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Genetic Interactions between *KAR7/SEC71*, *KAR8/JEM1*, *KAR5*, and *KAR2* during Nuclear Fusion in *Saccharomyces cerevisiae*

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During mating of *Saccharomyces cerevisiae,* two nuclei fuse to produce a single diploid nucleus. Two genes, *KAR7* and *KAR8*, were previously identified by mutations that cause defects in nuclear membrane fusion. *KAR7* is allelic to *SEC71*, a gene involved in protein translocation into the endoplasmic reticulum. Two other translocation mutants, *sec63-1* and *sec72*Δ, also exhibited moderate karyogamy defects. Membranes from *kar7/sec71*Δ and *sec72*Δ, but not *sec63-1,* exhibited reduced membrane fusion in vitro, but only at elevated temperatures. Genetic interactions between *kar7* and *kar5* mutations were suggestive of protein–protein interactions. Moreover, in *sec71* mutants, Kar5p was absent from the SPB and was not detected by Western blot or immunoprecipitation of pulse-labeled protein. *KAR8* is allelic to *JEMI*, encoding an endoplasmic reticulum resident DnaJ protein required for nuclear fusion. Overexpression of *KAR8/JEM1* (but not *SEC63*) strongly suppressed the mating defect of *kar2-1*, suggesting that Kar2p interacts with Kar8/Jem1p for nuclear fusion. Electron microscopy analysis of *kar8* mutant zygotes revealed a nuclear fusion defect different from *kar2*, *kar5*, and *kar7/sec71* mutants. Analysis of double mutants suggested that Kar5p acts before Kar8/Jem1p. We propose the existence of a nuclear envelope fusion chaperone complex in which Kar2p, Kar5p, and Kar8/Jem1p are key components and Sec71p and Sec72p play auxiliary roles.

INTRODUCTION

Nuclear fusion (karyogamy) is the last step in the mating pathway that culminates in the formation of a diploid cell. In preparation for mating in *Saccharomyces cerevisiae*, cells respond to the mating pheromone secreted by **a** and α cells, exit the mitotic cell cycle, and differentiate into mating-proficient cells. The mating cells grow directionally toward the selected mating partner, producing a cell with a characteristic mating projection often called a shmoo. Once contact between the partner cells is established, the mating pair undergoes cell fusion followed by nuclear fusion to form a diploid zygote (for review, see Sprague and Thorner, 1994; Herskowitz, 1995; Rose, 1996; Marsh and Rose, 1997).

The pathway of karyogamy in yeast proceeds by at least two major steps (Kurihara *et al.*, 1994). First, cytoplasmic microtubules emanating from the spindle pole body (SPB) are required to bring the nuclei into close proximity (Byers and Goetsch, 1975; Byers, 1981), a process called congression. The SPB is embedded in the nuclear envelope, which otherwise remains intact throughout all phases of the cell cycle (Byers, 1981). Upon pheromone induction, the cytoplasmic microtubules emanating from the SPB position the nucleus close to the mating projection (Byers and Goetsch, 1975; Miller and Rose, 1998). Immediately after cell

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fusion, the cytoplasmic microtubules interconnect, the two nuclei move together, and the two SPBs become closely apposed (Byers and Goetsch, 1975; Meluh and Rose, 1990; Beh *et al.*, 1997).

The second step of karyogamy entails the fusion of the nuclear membranes. Two membranes, the inner and outer nuclear envelopes, surround each nucleus. Therefore, the establishment of nuclear lumenal continuity requires that the two outer and two inner membranes become fused in register. Membrane fusion is also coupled to the fusion of the two SPBs, resulting in the formation of a single larger microtubule-organizing center (Byers and Goetsch, 1975). Based on ultrastructural analysis, Byers and Goetsch (1975) proposed that nuclear membrane fusion initiates along one edge of the two SPBs to generate a single diploid nucleus. Whether the SPB and the two sets of membranes fuse in one concerted event or in several stepwise events is not yet known (Rose, 1996).

Several mutations that block nuclear fusion have been isolated and characterized (Conde and Fink, 1976; Polaina and Conde, 1982; Kurihara *et al.*, 1994). The mutants fall into two distinct classes corresponding to the two major events in the karyogamy pathway. Class I mutants are defective for nuclear congression, and class II mutants are defective for nuclear membrane fusion (Kurihara *et al.*, 1994). All of the class I mutants are unable to bring the two nuclei into close proximity, and all contain mutations in genes that are involved with microtubule function (Kurihara *et al.*, 1994).

In the class II mutants, *kar2, kar5, kar7*, and *kar8*, the two nuclei congress normally, but the nuclear membranes do not fuse (Kurihara *et al.*, 1994). Mutations in these genes are also defective in an in vitro endoplasmic reticulum (ER)–nuclear envelope membrane fusion assay (Kurihara *et al.*, 1994; Latterich and Schekman, 1994). Kar2p is the yeast homologue of the mammalian BIP/GRP78, a member of the Hsp70 chaperone family (Rose *et al.*, 1989). Kar2p resides in the lumen of the ER–nuclear envelope and is essential for the translocation and folding of secretory precursors into the ER (Rose *et al.*, 1989; Vogel *et al.*, 1990; Sanders *et al.*, 1992). Two observations suggest that Kar2p has a direct role in nuclear membrane fusion, which is independent of its role in translocation (Vogel *et al.*, 1990; Vogel, 1993; Latterich and Schekman, 1994). First, temperature-sensitive *KAR2* mutants show temperature-sensitive ER–nuclear envelope membrane fusion in vitro. Second, there is a striking lack of correlation between nuclear fusion and translocation defects for various *kar2* alleles.

Kar5p is a novel integral ER–nuclear envelope membrane protein. Kar5p is predicted to have a carboxyl-terminal transmembrane domain, and protease protection analysis demonstrated that most of the protein is present in the lumen of the ER–nuclear envelope (Beh *et al.*, 1997). Consistent with its role in nuclear fusion, Kar5p is induced by pheromone and localizes near the SPB (Beh *et al.*, 1997). *KAR5* was also identified in a screen for pheromone-induced genes (Erdman *et al.*, 1998). A homologue of Kar5p, called Tht1p, has also been identified in *Schizosaccharomyces pombe* and shown to play a role in nuclear fusion (Tange *et al.*, 1998).

In addition, Ng and Walter (1996) found that certain mutations in *SEC63*, *SEC71*, and *SEC72* also result in zygotes with nuclear membrane fusion defects. These three genes all encode ER–nuclear envelope proteins with roles in protein translocation. Sec63p is an essential integral membrane protein with a large cytoplasmic domain and a smaller lumenal domain. The lumenal domain of Sec63p is composed of a DnaJ homology domain that interacts with Kar2p (Sadler *et al.*, 1989; Brodsky and Schekman, 1993; Feldheim *et al.*, 1993; Scidmore *et al.*, 1993). *SEC71* encodes a 206-residue, 31.5-kDa integral membrane glycoprotein in the Sec63p complex (Green *et al.*, 1992; Brodsky and Schekman, 1993; Feldheim *et al.*, 1993; Kurihara and Silver, 1993). Unlike *SEC63* and most other components of the translocation machinery, *SEC71* is not an essential gene; deletion mutations result in a temperature-sensitive growth defect and the accumulation of a subset of precursor proteins at the nonpermissive temperature (Feldheim *et al.*, 1993; Kurihara and Silver, 1993). Sec72p is a 23-kDa peripheral membrane protein that is also a component of the Sec63p complex (Green *et al.*, 1992; Brodsky and Schekman, 1993; Fang and Green, 1994; Feldheim and Schekman, 1994). *SEC72* is also not essential for life but is required for the translocation of a subset of protein precursors (Feldheim and Schekman, 1994).

Recently, another gene with homology to DnaJ, called *JEM1* (DnaJ-like protein of the ER membrane), was identified by the Yeast Genome Project. *JEM1* encodes a 645–amino acid peripheral membrane protein associated with the lumenal region of the ER (Nishikawa and Endo, 1997; Nishikawa and Endo, 1998). The carboxyl-terminal domain of *JEM1* contains a J domain with 47% identity to the *Escherichia coli* DnaJ protein. Disruption of *JEM1* results in a bilateral karyogamy defect reminiscent of other class II karyogamy mutants (Nishikawa and Endo, 1997).

Here we show that *KAR7* and *KAR8* are allelic to *SEC71* and *JEM1*, respectively. In agreement with Ng and Walter (1996), we find that other components of the translocation machinery, including *SEC63* and *SEC72*, but not *SEC61*, are required for efficient nuclear fusion in vivo. However, membranes that are devoid of Sec71p and Sec72p showed only a temperature-sensitive reduction in membrane fusion competence in vitro. To investigate *KAR7/SEC71*'s role in karyogamy, we analyzed the basis of a previously noted genetic interaction between *KAR7/SEC71* and

KAR5, a gene specifically required for nuclear fusion. We conclude that *KAR7/SEC71* is required for the synthesis and/or stability of Kar5p. To investigate *KAR8/JEM1*'s role we used dosage suppression experiments and electron microscopy (EM) analysis. We show that *KAR8/JEM1* has a unique karyogamy function that cannot be substituted for by *SEC63* or *SCJ1*. EM analysis showed that the nuclear fusion bridges seen in *kar8/jem1*Δ mutant zygotes are different from those observed in *kar2*, *kar5*, and *kar7* mutants. Analysis of *kar5*Δ *kar8/jem1*Δ double mutants suggests that Kar8p functions downstream of Kar5p. We propose the existence of a nuclear fusion complex in which Kar5p, Kar8/Jem1p, and Kar2p are key components and Sec63p, Sec71p, and Sec72p play auxiliary roles.

MATERIALS AND METHODS

Microbial Techniques, General Methods, and Strains

Yeast media and genetic techniques were as previously described (Rose *et al.*, 1990). Yeast and *E. coli* plasmid DNA minipreps were performed as described elsewhere (Rose *et al.*, 1990). Yeast strains were transformed by the lithium acetate method (Ito *et al.*, 1983). Limited plate matings were performed as described previously (Brizzio *et al.*, 1996). Filter matings for the microscopic analysis of zygotes were performed as described elsewhere (Brizzio *et al.*, 1996). Briefly, $\sim 5 \times 10^6$ cells in exponential phase from each parent were mixed onto a 45-μm nitrocellulose filter. The mating mixtures were then incubated for 2–3 h at 30°C. The cells were subsequently fixed in methanol:acetic acid (3:1) on ice for 1 h and washed two times with PBS. 4′,6′-Diamidino-2-phenylindole (DAPI) was added at 1 μg/ml for 5 min, and the cells were washed with PBS. Zygotes were then analyzed by differential interference contrast (DIC) and fluorescence microscopy (Axiophot; Carl Zeiss, Thornwood, NY). For *sec63-201* × *sec63-201* matings, zygotes were fixed in 3.7% formaldehyde diluted in PBS for 10 min and then washed two times with PBS before staining with DAPI.

Quantitative matings were performed as described previously (Rose *et al.*, 1990). In brief, $\sim 3 \times 10^6$ cells in midexponential phase from each parent were mixed onto a 45-μm nitrocellulose filter. The mating mixtures were then incubated for 4 h at 23, 30, and at 35°C. Several dilutions were plated on YEPD, on appropriate plates for diploid selection and on YPG containing cycloheximide at 3 μg/ml. Rho° cycloheximide-resistant strains (ρ° cyh2) were generated as described previously (Rose *et al.*, 1990).

The strains used in this study are listed in Table 1. Unless stated otherwise, all the strains are isogenic to S288C.

Strain Construction and Plasmids

Plasmid pMR3056, used for linkage analysis between the *SEC71* locus and *kar7-1039*, was constructed by cloning a 1.5-kb *Xba*I–*Sal*I restriction fragment from the *SEC71* locus (see Figure 2) into pRS405 YIp-*LEU2* vector (Sikorski and Hieter, 1989). Plasmid pMR3056 was linearized with *Pst*I before transformation of MS1554 to generate strain MS3908.

