	<i>EGFP</i>
pBS-Cg-LEU2	<i>Candida glabrata LEU2</i>
pYO2522 (pBS-ARP1-URA3)	Used for integrating <i>ARP1:URA3</i> in the <i>ARP1</i> locus
pYO2523 (pBS-wac1-URA3)	Used for integrating <i>wac1:URA3</i> in the <i>ARP1</i> locus
pYO2524 (pBS-arp1-2-URA3)	Used for integrating <i>arp1-2:URA3</i> in the <i>ARP1</i> locus
pYO2526 (pBS-arp1-31-URA3)	Used for integrating <i>arp1-31:URA3</i> in the <i>ARP1</i> locus
pYO2526 (pBS-arp1-35-URA3)	Used for integrating <i>arp1-35:URA3</i> in the <i>ARP1</i> locus
pYO2527 (pBS-arp1-46-URA3)	Used for integrating <i>arp1-46:URA3</i> in the <i>ARP1</i> locus
pYO2528 (pBS-arp1-67-URA3)	Used for integrating <i>arp1-67:URA3</i> in the <i>ARP1</i> locus
pYO2529 (pBS-arp1-73-URA3)	Used for integrating <i>arp1-73:URA3</i> in the <i>ARP1</i> locus
pYO2530 (pBS-arp1-79-URA3)	Used for integrating <i>arp1-79:URA3</i> in the <i>ARP1</i> locus
pYO2531 (pBS-arp1-84-URA3)	Used for integrating <i>arp1-84:URA3</i> in the <i>ARP1</i> locus
pYO2532 (pBS-arp1-108-URA3)	Used for integrating <i>arp1-108:URA3</i> in the <i>ARP1</i> locus
pYO2533 (pBS-arp1-122-URA3)	Used for integrating <i>arp1-122:URA3</i> in the <i>ARP1</i> locus
pYO2534 (pBS-arp1-133-URA3)	Used for integrating <i>arp1-133:URA3</i> in the <i>ARP1</i> locus
pYO2535 (pBS-arp1-162-URA3)	Used for integrating <i>arp1-162:URA3</i> in the <i>ARP1</i> locus
pYO2536 (pBS-arp1-185-URA3)	Used for integrating <i>arp1-185:URA3</i> in the <i>ARP1</i> locus
pYO2537 (pBS-arp1-192-URA3)	Used for integrating <i>arp1-192:URA3</i> in the <i>ARP1</i> locus
pYO2538 (pBS-arp1-202-URA3)	Used for integrating <i>arp1-202:URA3</i> in the <i>ARP1</i> locus
pYO2539 (pBS-arp1-214-URA3)	Used for integrating <i>arp1-214:URA3</i> in the <i>ARP1</i> locus
pYO2540 (pBS-arp1-222-URA3)	Used for integrating <i>arp1-222:URA3</i> in the <i>ARP1</i> locus
pYO2541 (pBS-arp1-233-URA3)	Used for integrating <i>arp1-233:URA3</i> in the <i>ARP1</i> locus
pYO2542 (pBS-arp1-236-URA3)	Used for integrating <i>arp1-236:URA3</i> in the <i>ARP1</i> locus
pYO2543 (pBS-arp1-251-URA3)	Used for integrating <i>arp1-251:URA3</i> in the <i>ARP1</i> locus
pYO2544 (pBS-arp1-256-URA3)	Used for integrating <i>arp1-256:URA3</i> in the <i>ARP1</i> locus
pYO2545 (pBS-arp1-263-URA3)	Used for integrating <i>arp1-263:URA3</i> in the <i>ARP1</i> locus
pYO2546 (pBS-arp1-266-URA3)	Used for integrating <i>arp1-266:URA3</i> in the <i>ARP1</i> locus
pYO2547 (pBS-arp1-282-URA3)	Used for integrating <i>arp1-282:URA3</i> in the <i>ARP1</i> locus
pYO2548 (pBS-arp1-294-URA3)	Used for integrating <i>arp1-294:URA3</i> in the <i>ARP1</i> locus
pYO2549 (pBS-arp1-300-URA3)	Used for integrating <i>arp1-300:URA3</i> in the <i>ARP1</i> locus
pYO2550 (pBS-arp1-317-URA3)	Used for integrating <i>arp1-317:URA3</i> in the <i>ARP1</i> locus
pYO2551 (pBS-arp1-326-URA3)	Used for integrating <i>arp1-326:URA3</i> in the <i>ARP1</i> locus
pYO2552 (pBS-arp1-336-URA3)	Used for integrating <i>arp1-336:URA3</i> in the <i>ARP1</i> locus
pYO2553 (pBS-arp1-344-URA3)	Used for integrating <i>arp1-344:URA3</i> in the <i>ARP1</i> locus
pYO2554 (pBS-arp1-368-URA3)	Used for integrating <i>arp1-368:URA3</i> in the <i>ARP1</i> locus
pYO2555 (pBS-arp1-374-URA3)	Used for integrating <i>arp1-374:URA3</i> in the <i>ARP1</i> locus
pYO2763 (pRS316-KAR9-URA3)	Used for testing the synthetic lethality between <i>arp1-x</i> and $\Delta kar9$

