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Genetic Interactions Between *KAR2* and *SEC63*, Encoding Eukaryotic Homologues of DnaK and DnaJ in the Endoplasmic Reticulum

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KAR2 encodes the yeast homologue of mammalian BiP, the endoplasmic reticulum (ER) resident member of the HSP70 family. Kar2p has been shown to be required for the translocation of proteins across the ER membrane as well as nuclear fusion. Sec63p, an ER integral membrane protein that shares homology with the *Escherichia coli* DnaJ protein, is also required for translocation. In this paper we describe several specific genetic interactions between these two proteins, Kar2p and Sec63p. First, temperature-sensitive mutations in *KAR2* and *SEC63* form synthetic lethal combinations. Second, dominant mutations in *KAR2* are allele-specific suppressors for the temperature-sensitive growth and translocation defect of *sec63-1*. Third, the *sec63-1*, unlike other translocation defective mutations, results in the induction of *KAR2* mRNA levels. Taken together, these genetic interactions suggest that Kar2p and Sec63p interact in vivo in a manner similar to that of the *E. coli* HSP70, DnaK, and DnaJ. We propose that the interaction between these two proteins is critical to their function in protein translocation.

INTRODUCTION

KAR2 is an essential member of the yeast *Saccharomyces cerevisiae* heat shock protein (HSP70) family (reviewed in Craig, 1985) that is localized to the lumen of the endoplasmic reticulum (ER) (Normington *et al.*, 1989; Rose *et al.*, 1989). Specifically, *KAR2* encodes the yeast homologue of BiP/GRP78 (Normington *et al.*, 1989; Rose *et al.*, 1989), which was originally identified in mammalian cells by its binding to immunoglobulin precursors (Morrison and Scharff, 1975; Haas and Wabl, 1983). BiP binds stably to misfolded (Sharma *et al.*, 1985; Copeland *et al.*, 1986; Gething *et al.*, 1986) or underglycosylated secretory proteins (Dorner *et al.*, 1987; Kassenbrock *et al.*, 1988) and transiently to assembly intermediates (Bole *et al.*, 1986; Gething *et al.*, 1986; Dorner *et al.*, 1987). BiP/GRP78 was independently identified as a protein (GRP78) induced in mammalian cells by glucose starvation (Pouysségur *et al.*, 1977; Shiu *et al.*, 1987) and a number of other treatments that perturb protein folding and modification in the ER (reviewed in Lee, 1987; Kozutsumi *et al.*, 1988). Models for BiP function in mammalian cells include the melting of protein-protein aggregates in the lumen of the ER (Munro and Pelham, 1986), the oligomerization of multimeric proteins (Bole *et al.*, 1986; Gething *et al.*, 1986; Munro and Pelham, 1986), and the scavenging of misfolded and unassembled secretory proteins (Bole *et al.*, 1986; Gething *et al.*, 1986; Hendershot *et al.*, 1987; Hurtley *et al.*, 1989). Although *KAR2* was originally identified in yeast by a mutation, *kar2-1*, that is defective for karyogamy (Polaina and Conde, 1982), *KAR2* is required for the translocation of secretory proteins across the ER membrane (Vogel *et al.*, 1990).

The translocation of secretory proteins across the ER has been studied intensively by a combination of genetic and biochemical approaches in yeast. In addition to Kar2p, a number of proteins resident in the cytoplasm and the ER membrane have been shown to function in translocation. The cytoplasmic family of HSP70 proteins encoded by the *SSA1-4* genes are required for efficient translocation in vivo and in vitro (Chirico *et al.*, 1988; Deshaies *et al.*, 1988) and have been proposed to act as chaperones to maintain precursors in a nonaggregated state (Pelham, 1986). Several components of a complex similar to the mammalian signal recognition particle (SRP) have been identified in yeast. Three genes, *SRP54*

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(Hann *et al.*, 1989; Amaya *et al.*, 1990), encoding the 54-kDa subunit; *SEC65* (Hann *et al.*, 1992; Stirling and Hewitt, 1992), encoding the 19-kDa subunit, and *SRP101* (Ogg *et al.*, 1992), an ER membrane protein, as well as *SCR1* (Felici *et al.*, 1989; Hann and Walter, 1991), an SRP RNA, are important but not essential for the translocation of secretory proteins.

Mutations in several essential genes including *SEC61* (Deshaies and Schekman, 1987), *SEC62* (Rothblatt *et al.*, 1989), and *SEC63* (Toyn *et al.*, 1988; Rothblatt *et al.*, 1989) lead to a block in translocation. Sec61p, Sec62p, and Sec63p are integral membrane proteins found in a complex with two proteins of molecular masses of 23 and 31.5 kDa (Deshaies and Schekman, 1990; Deshaies *et al.*, 1991) as demonstrated by DNA sequence (Deshaies and Schekman, 1989; Sadler *et al.*, 1989; Stirling *et al.*, 1992), genetic interactions (Rothblatt *et al.*, 1989), cross-linking studies (Deshaies *et al.*, 1991), and copurification data (Deshaies *et al.*, 1991). Consistent with their requirement in vivo, membranes from *kar2* (Sanders *et al.*, 1992), *sec62* (Deshaies and Schekman, 1989) and *sec63* (Toyn *et al.*, 1988; Rothblatt *et al.*, 1989) mutant strains are defective for translocation in vitro. In addition, Sec61p, Sec62p, and Kar2p can be crosslinked to modified secretory proteins trapped during translocation (Müsch *et al.*, 1992; Sanders *et al.*, 1992), providing evidence for the direct involvement of these proteins in translocation in yeast.