Generation of a *sec71*Δ allele (*sec71-Δ1*::*URA3*) was done by one-step gene replacement (Scherer and Davis, 1979). Plasmid pMR3057 was made by cloning 513-bp *Eco*RI–*Bam*HI and 286-bp *Bam*HI–*Xba*I restriction fragments from pMR3047 (see Figure 2) into pRS406 YIp-*URA3* vector (Sikorski and Hieter, 1989) cut with *Xba*I–*Eco*RI. Plasmid pMR3057 was linearized with *Bam*HI before transformation of MS1554 and MS1683 to generate MS3910 and MS3911, respectively. This construct results in a 577-bp deletion that removes the *SEC71* promoter regions and 173 amino acids of *SEC71* coding region, leaving just 34 carboxyl-terminal amino acids. Generation of the *sec71-Δ1*::*URA3* allele was confirmed by Southern blotting as previously described (Hoffman and Winston, 1987; Rose *et al.*, 1990).

For linkage analysis between *KAR8* and *JEM1*, plasmid pMR3133 was constructed to direct genomic integration at the *KAR8* locus. Plasmid pMR3133 was made by cloning a genomic 1.2-kb *Bgl*II–*Bam*HI restriction fragment from pMR2935 into pRS405, which had been cut with *Bam*HI (see Figure 2). Plasmid pMR3133 was linearized with *Mlu*I before transformation of MS52.

Generation of the *kar8/jem1*Δ::*LEU2* allele was done by one-step gene replacement. A disruption plasmid was generated by cloning 806-bp *Hin*dIII–*Apa*I and 647-bp *Avr*II–*Hin*dIII restriction fragments into pRS405 YIp-*LEU2* vector (Sikorski and Hieter, 1989), and subsequently linearized with *Hin*dIII before transformation of MS5, to generate strain MS4076. This allele removes 642 amino acids internal to *KAR8/JEM1*, leaving 24 amino-terminal and 26 carboxyl-terminal amino acids. Generation of the *kar8/jem1*Δ allele was confirmed by Southern blotting as previously described (Sambrook *et al.*, 1989; Rose *et al.*, 1990). Strains MS4338 and MS4342 were generated by sporulation of a cross between MS4076 and MS4326.

To construct a *KAR8/JEM1* 2μ plasmid (pMR3270), a 3.3-kb *Hin*dIII fragment containing *KAR8/JEM1* from pMR2935 was subcloned into pRS426 YEp-*URA3* (Sikorski and Hieter, 1989). Plasmids pDF14 (*LEU2* 2μ *SEC63*) and pDF15 (*URA3* 2μ *SEC63*) were kindly provided by the R. Schekman laboratory (University of California, Berkeley, CA) and have been previously described (Feldheim *et al.*, 1993). Plasmid pCen63, a *CEN URA3* plasmid containing *SEC63*, was obtained from the P. Silver laboratory (Harvard University, Boston, MA). The *KAR8/JEM1* 2μ plasmid pMR3352 and the *KAR8/JEM1-CEN* plasmid pMR3369 were made by subcloning a 3.3-kb *Hin*dIII fragment containing *KAR8/JEM1*from pMR2935 into pRS425 YEp-*LEU2* and pRS415 YCp-*LEU2*, respectively (Sikorski and Hieter, 1989). The *SCJ1* 2μ plasmid pPS720 (Silberstein et al., 1998) was kindly provided by Reid Gilmore (University of Massachusetts Medical School, Worcester, MA).

Cloning of KAR7

In addition to its mating defect, *kar7-1039* showed a temperature-sensitive defect for growth at 37°C. Linkage analysis was used to show that the mating and the temperature-sensitive phenotypes were tightly linked. A *kar7-1039* strain (MS3539) was crossed to a wild-type strain (MS10), and in 30 tetrads analyzed, there was cosegregation of the temperature-sensitive phenotype (Ts^-) with the mating defect (2 Kar^+:2 Kar^-Ts^-), indicating a genetic distance of <1.6 centimorgans (cM). The temperature-sensitive phenotype of *kar7-1039* was then used to clone *KAR7* by complementation. A yeast centromere-based (YCp50) genomic library (Rose *et al.*, 1987) was transformed into a *kar7-1039* strain (MS3259). Twenty thousand Ura^+ transformants were selected at 30°C and screened for growth at 37°C. Fourteen positives were isolated. They all shared DNA fragments in common and complemented both the temperature-sensitive growth defect and the mating defect when reintroduced in the *kar7-1039* strain (MS3259). A 3.9-Kb *Hin*dIII–*Hin*dIII restriction fragment present in all 14 clones was subcloned into pRS416 (Sikorski and Hieter, 1989). This plasmid (pMR3047) was then tested for its ability to complement the temperature-sensitive growth defect and the mating defect of *kar7-1039* (MS3259). As indicated in Figure 2A, this fragment still retained both complementing activities. The sequence of the ends of the insert in pMR3047 was determined using Sequenase (United States Biochemical, Cleveland, OH) and the T3 and T7 primers (from pBluescript; Stratagene, La Jolla, CA), following the manufacturer's instructions. Examination of the DNA sequence and consultation of the GenBank database showed that the cloned DNA contained the *SEC71* and *NPL4* genes and part of an uncharacterized gene, *SSE2*. To precisely define *KAR7*, several subclones were generated and tested for complementation of both defects (Figure 2A). Cloning of 2.9-kb *Hin*dIII–*Cla*I, 2.6-kb *Xba*I–*Xba*I, and 1-kb *Cla*I–*Hin*dIII restriction fragments into the pRS416

Table 1. Yeast strains used in this study

Strain	Genotype
MS5	*MAT***a** *ura3-52 leu2-3 leu2-112*
MS10	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101*
MS16	*MATα ade2-101 trp1-Δ1*
MS22	*MAT***a** *ade2-101 trp1-Δ1 lys2-801*
MS23	*MATα ade2-101 trp1-Δ1 lys2-801*
MS31	*MATα trp1-Δ1*
MS52	*MATα ura3-52 leu2-3 leu2-112 trp1-Δ1*
MS1111	*MAT***a** *ura3-52 leu2-3 leu2-112 ade2-101 kar2-1*
MS1554	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 his3-Δ200*
MS2705	*MATΔ::LEU2 ura3-52 leu2-3, leu2-112 trp1-Δ1 kar8-1333* [YCp*MATα*]
MS2706	*MATΔ::LEU2 ura3-52 leu2-3, leu2-112 trp1-Δ1 kar8-1333*
MS3259	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 his3-Δ200 kar7-1039*
MS3260	*MAT***a** *ura3-52 leu2-3 leu2-112 his3-Δ200 ade2-101 kar8-1333*
MS3534	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 his3-Δ200 lys2-801 kar5-1162*
MS3536	*MATα ura3-52 lys2-801 his3-Δ200 ade2-101 leu2-3 leu2-112 kar8-1333*
MS3537	*MATα ura3-52 ade2-101 his3-Δ200 lys2-801 kar5-486*
MS3538	*MATα ura3-52 ade2-101 his3-Δ200 lys2-801 kar5-1162*
MS3539	*MATα ura3-52 ade2-101 his3-Δ200 lys2-801 kar7-1039*
MS3577	*MATα ura3-52 leu2-3, leu2-112 trp1-Δ1 kar5-Δ2::LEU2*
MS3823	*MAT***a** *ura3-52 ade2-101 kar7-1039*
MS3826	*MATα ura3-52 leu2-3, leu2-112 ade2-101 kar7-1039*
MS3856	*MATα ura3-52 leu2-3, leu2-112 trp1-Δ1 ρ^o cyh2*
MS3908	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 his3-Δ200 SEC71::*YIp-*LEU2*
MS3910	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 his3-Δ200 sec71-Δ1::URA3*
MS3911	*MATα ura3-52 ade2-101 his3-Δ200 lys2-801 sec71-Δ1::URA3*
MS3927	*MATα ura3-52 leu2-3, leu2-112 trp1-Δ1 sec71-Δ1::URA3ρ^o cyh2*
MS3928	*MAT***a** *ura3-52 leu2-3 leu2-112 kar8-1333*
MS3986	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 his3-Δ200 lys2-801 kar5-Δ2::LEU2* (pRS426)
MS3987	*MAT***a** *ura3-52 leu2-3, leu2-112 his3-Δ200 kar5-Δ2::LEU2* (pMR3142)
MS3989	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 lys2-801 kar5-1039* (pMR3142)
MS3991	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 lys2-801 kar7-1039* (pMR3142)
MS4020	*MAT***a** *ura3-52 ade2-101 leu2-3, leu2-112 kar2-1* (pMR3142)
MS4021	*MAT***a** *ura3-52 leu2-3 kar1-1* (pMR3142)
MS4060	*MATα ura3-52 ade2-101 his3-Δ200 lys2-801 kar7-1039 ρ^o cyh2*
MS4076	*MAT***a** *ura3-52 leu2-3 leu2-112 kar8-Δ1::LEU2*
MS4326	*MATα ura3-52 leu2-3 leu2-112 his3-Δ200 ade2-101 trp1-Δ1*
MS4338	*MATα ura3-52 leu2-3 leu2-112 trp1-Δ1 his3-Δ200 kar8-Δ1::LEU2*
MS4342	*MAT***a** *ura3-52 leu2-3 leu2-112 his3-Δ200 kar8-Δ1::LEU2*
MS4359	*MATα ura3-52 leu2-3 leu2-112 ade2-101 his3-Δ200 kar5-Δ1::URA3 kar8-Δ1::LEU2*
MS4360	*MAT***a** *ura3-52 leu2-3 leu2-112 ade2-101 his3-Δ200 trp1-Δ1 kar5-Δ1::URA3 kar8-Δ1::LEU2*
MY2248[a]	*MATα ura3-52 leu2-3 leu2-112 ade2-101 trp1-Δ1 sec63-1*
MY2339	*MATα ura3-52 ade2-101 his4-539 sec61-2*
MY2341	*MAT***a** *ura3-52 leu2-3, leu2-112 ade2-101 sec61-2*
MY2653[a]	*MATα ura3-52 leu2-3 leu2-112 trp1-Δ1 sec63-4*
MY2808	*MAT***a** *trp1Δ sec63-1*

Table 1. Continued

Strain	Genotype
MY3564	*MATα ura3-52 sec63-1 ρ^o cyh2*
MY3594	*MAT***a** *ura3-52 ade2-101 his4-539 trp1-Δ1 sec62-1*
MY3676	*MATα ura3-52 ade2-101 his4-539 sec61-2 ρ^o cyh2*
MY3678	*MATα ura3-52 leu2-3, leu2-112 his4-539 sec62-1 ρ^o cyh2*
MY3917	*MATα ura3-52 ade2-101 his3-Δ200 lys2-801 trp1-Δ1 sec72-Δ::HIS3*
MY3918	*MAT***a** *ura3-52 leu2Δ1 ade2-101 his3-Δ200 sec72-Δ::HIS3*
MY3931	*MATα ura3-52 ade2-101 his3-Δ200 lys2-801 trp1-Δ1 sec72-Δ::HIS3 ρ^o cyh2*
MY4169[a]	*MATα ura3-52 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 sec63-Δ::HIS3* (*SEC63-URA* 2μ)
MLY1600[a]	*MATα ura3-52 leu2-3, leu2-112 pep4-Δ::URA3*
MLY1601[a]	*MAT***a** *ura3-52 leu2-3, leu2-112 pep4-Δ::URA3 gls1-1*
MLY1651[a]	*MATα ura3-52 leu2-3, leu2-112 pep4-Δ::URA3 sec63-1*
MLY1652[a]	*MATα ura3-52 leu2-3, leu2-112 pep4-Δ::URA3 gls1-1 sec63-1*
MLY1889[a]	*MAT***a** *ura3-52 leu2-3, leu2-112 pep4-Δ::URA3 gls1-1 sec71-Δ*
MLY1890[a]	*MATα ura3-52 leu2-3, leu2-112 pep4-Δ::URA3 sec71-Δ*
MLY1891[a]	*MAT***a** *ura3-52 leu2-3, leu2-112 pep4-Δ::URA3 gls1-1 sec72-Δ*
MLY1892[a]	*MATα ura3-52 leu2-3, leu2-112 pep4Δ::URA3 sec72-Δ*
DNY65[b]	*MAT***a** *sec63-201 ura3-Δ99 leu2-Δ99 his3-Δ200 leu2-Δ1 trp1-Δ99 ade2-101* (pDN106)
DNY66[b]	*MATα sec63-201 ura3-Δ99 leu2-Δ99 his3-Δ200 leu2-Δ1 trp1-Δ99 ade2-101* (pDN106)

[a] Obtained from R. Schekman laboratory.