Results and Discussion

Construction of *ARP1* charged cluster-to-alanine alleles

To investigate the structure-function relationship of Arp1p, we used a comprehensive mutagenesis method called “charged cluster-to-alanine scanning mutagenesis,” in which a cluster of charged amino acids in a target protein is replaced with alanines. In this procedure, it is expected that

the surfaces of functional domains are altered with minimal misfolding or structural alterations at a distance from the mutated site. The algorithm for selecting the target clusters was two or three charged amino acids (Glu, Asp, Arg, Lys, or His) in a window of five. As a result, 32 clusters of charged amino acids were identified in the amino acid sequence of Arp1p (Fig. 1). Using a PCR-based mutagenesis method, we constructed a series of mutated *arp1* alleles (designated *arp1-x*, where *x* indicates the number of the first

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MDQLSDSYALYNQPVVI DNGSGIIKAGFSGEEERPKALEYCLVGNTKYDKVMLEGLQGDTF
      2              31    35              46

IGNNAQKLRGLLKLRYPIKHGVVEDWDSMELIWSYVLNEVLQLQNIGEHPLLITEAPMNP
      67      73    79      84              108

LKNREQMAQVLFETFDVSALYVSNPAVLSLYASG RTTGCVVDCGEGYCSTVPIYDGFALP
      122      133              162

ASMMRMDIGGADDITEQLQFQLRKSAGVSLFSSSEREIVRTMKEKVCYLAKNIKKEEEKYL
      185      192      202      214      222      233 236

QGTQDLISTFKLPDGRCIEVGNDRYRAPEILFSPQIHGLGYDGLSDMCMQSIWKVDLDLRK
      251      256      263 266              282      294 300

PLLSSILSGGTTTLKGFGDRMLWDLEALTKGTSKIKIIAPSERKYTTWIGGSILTGLS
      317      326      336      344

TFQRLWTKKSDWLEDSTRVYSNLM
      368      374

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Fig. 1. Amino acid sequence of the yeast Arp1p. Charged clusters are underlined (with allele numbers below), and the residues that are replaced with alanine are in boldface. A previously identified checkpoint-defective mutation is shown with a white letter: *wac1*, P268L.

Fig. 2. Diagram of the strategy for integrating the *URA3*-linked *arp1-x* allele into the yeast genome at the *ARP1* locus. (1) Genomic *ARP1* was deleted via homologous recombination with a PCR product composed of *Cg-LEU2* with 45 bp of the sequence flanking the coding region. (2) Homologous recombination between the *Hind*III-*Xba*I fragment of pBS-*arp1-x*-*URA3* and the chromosome containing a *Cg-LEU2* replacement of *ARP1* ($\Delta arp1::LEU2$) results in a strain with *URA3*-linked *arp1-x* (*arp1-x:URA3*) at the *ARP1* locus.

amino acid mutated in a charged cluster) that have alanine mutations at the clusters of charged amino acids, and then integrated the mutated alleles in the yeast chromosome (Fig. 2).

Alleles affecting the cell wall integrity checkpoint

We examined the effect of alanine mutations in Arp1p on the cell wall integrity checkpoint in the *fks1-1154 arp1-x* cells (YOC3066-YOC3099). Synchronized G1 cells of each strain were prepared by centrifugal elutriation and were incubated in YPD medium at the restrictive temperature (37°C). Cell cycle progression was monitored at 30-min intervals by observing the assembly of the bipolar spindle. To activate the cell wall integrity checkpoint, we used the

fks1-1154 mutant, a temperature-sensitive mutant defective in 1,3- β -glucan synthase activity. In a control strain, which contained *URA3*-linked *wac1* at the *ARP1* locus, about 80% of the cells assembled bipolar spindles at 180 min after the temperature shift, as did checkpoint-defective *wac1* cells (Fig. 3). In nine of the 32 mutant strains (*fks1-1154 arp1*-73, -84, -162, -214, -222, -233, -236, -263, and -266), over 50% of the cells did not arrest at G2 phase and assembled bipolar spindles. The remaining mutant strains exhibited slight or almost no effect on the cell wall integrity checkpoint. These results imply that the amino acids mutated in the *arp1*-73, -84, -162, -214, -222, -233, -236, -263, and -266 alleles are required for the cell wall integrity checkpoint function of the Arp1p molecule.