[b] Obtained from Davis Ng (P. Walter Laboratory).

YCp-URA3 vector generated plasmids pMR3049, pMR3052, and pMR3054, respectively. Plasmid pMR3054 containing just *SEC71* retained both complementing activities, suggesting that *KAR7* was identical to the previously characterized gene *SEC71*. To verify that *KAR7* is identical to *SEC71*, linkage analysis was performed. A *KAR7 leu2* strain (MS1554) was transformed with the integration plasmid pMR3056 (see Figure 2A). A stable Leu$^+$ transformant (MS3908) was crossed to a *kar7-1039 leu2* strain (MS3826), and tetrad analysis was performed. The temperature-sensitive (Ts$^-$) phenotype of *kar7-1039* was used to follow *KAR7* in this cross. In all 24 tetrads analyzed, there was cosegregation of Leu$^-$ with the Ts$^-$ phenotype (2 Leu$^+$:2 Ts$^-$Leu$^-$), indicating a genetic distance between *SEC71* and *KAR7* of <2.1 cM.

Cloning of KAR8

KAR8 was cloned by complementation of the mating defect of *kar8-1333*. A *MAT***a** *kar8-1333* strain (MS3260) was transformed with a yeast centromere-based YCp50 genomic library (Rose *et al*., 1987). Twenty-four thousand Ura$^+$ transformants were replica printed onto lawns of the *MATα kar8-1333* strain (MS2705) and allowed to mate for 3 h at 30°C on rich media. The colonies were then replica printed to synthetic minimal medium plates to select for diploids. Nine positives were isolated. Complementing plasmids were isolated, amplified in *E. coli*, and retransformed into MS3260. Seven of these clones completely rescued *kar8-1333* and shared common genomic fragments, as determined by restriction digest analysis. Two other unlinked clones showed only partial suppression, and they were not studied further. To map *KAR8* on the yeast physical map, a 1.8-kb *Bgl*II genomic fragment from one of the positive clones was hybridized to a lambda prime yeast genomic grid (Riles *et al*., 1993). By this method *KAR8* was physically mapped to chro-

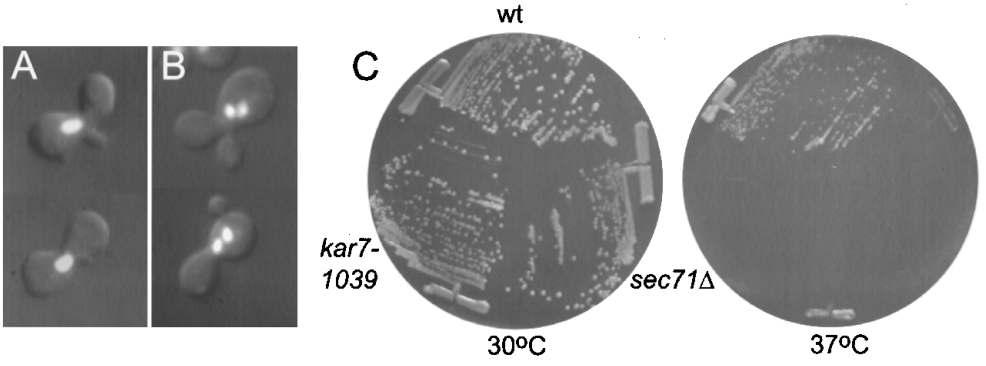


Figure 1. (A and B) Phenotype of class II Kar$^-$ zygotes. Shown are examples of wild-type (A) and class II Kar$^-$ (B) zygotes, respectively. Zygotes from filter matings between wild-type strains (MS1554 × MS23) or between *kar7-1039* strains (MS3259 × MS3539) were analyzed by microscopy. Each image shows the nucleus by DAPI fluorescence and the zygote morphology by DIC. (C) Temperature-sensitive defect of *kar7-1039*. Streaks of wild-type (MS1554), *kar7-1039* (MS3259), and *sec71*Δ (MS3910) strains were incubated at 30°C (left panel) or 37°C (right panel).

mosome X near *ARG3*. To further define *KAR8*, several subclones were generated and tested for the ability to rescue the *kar8-1333* mating defect (see Figure 2B). Plasmids pMR3252 and pMR3251 were derived from pMR2935 by deleting 5.6-kb *Hin*dIII and 8.5-kb *Mlu*I restriction fragments, respectively. Plasmids pMR3242 and pMR3260 were made by subcloning a 6.6-kb *Sal*I–*Sal*I and a 3.3-kb *Hin*dIII–*Hin*dIII into pRS416. Figure 2B shows that pMR3260, containing a single open reading frame recently identified as *JEM1* (Nishikawa and Endo, 1997), retained complementing activity. To further test whether *KAR8* is identical to *JEM1*, linkage analysis was performed. A *KAR8 leu2* strain (MS52) was transformed with the integration plasmid pMR3133. A stable Leu$^+$ transformant was crossed to a *kar8-1333 leu2* strain (MS3260), and tetrad analysis was performed. In 16 tetrads analyzed, the Leu$^-$ phenotype and the mating defect cosegregated (2 Leu$^+$:2 Mating$^-$Leu$^-$), indicating a distance between the *JEM1* and *KAR8* of <3.1 cM.

In Vitro ER Membrane Fusion Assay

Reagents used for measuring in vitro ER membrane fusion have been described before (Latterich and Schekman, 1994). Membranes isolated from wild-type, *sec63-1*, *sec71*Δ, and *sec72*Δ strains were tested for fusion competence as described before (Kurihara *et al.*, 1994; Latterich and Schekman, 1994). Microsomal membranes were isolated from the following strains grown at 24°C: wild-type *gls1* (MLY1601), wild-type *GLS1* (MLY1600), *sec71*Δ gls1 (MLY1889), *sec71*Δ *GLS1* (MLY1890), *sec72*Δ *gls1* (MLY1891), *sec72*Δ *GLS1* (MLY1892), *sec63-1 GLS1* (MLY1651), and *sec63-1 gls1* (MLY1652). These membranes were then tested for fusion competence by incubating donor and acceptor membranes (75 μg total protein each) at 24 and 37°C in the presence of an ATP regeneration system in a final volume of 50 μl for 1 h.

Immunological Techniques

Kar5p staining was performed by indirect immunofluorescence using polyclonal affinity-purified anti-Kar5p antibodies as previously described on strains MS3987, MS3986, MS4201, MS4020, MS3991, and MS3989 (Beh *et al.*, 1997). For Western analysis 10 ml of early exponential cultures (5×10^6-1.5×10^7 cells/ml) of MS3987 and MS3991 in synthetic complete media lacking uracil were treated with α-factor at 6 μM for 120 min. Total protein extracts were prepared as described elsewhere (Ohashi *et al.*, 1982). Proteins were electrophoretically separated using a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane. Western blotting using affinity-purified anti-Kar5p antibodies was performed as previously described (Beh *et al.*, 1997). For ^{35}S pulse analysis, strains MS3986, MS3987, and MS3991 were grown to early exponential phase in synthetic complete media lacking uracil and then treated as described by Gammie *et al.* (1999), the only modification being that the strains were grown at 23°C and pulse labeled for 5 min. For immunoprecipitation of pulse-labeled extracts, the anti-Kar5p antibody was used at a concentration of 1:10.

Rescue of SEC63 Temperature-sensitive and Null Alleles

sec63-1 (MY2248) and *sec63-4* (MY2653) mutant strains were transformed with pCen63, pMR3270, and pRS426 YEp-*URA3* (Sikorski and Hieter, 1989). Three transformants were grown on synthetic media lacking uracil at 23°C for 2 d and then tested for growth at 23, 30, and 37°C on plates. To determine whether a *sec63*Δ strain could be rescued by overexpression of *JEM1*, strain MY4169 containing a functional copy of *SEC63* on a *URA3* plasmid (obtained from the R. Schekman laboratory) was transformed with either *SEC63* 2μ *LEU2* (MR3253), *JEM1-CEN LEU2* (MR3369), *JEM1* 2μ *LEU2* (MR3352), or vector control *LEU2-CEN* plasmid (pRS415). The transformed strains were then patched on YPD and grown overnight and then replica plated to 5-fluoro-orotic acid, synthetic media lacking uracil, or leucine and incubated at 23, 30, and 37°C.

EM Analysis

For EM, mating mixtures were prepared as previously described (Kurihara *et al.*, 1994). Permanganate fixation used to enhance membranous structures was also performed as previously described (Kurihara *et al.*, 1994; Gammie *et al.*, 1998). Serial sections of 70 or 90 nm were stained with lead citrate and examined in a Jeol (Tokyo, Japan) 100C transmission electron microscope at 80 kV.

RESULTS

KAR7 Is Allelic to SEC71

The *kar7-1039* mutation was identified as a bilateral class II karyogamy mutant (Kurihara *et al.*, 1994). Diploid formation was reduced eightfold in a *kar7-1039* bilateral mating compared with wild type (Kurihara *et al.*, 1994). Figure 1B displays the typical class II karyogamy phenotype of *kar7-1039*, with zygotes containing two closely apposed unfused nuclei. In contrast, wild-type zygotes have a single fused nucleus (Figure 1A). Table 2 presents a quantitative analysis of the karyogamy phenotype. As expected, a wild-type × wild-type mating resulted mostly in zygotes with a single diploid nucleus. In contrast, the *kar7-1039* × *kar7-1039* mating, but not the *kar7-1039* ×

Table 2. Nuclear fusion defect of *kar7-1039* and *sec71*Δ by microscopic analysis of zygotes

mating	wt	Kar⁻
wild type x wild type	95	5
wild type x *kar7-1039*	89	11
kar7-1039 x *kar7-1039*	10	90
*sec71*Δ x *sec71*Δ	9	91
*sec71*Δ x *kar7-1039*	9	91

Mating mixtures were stained with DAPI, and the phenotypes of the zygotes were observed by microscopy. Numbers represent percentages of wild-type (wt) or karyogamy defective class II (Kar⁻) zygotes. At least 150 zygotes were analyzed. The matings were as follows: wild type × wild type (MS1554 × MS23); wild type × *kar7-1039* (MS22 × MS3539); *kar7-1039* × *kar7-1039* (MS3823 × MS3539); *sec71*Δ × *sec71*Δ (MS3910 × MS3911); and *sec71*Δ × *kar7-1039* (MS3910 × MS3539).

wild-type mating, resulted in mostly Kar⁻ zygotes (90%).

In addition to its mating defect, the *kar7-1039* mutant exhibited a tightly linked temperature-sensitive growth defect at 37°C (Figure 1C and MATERIALS and METHODS). The growth defect of *kar7-1039* was then used to clone *KAR7* by complementation (see MATERIALS and METHODS). Fourteen candidate plasmids were isolated. A subclone (pMR3047) containing a 3.9-kb *Hin*dIII–*Hin*dIII restriction fragment present in all 14 clones was able to complement both the temperature-sensitive growth defect and the mating defect of *kar7-1039* (Figure 2A). Sequencing analysis revealed that the subcloned DNA corresponded to a region of the genome that contains *SEC71, NPL4,* and part of *SSE2.* Further subcloning showed that the previously characterized gene *SEC71* alone on plasmid pMR3054 complemented both defects, suggesting that *KAR7* is allelic to *SEC71* (Figure 2A). Linkage analysis demonstrated that the *KAR7* locus is tightly linked to *SEC71,* further supporting the identity of *KAR7* as *SEC71* (see MATERIALS and METHODS). We therefore generated *MAT***a** and *MATα sec71*Δ

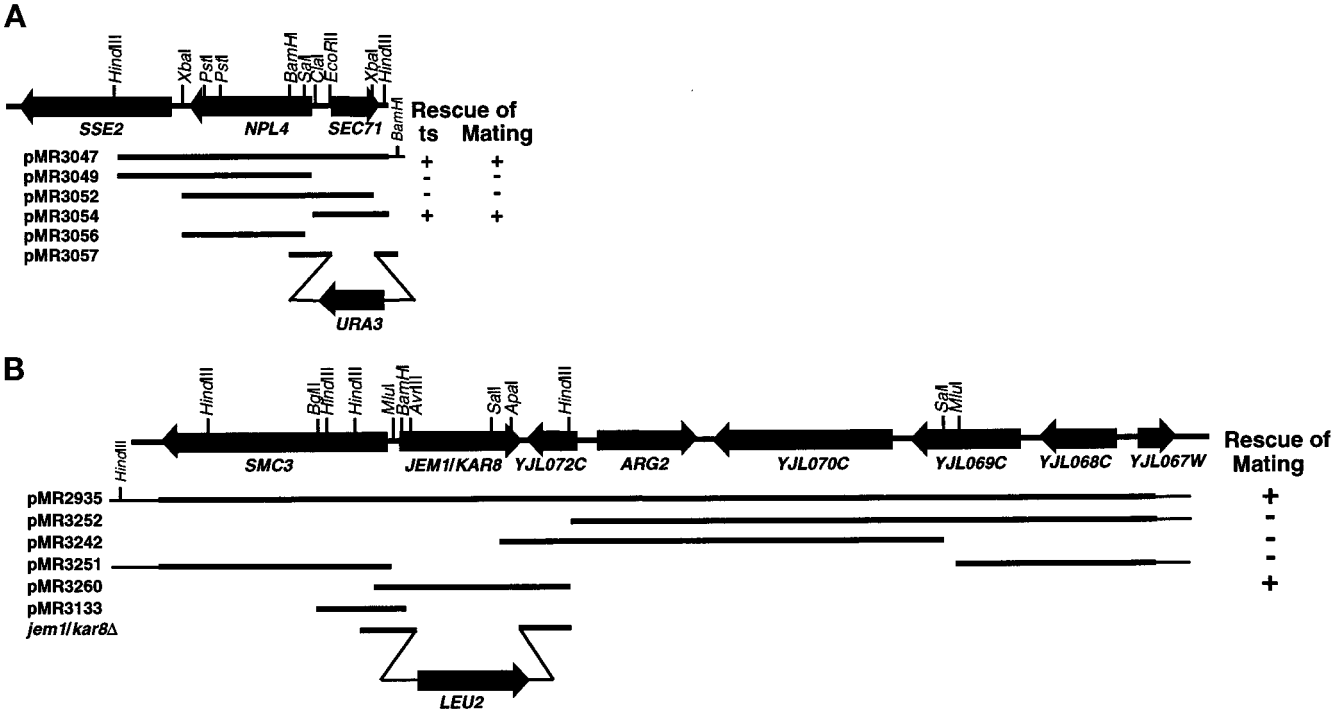


Figure 2. (A) Restriction map of *KAR7/SEC71* and surrounding region on chromosome II. Shown are several subclones generated to further define *KAR7*. Bars represent the DNA fragments present in the different plasmids. The ability (+) or inability (−) of the different plasmids to suppress the mating defect is indicated to the right. Shown at the bottom is the structure of plasmids pMR3056 and pMR3057 used in the linkage analysis and the generation of *sec71*Δ by one-step gene replacement respectively. (B) Restriction map of *KAR8/JEM1* and surrounding region on chromosome X. One of the original plasmids (pMR2935) able to rescue the *kar8-1333* mating defect is depicted. Several subclones were generated and tested for rescuing activity. Bars represent the DNA fragments present in the different plasmids. The ability (+) or inability (−) of the different plasmids to suppress the mating defect is indicated to the right. Shown at the bottom is the structure of plasmid pMR3133 used in the linkage analysis of *JEM1* and the structure of the *jem1/kar8*Δ allele generated by one-step gene replacement.

strains and found that they exhibited a karyogamy defect identical to that of *kar7-1039* (Table 2). In addition, as previously reported, *sec71*Δ was viable, resulting only in a temperature-sensitive growth defect (Figure 1C; Feldheim *et al.*, 1993; Kurihara and Silver, 1993).

Mutations in a Subset of ER Translocation Proteins Result in a Karyogamy Defect

Given that *KAR7* is allelic to *SEC71*, which encodes a component of the protein translocation machinery that includes Kar2p, we independently tested whether mutations in other components of the translocation machinery resulted in karyogamy defects.

The results of quantitative matings with mutants defective for protein translocation are shown in Table 3. In each case, (*sec61-2*, *sec62-1*, *sec63-1*, *kar7-1039*, *sec71*Δ, and *sec72*Δ), the mutant strains were mated against strains harboring the same mutations. All the mutants tested showed a reduction in the mating efficiency indicated by the reduction in the percentage of diploids formed (Table 3). To determine whether the reduction in mating efficiency was due to a defect in nuclear fusion, cytoductant analysis and microscopic analysis of the zygotes were performed. Cytoductants are haploid cells with the nucleus of one parent but the cytoplasm from both. In wild-type matings, >95% of the zygotes have a diploid nucleus, and the number of cytoductants produced is very low. In contrast, the presence of two unfused nuclei in Kar$^-$ zygotes greatly increases the frequency of cytoductant buds. As shown in Table 3, the *sec63-1*, *kar7-1039*, and *sec71*Δ matings were the only matings that showed a cytoductant:diploid ratio significantly higher than the wild-type × wild-type mating. The nuclear fusion defect was considerably more severe for the *sec71* alleles than for *sec63-1*. In addition, it is striking that the frequency of cytoductants was correlated with temperature in inverse ways for the two mutants. For *sec71*Δ and especially for *kar7-1039*, the frequency of cytoductants increased with temperature, in parallel with the decrease in diploid formation. In contrast, for *sec63-1* the frequency of cytoductants decreased with increasing temperature, as the defect in translocation becomes more severe. One interpretation of this observation is that Sec63p's role in nuclear fusion is not related to its function in translocation.

To examine nuclear fusion directly, we stained zygotes with DAPI and examined the nuclei microscopically (Table 4). By this assay only the *sec71* mutants showed a very strong defect (90% Kar$^-$ zygotes). The *sec63-1* mating showed an intermediate defect (60% Kar$^-$ zygotes). The *sec72*Δ mating showed ~32% mutant zygotes, indicating a mild defect in nuclear fusion, although the cytoductant:diploid ratio was not significantly higher than the wild-type mating (Tables 3 and 4). The difference between the two assays is not clear but could be explained if the zygotes that exhibited the nuclear fusion defect were inviable. Alternatively, the nuclear fusion defect might be temporary, and the nuclei eventually fuse. Finally, the *sec61-2* and *sec62-1* matings showed only 10–15% Kar$^-$ zygotes. These data demonstrate that mutations in *SEC71* and, to a lesser extent, *SEC63* and *SEC72* lead to defects in nuclear fusion.

Table 3. Quantitative mating experiments of various protein translocation-defective mutants

	Diploids (%)	C:D ratio
Wild type		
23°C	49	0.0009
30°C	49	0.0008
35°C	21	0.0003
sec61-2		
23°C	27	0.0018
30°C	7	0.0018
35°C	2	0.0017
sec62-1		
23°C	38	0.0038
30°C	6	0.076
35°C	6	0.0083
sec63-1		
23°C	5	0.22
30°C	3	0.028
35°C	0.2	0.011
kar7-1039		
23°C	9	0.02
30°C	3	0.52
35°C	0.1	1.2
*sec71*Δ		
23°C	5	0.19
30°C	2	0.25
35°C	1	0.53
*sec72*Δ		
23°C	24	0.0013
30°C	15	0.0041
35°C	11	0.001

Quantitative mating experiments performed at 23, 30, and 35°C. Percent diploid formation represents the number of diploids measured on appropriate selection plates divided by the number of viable cells measured on YPD plates × 100. C:D ratio reports the number of cytoductants measured on YPG cycloheximide plates divided by the number of diploids formed. The matings were as follows: wild type (MS1554 × MS3856); *sec61-2* (MY2341 × MY3676); *sec62-1* (MY3594 × MY3678); *sec63-1* (MY2808 × MY3564); *kar7-1039* (MS3259 × MS4060); *sec71*Δ (MS3910 × MS3927); and *sec72*Δ (MY3918 × MY3931). In general, matings against a wild-type strain gave a greater mating efficiency than mating involving the mutants themselves and a C:D ratio comparable to the wild-type × wild-type mating. However, *sec63-1*, *kar7-1039*, and *sec71*Δ strains resulted in a C:D ratio of 10^{-2}–10^{-3} even when these mutants were mated against wild type.

Membranes Defective in Sec71p or in Sec72p, but Not Sec63p, Show a Membrane Fusion Defect In Vitro

Previous studies demonstrated that the class II karyogamy genes are required for membrane fusion in

Table 4. Nuclear fusion defect of protein translocation mutants by microscopic analysis of zygotes

	wt	Kar⁻
mating		
wild type x wild type	95	5
sec61-2 x *sec61-2*	90	10
sec62-1 x *sec62-1*	90	10
sec63-1 x *sec63-1*	40	60
kar7-1039 x *kar7-1039*	10	90
*sec71*Δ x *sec71*Δ	9	91
*sec72*Δ *x sec72*Δ	68	32

Mating mixtures were stained with DAPI, and the phenotypes of the zygotes were observed by microscopy. Numbers represent percentages of wild-type (wt) or karyogamy-defective class II (Kar⁻) zygotes. At least 100 zygotes were analyzed. The *sec61-2*, *sec62-1*, and *sec63-1* strains were pregrown at 23°C. Filter matings were performed for 2.5 h at 30°C except for *sec63-1*, which was performed for 4 h at 23°C. The matings were as follows: wild type × wild type (MS1554 × MS23); *sec61-2* × *sec61-2* (MY2341 × MY2339); *sec62-1* × *sec62-1* (MY3594 × MY3678); *sec63-1* × *sec63-1* (MY2808 × MY3564); *kar7-1039* × *kar7-1039* (MS3823 × MS3539); *sec71*Δ × *sec71*Δ (MS3910 × MS3911); and *sec72*Δ × *sec72*Δ (MY3918 × MY3917).