Fig. 3. Effect of the alanine-replaced mutations on the cell wall integrity checkpoint.

(A) Synchronized G1 cells of *fks1-1154* (YOC1087), *fks1-1154 wac1* (YOC2858), *fks1-1154 Δarp1* (YOC2889), *fks1-1154 arp1-31* (YOC3069), *fks1-1154 arp1-236* (YOC3086), *fks1-1154 arp1-266* (YOC3090), and *fks1-1154 arp1-374* (YOC3099) were prepared by centrifugal elutriation and then incubated at the restrictive temperature (37°C). The percentage of cells with bipolar spindles was examined every 30 min after the temperature shift. At least 200 cells were counted. (B) Fluorescence microscopy of tubulin, the spindle polar body, and the nucleus. Photographs were taken at 180 min after the temperature shift.

Alleles affecting nuclear migration

We examined the effect of alanine mutations in Arp1p on nuclear migration by observing the accumulation of cells that did not segregate a nucleus into the daughter cell in *FKS1 arp1-x* cells (YOC3032-YOC3065). In 14 of the 32 mutant strains (*FKS1 arp1-46*, *-73*, *-84*, *-108*, *-162*, *-214*, *-222*, *-251*, *-266*, *-294*, *-326*, *-344*, *-368*, and *-374*), over 20% of the cells accumulated as mother cells with two nuclei, as in the nuclear migration-defective *FKS1 Δarp1* cells (Fig. 4). In the remaining mutant strains, including the

wac1 mutant, most of cells properly segregated one nucleus into each daughter cell (Fig. 4). These results suggest that the amino acids mutated in *arp1-46*, *-73*, *-84*, *-108*, *-162*, *-214*, *-222*, *-251*, *-266*, *-294*, *-326*, *-344*, *-368*, and *-374* are required for the nuclear migration function of the Arp1p molecule.

Most of the *arp1-x* alleles could be classified into one of four groups based on these phenotypes (Table III). The first group of mutants (*arp1-2*, *-31*, *-35*, *-79*, *-133*, *-185*, *-192*, *-202*, *-256*, *-282*, *-300*, and *-317*) shows almost no difference from wild type. The second group of mutants (*arp1-*

Fig. 4. Effect of the alanine-replaced mutations on nuclear migration.

(A) Synchronized G1 cells of *FKS1 ARP1:URA3* (YOC3032), *FKS1 wac1:URA3* (YOC3033), *FKS1 Δarp1* (YOC2888), *FKS1 arp1-31* (YOC3035), *FKS1 arp1-236* (YOC3052), *FKS1 arp1-266* (YOC3056), and *FKS1 arp1-374* (YOC3065) were prepared by centrifugal elutriation and then incubated at 37°C. The percentage of cells with two nuclei in the mother cell was examined after anaphase. At least 200 cells that had completed nuclear division were counted. (B) Fluorescence microscopy of the nucleus. Photographs were taken at the time when most of the cells had completed nuclear division. (C) The indicated cells were streaked on a plate containing 1 μg/ml 5-FOA and were incubated for 2 days at 25°C.