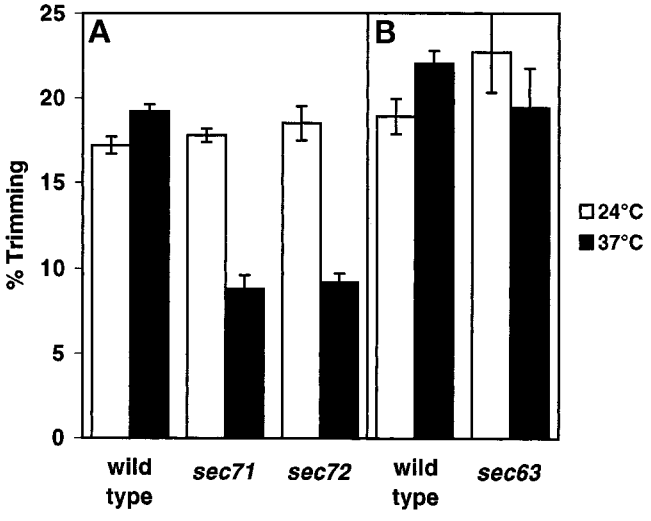


Figure 3. *sec71*Δ and *sec72*Δ, but not *sec63-1*, membranes show a defect in the in vitro ER–nuclear membrane fusion assay at 37°C. (A) Donor and acceptor membranes (75 μg protein each) prepared from wild-type strains (MLY1601 and MLY1600), strains deleted for the *SEC71* gene (MLY1889 and MLY1890), or strains deleted for the *SEC72* gene (MLY1891 and MLY1892) were combined in the presence of an ATP regeneration system in a total volume of 50 μl and held on ice. Reactions were incubated for 60 min at 24 or 37°C. (B) In a separate experiment, donor and acceptor membranes prepared from wild-type strains MLY1600 and MLY1601 and *sec63-1* strains MLY1651 and MLY1652) were combined and incubated as above. In all cases, the experiments were repeated three times, and the mean values and SDs are shown. All strains were grown at 24°C before membrane isolation. The amount of glucose trimming, indicative of the successful fusion of membranes, was assessed as described previously (Latterich and Schekman, 1994).

vitro (Kurihara *et al.*, 1994; Latterich and Schekman, 1994). We therefore decided to analyze the fusion competence of membranes isolated from strains deleted for *SEC71* and *SEC72* or containing the *sec63-1* mutation. In the ER membrane fusion assay, microsomal membrane fractions are derived from strains that either lack or contain glucosidase I (Gls1p), the enzyme that is responsible for initiating deglucosylation of newly synthesized glycoproteins (Latterich and Schekman, 1994). Yeast prepro-α-factor translocated into the lumen of the glucosidase I–deficient microsomal membranes (*gls1*; donor membrane) becomes processed to the deglucosylated form only when donor membranes fused with glucosidase-containing (*GLS1*) ER membranes. Quantifying the amount of trimmed and untrimmed pro-α-factor serves as a direct measure of membrane fusion (Latterich and Schekman, 1994). Microsomal membranes from wild-type, *sec71*Δ, *sec72*Δ, and *sec63-1* strains were tested for fusion competence by incubating donor and acceptor membranes at 24 and 37°C in the presence of an ATP regeneration system for 1 h.

Both the wild-type and the *sec63-1* membranes exhibited similar levels of fusion at 24 and 37°C (Figure 3B). In contrast, both *sec71*Δ and *sec72*Δ membranes exhibited a similar temperature-sensitive fusion defect, approximately half of that of wild-type at 37°C (Figure 3A). The partial fusion defect was not caused by membrane rupture, because the glycosylated pro-α-factor translocated into the *sec71*Δ or *sec72*Δ mutant membranes remained protease protected after the incubation at 37°C. The fusion defect of the *sec71*Δ strain was comparable to that of the *kar7-1039* strain tested before (Kurihara *et al.*, 1994). Therefore, we concluded that both Sec71p and Sec72p, but not Sec63p, are necessary for efficient membrane fusion at elevated temperature. However, the two gene products are not required for in vitro membrane fusion at the lower temperature of 24°C. These results suggest that Sec71p and Sec72p play a role in stabilizing the fusion machinery rather than being directly required for the fusion reaction. In addition, because the assay is done under conditions in which there is neither protein synthesis nor translocation, we concluded that the role of Sec71p and Sec72p in membrane fusion is independent of their role in translocation.

Kar5p, a Protein Specifically Required for Nuclear Fusion, Is Absent in kar7-1039

KAR5 encodes a pheromone-inducible ER–membrane protein that is important for nuclear membrane fusion (Beh *et al.*, 1997). Interestingly, one allele of *KAR5*, *kar5-1162*, showed unlinked noncomplementation with *kar7-1039* (Kurihara *et al.*, 1994). That is, a mating diploid of the form *kar5-1162/KAR5 SEC71/kar7-1039* has a mating defect, even though both mutations are recessive. Unlinked noncomplementation is indicative of two proteins that functionally interact. In a few well-documented examples, the proteins were shown to physically interact (e.g., α- and β-tubulin; Stearns and Botstein, 1988).

To further characterize *KAR5* and *SEC71* interaction, we performed matings between various *kar5* and *sec71* mating partners and analyzed the karyogamy phenotype by microscopy. As shown in Table 5, any *kar5* × *sec71* mutant mating is worse than wild-type × wild-type, *kar5* × wild-type, or *sec71* × wild-type matings. In particular, when *kar5-1162* was mated to either *kar7-1039* or *sec71*Δ, the result was a very strong karyogamy defect, similar to that of each mutant mated by itself (~90% Kar$^-$ zygotes; Table 5). This behavior is in contrast to that of other *kar* mutations wherein crosses between the different bilateral mutants yielded wild-type zygotes (Kurihara *et al.*, 1994). Therefore, we concluded that mutations in *KAR5* and in *SEC71* are "synthetic bilateral"; that is, mutation in one gene appears to cause a defect in the function of both proteins. One interpretation of these genetic data is that there is a functional interaction between Kar5p and Sec71p.

To examine the nature of the interaction between Kar5p and Sec71p, we analyzed the localization of Kar5p in *kar7-1039* and other *kar* mutants (Figure 4). In wild-type cells that have been induced with mating pheromone, Kar5p localizes in the vicinity of the SPB (Figure 4A; Beh *et al.*, 1997). Surprisingly, we found that Kar5p did not localize to the SPB in *kar7-1039* (Figure 4E). Of 51 *kar7-1039* shmoos examined, 49 did not exhibit the characteristic Kar5p staining. Kar5p was present and correctly localized in the *kar1-1*, *kar2-1*, and *kar8-1333* mutants, indicating that the defect is specific for *kar7-1039* (Figure 4, C, D, and F). The presence or absence of Kar5p in *kar7-1039* was further investigated by Western analysis of total protein extract (Figure 5A). Kar5p was detected after pheromone induction of a *KAR7* strain containing a *KAR5* 2μ plasmid (wild-type control; Figure 5A, lane 2). In contrast, no Kar5p was detected in isogenic pheromone-induced *kar7-1039* cells containing the *KAR5* 2μ plasmid (Figure 5A, lane 4). This result suggested that either Kar5p is not synthesized under these conditions or that Kar5p is rapidly degraded. To determine whether *KAR7* is required for the transcription of *KAR5*, we performed a Northern blot of a *kar7-1039* strain containing the *KAR5* 2μ plasmid. We found that *KAR5* mRNA was made in the *kar7-1039* strain upon induction with α-factor, equivalent to the *KAR7* control strain. Thus, *KAR7* is not required for transcription of *KAR5*. To address whether Kar7p is required for the synthesis or stability of Kar5p, *KAR7* and *kar7-1039* strains were pulse labeled with ^{35}S, and Kar5p was immunoprecipitated. In the pheromone-induced *KAR7* strain, Kar5p was readily detected after 5 min of pulse labeling (Figure 5B, lane 2). In contrast, in the pheromone-induced *kar7-1039* mutant, Kar5p could not be detected under equivalent conditions (Figure 5B, lane 4). Thus *KAR7/SEC71* appears to be required for the normal synthesis of Kar5p. From these experiments, we cannot distinguish whether Kar5p is synthesized at a very decreased rate or very rapidly degraded after synthesis. These results also do not rule

Table 5. *sec71* and *kar5* mutants show synthetic bilateral nuclear fusion defects

mating	wt	Kar$^-$
wild type x wild type	97	3
*sec71*Δ x wild type	89	11
*kar5*Δ x wild type *	80	20
kar5-1162 x wild type	79	21
*sec71*Δ x *sec71*Δ	11	89
*kar5*Δ x *kar5*Δ *	5	95
kar5-1162 x *kar5-1162*	10	90
*kar7*Δ x *kar5*Δ	50	50
*kar7*Δ x *kar5-486*	41	59
*kar7*Δ x *kar5-1162*	9	91
kar7-1039 x *kar5-1162*	9	91

Mating mixtures were stained with DAPI, and the phenotypes of the zygotes were observed by microscopy. Numbers represent percentages of wild-type (wt) or karyogamy-defective class II (Kar$^-$) zygotes. At least 100 zygotes were analyzed. The matings were as follows: wild type × wild type (MS1554 × MS23); *sec71*Δ × wild type (MS3910 × MS16); *kar5-1162* × wild type (MS3534 × MS23); *sec71*Δ × *sec71*Δ (MS3910 × MS3911); *kar5-1162* × *kar5-1162* (MS3534 × MS3538); *kar7*Δ × *kar5*Δ (MS3910 × MS3577); *kar7*Δ × *kar5-486* (MS3910 × MS3537); *kar7*Δ × *kar5-1162* (MS3910 × MS3538); and *kar7-1039* × *kar5-1162* (MS3823 × MS3538).

* Data taken from Beh *et al.* (1997).

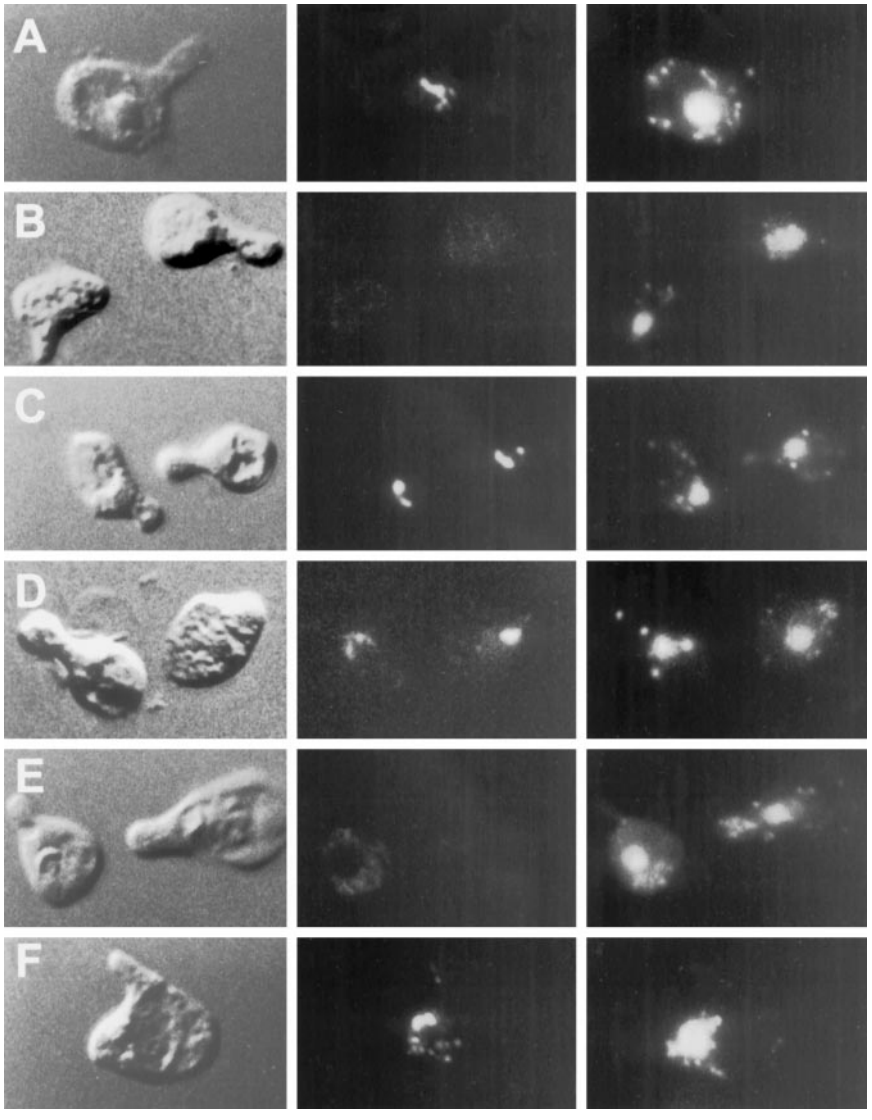


Figure 4. Kar5p is mislocalized in *kar7-1039* but not in other karyogamy mutants. In each series (A–F), the left panel shows the morphology of the cell (shmoo) by DIC. The middle panel shows the Kar5p immunofluorescence, and the right panel shows the nucleus stained with DAPI. (A) Kar5 shmoos (MS3987); (B) *kar5*Δ*2* shmoos (MS3986); (C) *kar1-1* shmoos (MS4021); (D) *kar2-1* shmoos (MS4020); (E) *kar7-1039* shmoos (MS3991); (F) *kar8-1333* shmoos (MS3989). Cells were treated with α-factor for 2–2.5 hr before preparation for immunofluorescence. A and B are reproduced from Beh *et al*. (1997) J. Cell. Biol. *139*, 1063–1076, by copyright permission of The Rockefeller University Press.

out the possibility, suggested from other experiments, that some functional Kar5p is made in the *kar7* mutant. We conclude that *KAR7/SEC71* is required for the synthesis and/or stability of Kar5p.

KAR8 Is Identical to JEM1

The *kar8-1333* mutation was identified as a class II mutation in the same screen as *kar7-1039* (Kurihara *et al*., 1994). The *kar8-1333* mutation results in a strong bilateral mating defect in which diploid formation is reduced at least 270-fold compared with wild type (Kurihara *et al*., 1994). The *kar8-1333* mutation also results in membrane fusion defects assayed by the in vitro ER membrane fusion assay (Kurihara *et al*., 1994).

KAR8 was cloned by complementation of the *kar8-1333* mating defect (see MATERIALS and METHODS). Seven candidate plasmids that completely restored mating ability were isolated. These plasmids shared common restriction fragments from a genomic location that was physically mapped to chromosome X near *ARG3*. Subcloning and deletion analysis showed that a plasmid (pMR3260) containing a recently characterized gene, *JEM1*, was able to rescue the *kar8-1333* mating defect (Figure 2B). Linkage analysis showed a tight linkage between the isolated complementing DNA containing *JEM1* and the *KAR8* locus, indicating that *KAR8* is allelic to *JEM1* (see MATERIALS and METHODS). To confirm this, we generated *MAT***a** and *MATα kar8/jem1*Δ strains and found that they exhibited a karyogamy defect indistinguishable from that of *kar8-1333* (Table 6).

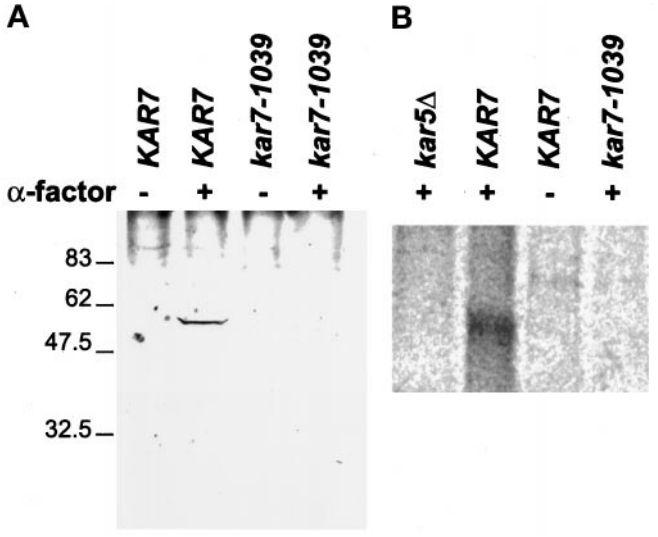
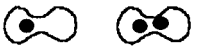


Figure 5. Kar5p was not detected in the *kar7-1039* mutant. (A) Western blot analysis. The strains *kar5*Δ*2* and *kar7-1039* transformed with 2μ *KAR5* plasmid (MS3987 and MS3991) were treated (+) or left untreated (−) with α-factor for 2 hr. Total protein extracts were analyzed by Western blot using affinity-purified anti-Kar5p antibodies. (B) Pulse labeling. MS3986 and MS3987, *kar5*Δ*2* strains transformed with either vector (lane 1) or 2μ *KAR5* plasmid (lanes 2 and 3), and MS3991, a *kar7-1039* strain transformed with 2μ *KAR5* plasmid (lane 4), were ^{35}S pulsed for 5 min at 23°C after treatment (+) or no treatment (−) with α-factor. Pulse-labeled extracts were immunoprecipitated with crude anti-Kar5p antibodies, run on a polyacrylamide gel, and visualized by PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Table 6. Nuclear fusion defect of *kar8-1333* and *jem1/kar8*Δ by microscopic analysis of zygotes

	wt	Kar⁻
mating		
wild type x wild type	98	2
wild type x *kar8-1333*	92	8
kar8-1333 x *kar8-1333*	8	92
*jem1*Δ x *jem1*Δ	8	92
*jem1*Δ x *kar8-1333*	2	98

Mating mixtures were stained with DAPI, and the phenotypes of the zygotes were observed by microscopy. Numbers represent percentages of wild-type (wt) or karyogamy-defective class II (Kar⁻) zygotes. At least 200 zygotes were analyzed. The matings were as follows: wild type × wild type (MS1554 × MS52); wild type × *kar8-1333* (MS1554 × MS3536); *kar8-1333* × *kar8-1333* (MS3260 × MS3536); *jem1*Δ × *jem1*Δ (MS4342 × MS4338); and *jem1*Δ × *kar8-1333* (MS4342 × MS3536).

KAR8/JEM1 Genetically Interacts with KAR2

Because *KAR8/JEM1* is a DnaJ homologue, and DnaJ proteins are predicted to interact with Hsp70/DnaK, we investigated whether Kar8/Jem1p performs its karyogamy function by interacting with Kar2p, the ER-lumenal BiP/Hsp70 of yeast. To this end, we examined whether overexpression of *KAR8/JEM1* from a 2μ plasmid could relieve the mating defect of *kar2-1*, a point mutant in *KAR2*, which specifically results in a strong unilateral karyogamy defect (Table 7). A *kar2-1* × wild-type mating resulted in mostly Kar⁻ zygotes (74%). When a *kar2-1* strain transformed with a *KAR8/JEM1* 2μ plasmid was mated to wild type, we observed a greater than twofold increase in the number of Kar⁺ zygotes (from 26 to 62%). Thus, overexpression of *KAR8/JEM1* suppressed the unilateral mating defect of a *kar2-1* mutant.

Given that Sec63p interacts with Kar2p during protein translocation, we asked whether overexpression of *SEC63* could similarly relieve the karyogamy defect of *kar2-1*. As shown in Table 7, a *kar2-1* strain containing a *SEC63* 2μ plasmid showed only a slight increase in the occurrence of Kar⁺ phenotype (33% Kar⁺ compared with 26% for vector control). We then asked whether overexpression of *SEC63* could relieve the karyogamy defect of *kar8-1333*. As shown in Table 7, a *kar8-1333* × *kar8-1333* mating in which both mating partners carry a *SEC63* 2μ plasmid did not show a significant increase in the number of Kar⁺ zygotes (13% Kar⁺ compared with 10% for the vector control). We therefore concluded that *SEC63* overexpression did not relieve the bilateral *kar8-1333* karyogamy defect. To further investigate whether *SEC63* and *JEM1* have overlapping karyogamy functions, we also tested whether *JEM1* overexpression could relieve the karyogamy defect of a *sec63-201* × *sec63-201* mating. The *sec63-201* allele is a truncation mutant of *SEC63* that results in a strong mating defect (Ng and Walter, 1996). As shown in Table 7, *JEM1* overexpression had no effect on the karyogamy phenotype of *sec63-201* × *sec63-201* zygotes (48% Kar⁺ zygotes when *JEM1* was overexpressed compared with 50% for the vector control). In contrast, 80% of zygotes were Kar⁺ when *SEC63* was overexpressed. Taken together, these results suggest that *SEC63* and *KAR8/JEM1* perform distinct and different roles in karyogamy. Moreover, because *KAR8/JEM1* overexpression suppresses the *kar2-1* mating defect to a much greater degree than *SEC63*, we propose that Kar2p specifically interacts with Kar8/Jem1p for nuclear fusion.

Cells deleted for *KAR8/JEM1* did not show a detectable growth defect (Nishikawa and Endo, 1997; our unpublished observations). However, deletion of *JEM1* in combination with a deletion of *SCJ1*, encoding another lumenal ER DnaJ homologue, resulted in a temperature-sensitive phenotype, suggesting that

Table 7. Dosage suppression of *kar2*, *kar8*, and *sec63* mutants

mating	plasmid	wt 	Kar⁻ 
wild type x wild type	**vector**	98	2
kar2-1 x wild type	vector	**26**	**74**
kar2-1 x wild type	*JEM1-CEN*	24	76
kar2-1 x wild type	*JEM1*-2μ	**62**	**38**
kar2-1 x wild type	*SEC63-CEN*	19	**81**
kar2-1 x wild type	*SEC63*-2μ	33	67
***kar2-1* x wild type**	***SCJ1*-2μ**	**32**	**68**
kar8-1333 x *kar8-1333*	vector	10	90
kar8-1333 x *kar8-1333*	*SEC63-CEN*	14	86
kar8-1333 x *kar8-1333*	*SEC63*-2μ	13	87
kar8-1333* x *kar8-1333	***SCJ1*-2μ**	**13**	**87**
sec63-201* x *sec63-201	**vector**	**50**	**50**
sec63-201* x *sec63-201	***JEM1-CEN***	**54**	**46**
sec63-201* x *sec63-201	***JEM1*-2μ**	**48**	**52**
sec63-201* x *sec63-201	***SEC63*-2μ**	**80**	**20**

Mating mixtures were stained with DAPI, and the phenotypes of the zygotes were observed by microscopy. Numbers represent percentages of wild-type (wt) or karyogamy-defective class II (Kar⁻) zygotes. At least 200 zygotes were analyzed. The matings were as follows: wild type × wild type (MS1554 × MS52) transformed with a vector control (pRS426); *kar2-1* × wild type (MS1111 × MS31) in which MS1111 was transformed with vector (pRS426), *JEM1-CEN* (pMR3260), *JEM1* 2μ (pMR 3270), *SEC63-CEN* (pCen63), *SEC63* 2μ (pDF15), or *SCJ1* 2μ (pPS720); *kar8-1333* × *kar8-1333* (MS 3928 × MS3536) transformed with vector (pRS426), *SEC63-CEN* (pCen63), *SEC63* 2μ (pDF15), or *SCJ1* 2μ (pPS720); and *sec63-201* × *sec63-201* (DNY65 × DNY66) transformed with vector (pRS425), *JEM1-CEN* (pMR 3369), *JEM1* 2μ (pMR3352), or *SEC63* 2μ (pDF14). In the *kar2-1* × wild-type cross, only the *kar2-1* parent was transformed with the indicated plasmids. In the case of the *kar8-1333* × *kar8-1333* and *sec63-201* × *sec63-201* matings, both parents were transformed with the indicated plasmids. Data for the table were gathered from several independent experiments.