TABLE III. EXPERIMENTAL SUMMARY

<i>arp1-x</i> alleles	Amino acid replacement	Spindle formation (%) ^{a)}	Cells with two nuclei in mother cell (%) ^{b)}
<i>ARP1</i>	None	14.5	1.2
<i>wac1</i>	P268L	70.1	4.5
<i>Δarp1</i>	Deleted	72.1	30.2
<i>arp1-2</i>	D2A, D6A	12.4	1.2
<i>arp1-31</i>	E31A, E32A, R33A	19.3	1.8
<i>arp1-35</i>	K35A, E38A	11.0	2.2
<i>arp1-46</i>	K46A, D48A, K49A	19.6	28.9
<i>arp1-67</i>	K67A, R69A	35.4	13.7
<i>arp1-73</i>	K73A, R75A	62.2	3.0
<i>arp1-79</i>	K79A, H80A	19.5	25.1
<i>arp1-84</i>	E84A, D85A, D87A	54.3	31.3
<i>arp1-108</i>	E108A, H109A	30.2	28.6
<i>arp1-122</i>	K122A, R124A, E125A	11.3	9.8
<i>arp1-133</i>	E133A, D136A	13.6	2.0
<i>arp1-162</i>	D162A, E165A	57.9	29.8
<i>arp1-185</i>	R185A, D187A	12.3	3.4
<i>arp1-192</i>	D192A, E195A	11.9	1.0
<i>arp1-202</i>	R202A, K203A	11.1	2.7
<i>arp1-214</i>	E214A, R215A, E216A	50.1	28.8
<i>arp1-222</i>	K222A, E223A, K224A	51.7	25.4
<i>arp1-233</i>	K233A, K234A, E235A	63.3	2.2
<i>arp1-236</i>	E236A, E237A, K238A	64.7	1.4
<i>arp1-251</i>	K251A, D254A	11.2	23.7
<i>arp1-256</i>	R256A, E259A	17.1	4.5
<i>arp1-263</i>	D263A, R264A	58.6	2.6
<i>arp1-266</i>	R266A, E269A	57.4	25.1
<i>arp1-282</i>	D282A, D286A	14.0	1.6
<i>arp1-294</i>	K294A, D296A, D298A	13.6	25.0
<i>arp1-300</i>	R300A, K301A	15.8	2.5
<i>arp1-317</i>	K317A, D321A	10.0	1.5
<i>arp1-326</i>	D326A, E328A	12.3	30.5
<i>arp1-336</i>	K336A, K338A	15.5	12.1
<i>arp1-344</i>	E344A, R345A, K346A	8.3	30.2
<i>arp1-368</i>	K368A, K369A, D371A	8.5	27.1
<i>arp1-374</i>	E374A, D375A, R378A	10.1	31.9

^{a)} Bipolar spindle formation (percentage) was measured in the *fls1-1154* background. Strain: YOC2889 and YOC3066-3099.

^{b)} Cells with two nuclei in mother cell (percentage) was measured in *FKS1* background. Strain: YOC2888 and YOC3034-3065.

Mean values of at least two independent experiments are presented. Values judged to be defective in checkpoint or nuclear migration are represented in boldface.

233, -236, and -263) has a defect only in the cell wall integrity checkpoint and shows an intact nuclear migration function, as do cells with the *wac1* allele. The third mutant group (*arp1-46*, -251, -294, -326, -344, -368, and -374) has a defect only in nuclear migration and has an intact cell wall integrity checkpoint function. The fourth group of mutants (*arp1-73*, -84, -162, -214, -222, and -266) is defective in both cell wall integrity checkpoint and nuclear migration functions, as is the deletion mutant of *ARP1*.

Alleles affecting the genetic interaction with kar9

KAR9 is a component of the kinesin-mediated nuclear migration pathway. A mutation in *KAR9* can cause a nuclear migration defect that conveys synthetic lethality in conjunction with mutations in genes of the dynein/dynactin pathway (Miller and Rose, 1998). To confirm whether mutants representative of each of the four mutant groups (*arp1-31*, -236, -266, and -374) have defects in dynein/dynactin-mediated nuclear migration, we tested for synthetic lethality between *arp1* mutants and *Δkar9*. Cells of the strains

YOC4106, YOC4106, YOC4108, YOC4109, YOC4110, YOC4111, and YOC4112 were streaked on a plate containing 1 μ g/ml 5-FOA and were incubated for 2 days at 25°C. The mutation combinations of $\Delta arp1 \Delta kar9$, $arp1$ -266 $\Delta kar9$, and $arp1$ -374 $\Delta kar9$ showed obvious growth defects, whereas the $ARP1 \Delta kar9$, $wac1 \Delta kar9$, $arp1$ -31 $\Delta kar9$, and $arp1$ -236 $\Delta kar9$ strains formed colonies (Fig. 4C). These results further indicate that strains containing $arp1$ -266 and $arp1$ -374 have defects in dynein/dynactin-mediated nuclear migration, and that strains containing $wac1$, $arp1$ -31, and $arp1$ -236 do not.