KAR8/JEM1 has a vegetative function that is partially redundant with that of *SCJ1* (Nishikawa and Endo, 1997; our unpublished observations). Recent evidence suggests that *SCJ1* and to a lesser extent *JEM1* are required for protein folding in the ER lumen under stress conditions (Silberstein *et al*., 1998). Given that *SCJ1* and *KAR8/JEM1* may have overlapping functions during vegetative growth, we next determined whether they might interact during mating. Because *SCJ1* is not required for karyogamy (Nishikawa and Endo, 1997), and the *kar8/jem1* defect is very strong, an increased severity of the karyogamy defect in the double mutant might not be readily apparent. Therefore, we determined whether increased dosage of *SCJ1* suppresses the Kar⁻ phenotype of *kar2* and *kar8* mutations. As shown in Table 7, overexpression of *SCJ1* did not suppress the karyogamy defect of either *kar2* or *kar8* mutants. These results imply that *SCJ1* cannot substitute for *KAR8/JEM1* and that Kar8/Jem1p's suppression of *kar2* mutations is specific.

*scj1*Δ has been shown to be synthetically lethal with *kar2-159*, suggesting that *SCJ1* may have a role in protein translocation (Schlenstedt *et al*., 1995) like *SEC63*. Given the apparent overlap of vegetative function between Kar8/Jem1p and Scj1p, we next wanted to determine whether *KAR8/JEM1* also has vegetative functions in common with *SEC63*. To address this question we tested whether overexpression of *KAR8/JEM1* could substitute for *SEC63* or alleviate the temperature sensitivity of two *sec63* alleles. The growth of *sec63-1* and *sec63-4* strains transformed with vector control, *KAR8/JEM1* 2μ, or a *SEC63 CEN* plasmid was assessed at 23, 30, and 37°C. Whereas *sec63* mutants containing a *SEC63 CEN* plasmid grew at all temperatures, strains containing the *KAR8/JEM1* 2μ plasmid or vector control did not grow at 37°C, indicating that *KAR8/JEM1* function could not substitute for *SEC63*. In addition, we found that high-copy *KAR8/JEM1* could not restore viability to a *sec63* null at any temperature (our unpublished results). These data suggest that *SEC63* and *JEM1* have different vegetative functions.

Ultrastructural Analysis of Class II Mutants Reveals Two Distinct Phenotypic Classes

EM examination of class II mutants showed that although the nuclei did not fuse, they made direct contact through one or two membranous bridges that spanned the gap between the two nuclei (Kurihara *et al*., 1994). To extend the data reported by Kurihara *et al.* (1994), we carefully examined the phenotype of *kar8* mutant zygotes by EM. In 35 *kar8*Δ zygotes in which the nuclei were closely apposed, 12 had a bridge in which a lumen could be easily seen. Figure 6 shows electron micrographs from serial sections of two *kar8*Δ zygotes. The bridges that connected the two nuclei contained a significant lumen that traversed as many as six serial sections, as much as 400 nm (Figure 6, A–C and D–F). The lumen of the bridges also appeared to be continuous with the lumen between the inner and outer nuclear envelopes. In contrast, the morphology of the bridges in *kar2*, *kar5*, and *kar7/sec71* mutants was different from that in the *kar8/*

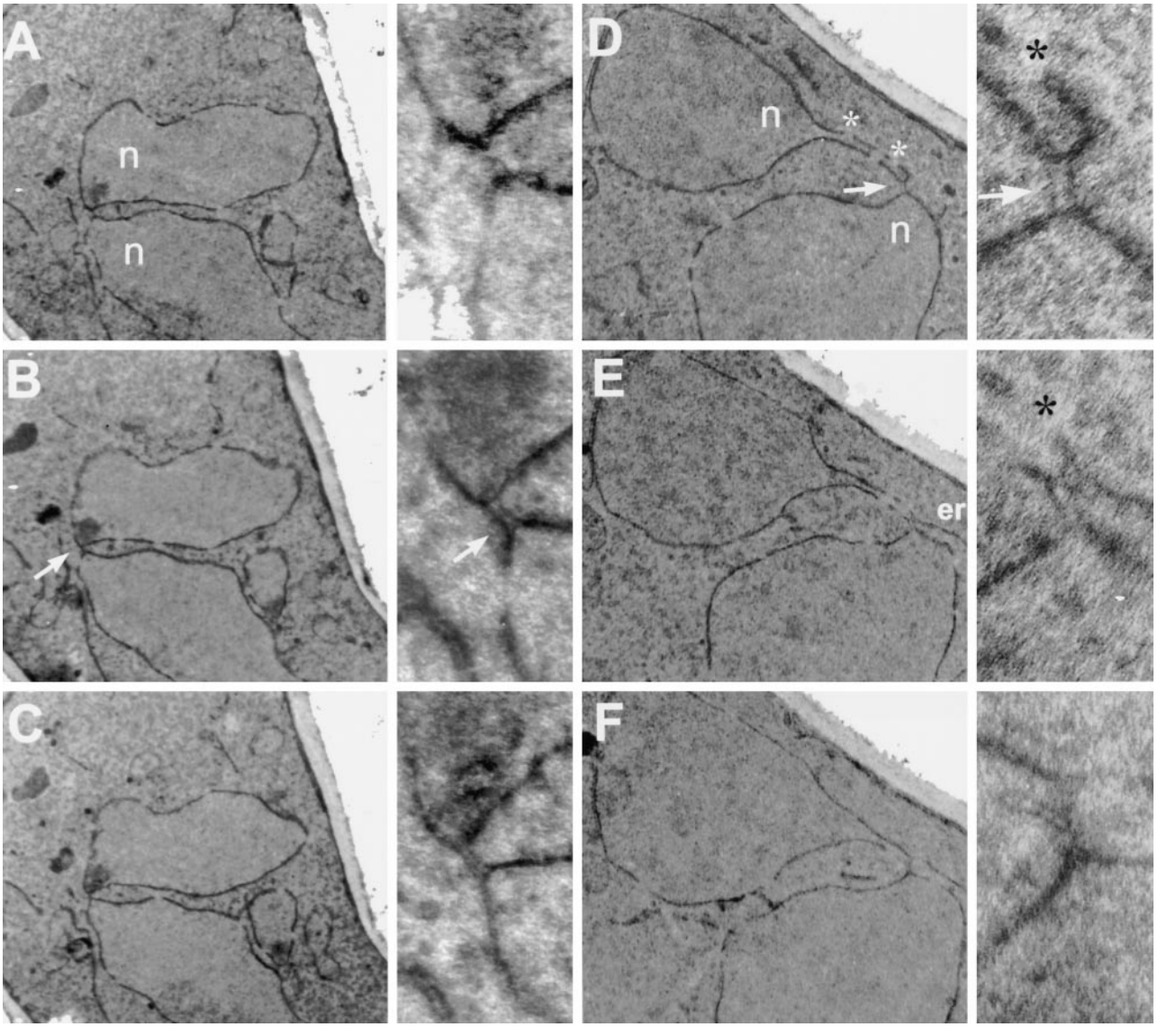


Figure 6. Electron micrographs of serial section of two *kar8-1333* zygotes. The *kar8-1333* mating partners used in this study were MS2705 and MS2706. (A–C) Micrographs of three consecutive serial sections (of seven) through a *kar8-1333* mutant zygote shown at two different magnifications. (D–F) Micrographs of sections 1, 3, and 5 (of five) of another *kar8-1333* mutant zygote also shown at two different magnifications. Each section is 70 nm thick. n, nuclei; *, nuclear pores; arrows, *kar8-1333* bridges between the two nuclei.

jem1 mutants. In *kar2*, *kar5*, and *kar7* mutant zygotes, the bridges had no apparent lumen and were entirely contained within a single section of 70 nm (Kurihara *et al.*, 1994; Beh, 1996). Based on the different morphologies of fusion bridges, we reasoned that Kar2p, Kar5p, Kar7p, and Kar8p might be required at different steps during nuclear fusion, and more specifically, that Kar8p function is required later in the nuclear fusion pathway. To test this idea, we constructed a *kar5*Δ *kar8*Δ double mutant and examined the morphology of the membranous bridges in the mutant zygotes. If Kar5p acts before Kar8p, then the bridges in the *kar5*Δ *kar8*Δ double mutant zygotes should resemble those in the *kar5*Δ mutant. If Kar8p acts before Kar5p, then the double mutant should resemble the *kar8*Δ mutant. Of 100 *kar5*Δ *kar8*Δ mutant zygotes examined, 5 had fusion bridges, all of which resembled that of *kar5*Δ single mutants (Figure 7, A–C). These observations are consistent with the idea that Kar8p acts downstream of Kar5p.

DISCUSSION

We cloned two genes, *KAR7* and *KAR8*, involved in the fusion of the nuclear envelopes during karyogamy, and

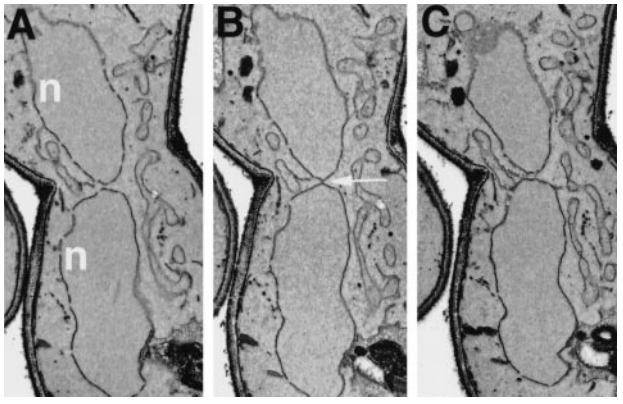


Figure 7. Electron micrographs of serial sections through a *kar5*Δ *kar8*Δ double mutant zygote. The *kar5*Δ *kar8*Δ mating partners used in this study were MS4359 and MS4360. (A–C) Micrographs of three consecutive 90-nm-thick serial sections through a *kar5*Δ *kar8*Δ mutant zygote. n, nuclei; arrows, morphology of the bridges observed in *kar5*Δ *kar8*Δ zygotes.

describe their genetic interactions with *KAR2* and *KAR5*. We found *KAR7* to be allelic to *SEC71*, a gene important for the posttranslational transport of a subset of protein precursors into the ER. In addition to *KAR2* and *SEC71*, we and others have found that two other components of the translocation machinery, *SEC63* and *SEC72*, are also required for efficient nuclear fusion in vivo (Ng and Walter, 1996). However, in vitro, membranes from the *sec63-1* mutant showed no defect, and membranes lacking Sec71p and Sec72p showed reduced fusion competence only at elevated temperature. These results suggest that Sec63p is not directly required for nuclear membrane fusion and that Sec71p and Sec72p are required to stabilize a protein or complex required for nuclear membrane fusion. Genetic interactions between *kar7/sec71* and *kar5* mutations suggested that Kar7/Sec71p and Kar5p interact during nuclear fusion. Characterization of Kar5p in the *kar7/sec71* mutants suggests that Kar7/Sec71p is required for the synthesis and/or stability of Kar5p. Cloning of *KAR8* revealed that it is allelic to *JEM1*, which encodes an ER DnaJ-like protein shown recently to be required for nuclear fusion (Nishikawa and Endo, 1997). *KAR8/JEM1* is therefore the second DnaJ homologue involved in nuclear fusion. Suppression experiments suggest that *KAR8/JEM1* has a unique function in karyogamy acting in conjunction with *KAR2*, which cannot be substituted by *SEC63* or *SCJ1*.

Protein Translocation Components and Nuclear Fusion

A combination of genetic and biochemical approaches have been used to identify factors involved in protein translocation across the ER in yeast (see review of Lyman and Schekman, 1996). *SEC61, SEC62*, and *SEC63* encode essential ER integral membrane proteins with multiple membrane-spanning domains (Deshaies and Schekman, 1987, 1989; Rothblatt *et al*., 1989; Sadler *et al*., 1989; Stirling *et al*., 1992). In contrast, *SEC71* encodes an ER transmembrane protein required for growth at 37°C, and *SEC72* encodes a peripheral ER membrane protein that is not essential at any temperature (Feldheim *et al*., 1993; Kurihara and Silver, 1993; Feldheim and Schekman, 1994). Although *SEC71* and *SEC72* are nonessential genes, they are important for the translocation of a subset of protein precursors (Green *et al*., 1992; Feldheim *et al*., 1993; Kurihara and Silver, 1993).