A structural model for the interpretation of Arp1p mutations

To clarify the structure-function relationship of the Arp1p molecule, the three-dimensional structure of the Arp1p monomer was predicted using the homology modeling software MODELLER (Marti-Renom *et al.*, 2000; Sali and Blundell, 1993). The prediction was performed with the three-dimensional structure of yeast conventional actin as a template, because Arp1p has about 50% amino acid identity and 70% similarity with yeast conventional actin and is considered to share a conserved ultrastructure (Goodson and Hawse, 2002; Kabsch and Holmes, 1995; Poch and Winsor, 1997). The putative three-dimensional structure of Arp1p was visualized with RasMol software (Sayle and Milner-White, 1995), and the positions of the corresponding alanine-replaced mutations were labeled on the structure (Fig. 5A and B). The amino acids labeled in red are essential only for the cell wall integrity checkpoint function ($arp1$ -233, -236, -263, and $wac1$). The positions of these amino acids are concentrated in one domain and are on the surface of the Arp1p molecule, with the exception of the $wac1$ mutation, which is buried in the core of the structure. The amino acids essential for only nuclear migration are shown in green ($arp1$ -46, -251, -294, -326, -344, -368, and -374) and are located on the overall surface of the Arp1p molecule. The positions of the mutations suggest that these residues exposed on the surface of the molecule may be required for physical interactions with Arp1p binding proteins. These physical interactions may determine the functional specificity of Arp1p related to the cell wall integrity checkpoint or nuclear migration. The amino acids colored yellow are essential for both the cell wall integrity checkpoint and nuclear migration functions ($arp1$ -73, -84, -162, -214, -222, and -266). Several of these residues are concentrated close to the ATP binding cleft that is required for Arp1p monomer polymerization (Bingham and Schroer, 1999). Therefore, the mutation of these amino acids may inhibit Arp1p polymerization, resulting in the destruction of the dynactin complex.

Arp1p forms an actin-like short filament composed of nine or ten Arp1p monomers (Bingham and Schroer, 1999; Schafer *et al.*, 1994). Therefore, the ultrastructure of the

Fig. 5. Positions of the functional residues on the monomer and polymer forms of Arp1p.

(A) The three-dimensional structure of the yeast Arp1p structure is presented with RasMol molecular visualization software (Sayle and Milner-White, 1995). This view is designated as the “front view” of Arp1p. The alanine mutations associated with the different effects are indicated according to the following color scheme: red, cell wall integrity checkpoint defective (over 50% cells formed bipolar spindle at 180 min); green, nuclear migration defective (over 20% cells have two nuclei in mother cell); and yellow, both cell wall integrity checkpoint and nuclear migration defective. Alleles are represented by number. (B) Same as A, except back view. (C) Ultrastructure of the rabbit muscle actin 8-mer (Lorenz *et al.*, 1993). The colored residues correspond to the equivalent residues in yeast Arp1p. Color scheme is the same as A and B.

rabbit muscle actin 8-mer was used for predicting the positions of the mutations on the filamentous form of Arp1p (Fig. 5C) (Lorenz *et al.*, 1993). Most of the amino acids colored red and green are located on the surface of the Arp1p polymer. Thus, mutations in these amino acids are unlikely to inhibit the formation of the Arp1p filament. On the other hand, the amino acids colored yellow tend to lie on the hidden surface of the filament. This supports the possibility that mutations of these residues may cause the destruction of the Arp1p filament, thereby abolishing both the cell wall integrity checkpoint and dynein-mediated nuclear migration functions in these cells. Alternatively, these mutated Arp1p molecules may be rapidly degraded, producing the same phenotype as that of the $arp1$ deletion.

Recently, Clark and Rose (Clark and Rose, 2005) reported that an $arp1$ mutant with the same mutations as our $arp1$ -214 mutant (E214A, R215A, E216A) has a defect in the polymerization of the Arp1p filament. In our study, we

showed that the *arp1-214* mutant is defective in both cell wall integrity checkpoint and nuclear migration. These data suggest that the precise formation of the Arp1p filament is essential not only for nuclear migration but also for the cell wall integrity checkpoint. Clark and Rose (Clark and Rose, 2005) proposed that residues K369, D371, E374, and D375 play an important role in the interaction with Jnm1p. We showed that the *arp1-368* (K368A, K369A, D371A) and *arp1-374* (E374A, D375, R378A) mutants were defective only in nuclear migration, suggesting that the interaction of Arp1p with Jnm1p is not critical for its checkpoint function.

In summary, the deletion of *arp1* causes defects in both cell wall integrity checkpoint and nuclear migration functions, whereas the *wac1* mutation causes a defect only in the cell wall integrity checkpoint. One possible explanation for the phenotypic difference between *wac1* and *arp1* deletion mutants is that the cell wall integrity checkpoint and nuclear migration are based on distinct molecular functions of Arp1p, and that these two functions are separable in the Arp1p molecule. These results, together with our previous results showing that dynein-related proteins, except dynactin subunits, are not required for the cell wall integrity checkpoint, indicate that the molecular function of the cell wall integrity checkpoint may be independent of the dynein-based nuclear migration mechanism.

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