Together with Sss1p and Sbh1p, Sec61p forms the core translocation pore complex (Esnault *et al*., 1994; Panzner *et al*., 1995). Sec63p is found in complexes with Sec62p, Sec71p, and Sec72p (called the Sec62/Sec63p complex) and with Sec71p, Sec72p, and Kar2p (called the Sec63p–BiP complex; Deshaies *et al*., 1991; Brodsky and Schekman, 1993). In translocation, Sec62p, Sec71p, and Sec72p seem to act early, probably as a membrane-bound receptor that binds the precursor proteins before the precursor–Sec61p complex interaction (Sanders *et al*., 1992; Feldheim *et al*., 1993; Fang and Green, 1994). Sec63p interacts with Kar2p through its DnaJ domain, and together they act both early, to activate the pore to receive precursor, and late, to facilitate the release of the translocating precursor into the lumenal side of the ER (Lyman and Schekman, 1995, Lyman and Schekman 1997).

Kar2p was the first protein shown to be required for both ER protein translocation and nuclear fusion (Polaina and Conde, 1982; Rose *et al*., 1989; Vogel *et al*., 1990; Sanders *et al*., 1992). We found that *KAR7* is allelic to *SEC71* and that mutations affecting two other translocation components, *sec63-1*, and to a lesser extent *sec72*Δ, resulted in moderate defects in nuclear fusion. These results confirm the basic observations of Ng and Walter (1996) that a truncation mutation of *SEC63* caused a severe karyogamy defect and that *SEC71* and *SEC72* are also required for nuclear fusion. However, we found that *sec72*Δ had a much less severe defect in karyogamy than mutations in *KAR7/SEC71*. Furthermore, we found that deletions of *kar7/sec71* and *sec72* resulted in only a moderate temperature-sensitive defect in vitro. These results suggest that nuclear membrane fusion does not specifically require either of these proteins, but instead they may serve to stabilize a fusion complex. Furthermore, the finding that membranes from the *sec63-1* mutant were not defective in vitro suggests that this protein is also not directly required.

In contrast to the other genes required for translocation, we did not detect significant karyogamy defects in mutant strains containing *sec61-2* or *sec62-1*. This was true even though the matings were performed under semipermissive conditions in which the temperature-sensitive mutations were clearly reduc-

ing the overall efficiency of mating. Therefore, it is very unlikely that the nuclear fusion defects arise from defects in translocation per se. We conclude that some, but not all, of the components of the translocation machinery are used in the nuclear membrane fusion pathway.

Sec71p's Role in Nuclear Fusion Might Be Mediated through Kar5p

Why does nuclear fusion require components of the protein translocation machinery? Do the shared components directly participate in the fusion process, or are they required to assemble or stabilize the real fusogenic apparatus? To address some of these issues, we investigated the genetic interaction between mutations in *KAR7/SEC71* and *KAR5*, a pheromone-inducible gene that seems to be specifically required for nuclear fusion (Beh *et al.*, 1997). *KAR7/SEC71* and *KAR5* mutants exhibit a "synthetic bilateral" mating defect. That is, in matings between the two different mutants, one or the other mutant behaves as if it were defective for both proteins. Kar5p, which normally localizes to the SPB (the site for nuclear fusion), is absent in *kar7-1039* but not in other *kar* mutant strains. Furthermore, both Western blot and pulse label analysis could not detect Kar5p in *kar7-1039*. Therefore we concluded that the karyogamy defect of *kar7/sec71* mutants is most likely due to reduced levels of Kar5p, consistent with the genetic data.

The failure to detect Kar5p in the *kar7-1039* mutant after pulse labeling at the permissive temperature suggests that Kar7/Sec71p is required for the synthesis of Kar5p. One obvious possibility is that Kar7p/Sec71p is required for the translocation of Kar5p. Our inability to detect a precursor is consistent with two models. First, Kar5p might be synthesized, but not translocated, resulting in its rapid degradation in the mutant. Alternatively, translation and translocation might be tightly coupled, such that Kar5p is not synthesized in the *kar7/sec71* mutant. If so, the synthesis and translocation of Kar5p would be unusual in being strongly dependent on Sec71p and not on other components of the translocation machinery.

However, other data suggest a more complex role for Kar7/Sec71p in nuclear fusion. First, in vivo, the *kar5*Δ mutant exhibited a somewhat more severe nuclear fusion defect than *kar7/sec71*Δ. Second, in the nuclear envelope–ER membrane fusion assay, the *kar5*Δ mutant exhibited a much more severe defect than *kar7/sec71*Δ. In both cases the *kar7/sec71*Δ defect was temperature sensitive. Taken together these data suggest that residual Kar5p was present and functional in the *kar7/sec71*Δ mutant, but its activity was compromised at the higher temperature. This would be consistent with a separate assembly and stabilization function for Kar7/Sec71p.

A role for Kar7/Sec71p in assembly and stabilization during nuclear fusion would be similar to its role in protein translocation. One of the functions of the Sec62p/Sec63p/Sec71p/Sec72p complex is to stimulate the formation of the Sec61p complex required for protein translocation (Hanein *et al.*, 1996). Likewise, a subset of these proteins including Sec71p and Sec72p might be required for the assembly of a protein complex, including Kar5p, which mediates nuclear membrane fusion. Alternatively, Kar7/Sec71p and Sec72p might function as auxiliary components of a complex that is directly involved in the fusion mechanism. The in vitro assay identified Kar2p, Kar5p, and Kar8p as being required for ER–nuclear envelope membrane fusion (Kurihara *et al.*, 1994; Latterich and Schekman, 1994). Possibly these proteins form a chaperone complex, stabilized by Kar7/Sec71p and Sec72p, that help mediate membrane fusion. A chaperone complex might be required to facilitate conformational changes of the nuclear fusion complex during membrane fusion.

Kar2p and DnaJ Partners

The interaction between Kar2p and Sec63p through the DnaJ homology domain has been well documented genetically and biochemically (Feldheim *et al.*, 1992; Brodsky and Schekman, 1993; Scidmore *et al.*, 1993). The *sec63-1* mutation maps to the DnaJ loop and disrupts the interaction with Kar2p (Nelson *et al.*, 1993; Brodsky and Schekman, 1993). Accordingly, *sec63-1* causes a strong protein translocation defect both in vivo and in vitro. Although *sec63-1* caused a moderate defect in karyogamy, in vivo, it had no effect on nuclear envelope–ER fusion, in vitro. The specificity of the genetic interaction between *sec63-1* and *kar2* mutations has been extensively explored (Scidmore *et al.*, 1993). Remarkably, *sec63-1* was synthetically lethal with *kar2* alleles that have a severe defect in translocation but not with alleles that have a more severe defect in nuclear fusion. Taken together with the biochemical data, these results suggests that Sec63p is the DnaJ partner for Kar2p's role in protein translocation, but that Sec63p is not likely to be the major DnaJ partner for Kar2p's role in nuclear fusion.

In contrast to Sec63p, there is strong evidence in favor of Kar8/Jem1p playing a major role in nuclear fusion. First, unlike *sec63-1*, *kar8/jem1* mutants exhibit a strong defect in nuclear fusion both in vivo and in vitro (Kurihara *et al.*, 1994; Nishikawa and Endo, 1997). Second, overexpression of Kar8/Jem1p, but not Sec63p, suppressed the karyogamy defect of *kar2-1*. Taken together, these results suggest that Kar8/Jem1p is the major DnaJ partner for Kar2p in nuclear fusion.

Nevertheless, the defects observed for both *sec63-1* and *sec63-201* (Ng and Walter, 1996) suggest that Sec63p does play a significant role in nuclear fusion.

Given that Sec63p interacts with Kar2p for translocation, it is possible that the mutant proteins interact with Kar2p in a way that then interferes with Kar2p's later interaction with Kar8p. This seems unlikely given that the *sec63-1* mutation blocks the interaction between Sec63p and Kar2p (Brodsky and Schekman, 1993). Interestingly, *sec63-201* and *sec63-1* affect different domains of Sec63p, with *sec63-201* truncating the carboxyl-terminal 27 residues of the cytoplasmic domain and *sec63-1* mapping to the lumenal DnaJ domain. In vitro experiments suggest that these two domains of Sec63p have different functions during translocation (Lyman and Schekman, 1995). An intriguing possibility is that Sec63p's role in nuclear fusion is mediated through its cytoplasmic domain and not through its DnaJ domain. If so, Sec63p's role in nuclear fusion would likely be independent of Kar2p, consistent with our genetic data. In addition, Ng and Walter (1996) reported a mutation, *sos1-1*, which suppressed the translocation, but not the karyogamy, defect of *sec63-201.*

Regardless of the specific function of Sec63p, a second issue concerns when each DnaJ protein might act during nuclear fusion. Based on the genetic data, it seems unlikely that Kar8/Jem1p and Sec63p interact with Kar2p to perform similar functions at the same step in the nuclear fusion process. More likely Kar8/Jem1p and Sec63p have different roles, and each may be required at different steps in the karyogamy pathway. In this model, Kar2p may act at more than one step in nuclear fusion, possibly interacting first with one DnaJ and then the other. In this model, mutations in each DnaJ homologue might have more or less severe effects depending on the stringency of the requirement for that step in vivo and in vitro.

In this regard, it is striking that the membrane bridges observed by EM in *kar2* and in *kar5* mutants are quite different from the bridges observed for the *kar8/jem1* mutant. The *kar8/jem1* bridges had distinct lumens extending >400 nm of the nuclear surface. In contrast, *kar2* and *kar5* bridges had no detectable lumen and extended over less than one section, most likely no more than 10 nm. Given the morphology of the *kar8/jem1* membranes, it is tempting to propose that Kar8/Jem1p acts later in the pathway, after the initial stages of membrane fusion. The presence of large bridges in *kar8* mutants may indicate that Kar8/Jem1p is required to resolve such structures at a late step in nuclear membrane fusion. Kar2p, Kar5p, and Kar7/Sec71p may be required for the formation of the initial structures leading to *kar8/jem1* bridges. If Kar2p acts at more than one step in the nuclear fusion pathway, as it does in translocation, then the EM analysis would only reveal its earliest point of action. Alternatively, the *kar8/jem1* bridges may reflect aberrant intermediates that form as a result of the loss of Kar8/Jem1p function. In either case, the fact that the *kar8/jem1* bridges do not form in a *kar5* mutant suggests that the *kar8/jem1* bridges arise from a later step in the fusion pathway.

Conclusion

Several ER–nuclear envelope proteins are required for the fusion of the nuclear envelope during conjugation of *S. cerevisiae*. Three proteins, Kar2p, Kar5p, and Kar8/Jem1p, are clearly required for nuclear fusion, both in vitro and in vivo. Three other proteins, Kar7/Sec71p, Sec72p, and Sec63p, appear to play secondary roles, with Kar7/Sec71p and Sec72p acting to stabilize a complex required for nuclear fusion. Of these, Kar2p, Kar7/Sec71p, Sec72p, and Sec63p have dual roles in protein translocation and nuclear fusion. In contrast, two proteins with major roles in protein translocation, Sec61p and Sec62p, were not required for nuclear fusion. These results suggest the existence of a novel chaperone complex, including Kar5p, Kar2p, and Kar8/Jem1p, and possibly Kar7/Sec71p and Sec72p required for nuclear fusion, which is distinct from the chaperone complexes mediating protein translocation and folding.

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