The *Escherichia coli* HSP70 homologue, DnaK, acts in concert with two low molecular weight proteins DnaJ and GrpE (reviewed in Georgopoulos *et al.*, 1990) in a variety of cellular functions including the initiation of bacteriophage λ DNA replication (Liberek *et al.*, 1988; Alfano and McMacken, 1989), P1 replication (Tilly and Yarmolinsky, 1989; Wickner, 1990), and protein export (Wild *et al.*, 1992). Purified DnaJ and GrpE stimulate the ATPase of DnaK and form a stable complex in vitro (Liberek *et al.*, 1991).

Several proteins sharing significant homology to *E. coli* DnaJ have been identified in yeast: Sec63p, Scj1p, Ydj1/Mas5p, and Sis1p. Sec63p's homology to DnaJ is restricted to a region of Sec63p localized to the lumen of the ER (Sadler *et al.*, 1989; Feldheim *et al.*, 1992) where it potentially interacts with Kar2p. Scj1p is thought to be localized to the mitochondria (Blumberg and Silver, 1991). Ydj1p/Mas5p is a cytosolic protein (Caplan and Douglas, 1991; Atencio and Jaffe, 1992), and Sis1p is a protein found both in the cytosol and nucleus (Luke *et al.*, 1991). Interaction between Ydj1p and Ssa1p has been demonstrated; purified Ydj1p stimulates the ATPase activity of purified Ssa1p (Cyr *et al.*, 1992). In addition, the affinity of Ssa1p for an in vitro substrate, carboxymethylated-lactalbumin, is regulated by Ydj1p (Cyr *et al.*, 1992). Given the interaction between DnaK and DnaJ in *E. coli* and the interaction between Ssa1p and Ydj1p, the question arises as to whether the other various yeast HSP70s interact with their cognate DnaJ homologues.

This paper addresses whether *KAR2* and *SEC63* interact in vivo in the process of translocation. Numerous temperature sensitive (Ts^-) mutations have been isolated in *KAR2* (Vogel *et al.*, 1990; Misra and Rose, unpublished data) and in *SEC63* (Rothblatt *et al.*, 1989). In addition, a number of temperature sensitive alleles of *SEC63* (*np11* alleles) were isolated as mutants defective in the nuclear localization of hybrid proteins containing both nuclear and mitochondrial targeting signals (Sadler *et al.*, 1989). Using the existing collection of Ts^- mutations in both genes as well as new *KAR2* mutations, we have determined that *KAR2* and *SEC63* show a number of specific genetic interactions. Based upon these data, we propose that Sec63p and Kar2p physically interact in vivo and that their interaction is critical to their function in translocation.

MATERIALS AND METHODS

Microbial Techniques and Strains

Media and genetic techniques were as described in Rose *et al.* (1990). Plasmids were recovered from yeast for transformation into *E. coli* by the method of Hoffman and Winston (1987). Small scale DNA preparations were made by the boiling method of Holmes and Quigley (1981). Yeast transformations were done by the lithium acetate method of Ito (1983) with 50 μg of sheared salmon sperm DNA as a carrier. Yeast strains are listed in Table 1.

Hydroxylamine Mutagenesis and Suppressor Isolation

Dominant *KAR2* suppressors of *sec63-1* were obtained by in vitro hydroxylamine mutagenesis of *KAR2* carried on a *URA3*-based centromere plasmid (pMR397) as described in Rose *et al.* (1990). Mutagenized plasmid was transformed into the *sec63-1* mutant strain (MY2248), and transformants were selected on synthetic complete plates lacking uracil at 23°C. Transformants were replica plated onto plates lacking uracil at 37°C, and suppressors were identified by those transformants now capable of growing at 37°C. Suppressor-containing plasmids were recovered into *E. coli* and retransformed into yeast to confirm the ability to suppress the *sec63-1* allele.

Plasmid Constructions and DNA Sequencing

Using double-stranded plasmid DNA as template and *KAR2*-specific primers, the *Bst*EII-*Bst*EII fragment was sequenced using Sequenase (United States Biochemical, Cleveland, OH) as described by the manufacturer.

Radiolabeling and Immunoprecipitation

Yeast strains were grown in synthetic complete media lacking uracil at 23°C to an OD_{600} = 0.5–1.0. An equivalent of 3 OD units of cells were washed in synthetic complete media lacking uracil, methionine, and cysteine and resuspended in 1 ml of the same media. Cells were grown overnight at 23°C, shifted to 37°C for 2 h, and then pulse-labeled with 150 μC of ^{35}S-Translabel (ICN Radiochemicals, Irvine, CA)/3 OD_{600} for 5 min at 37°C. Labeling was stopped by the addition of NaN_3 to 10 mM and cycloheximide to 100 μg/ml on ice. Cells were washed and resuspended in 0.2 ml of breaking buffer (50 mM tris(hydroxymethyl)aminomethane [Tris]-HCl pH 7.4, 100 mM NaCl, 2 mM EDTA, 1% sodium dodecyl sulfate [SDS], and 1 mM phenyl-

Table 1. Yeast strains used in this study

Strain	Genotype	Source[a]
MS4	*MATa leu2-3 leu2-112 trp1-Δ1*	
MS17	*MATα ura3-52 ade2-101 trp1-Δ1*	
MS21	*MATα ura3-52 trp1-Δ1*	
MS177	*MATα ura3-52 ade2-101 kar2-159*	
MS192	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-113*	
MS193	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-133*	
MS195	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-157*	
MS197	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-190*	
MS945	*MATα ura3-52 ade2-101 kar2-159* [pMR79:*CEN URA3*]	
MS958	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-191*	
MS961	*MATα ura3-52 ade2-101 trp1-Δ1* [pMR79:*CEN URA3*]	
MS965	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-165*	
MS1028	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-203*	
MS1111	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-1*	
MS1379	*MATa ura3-52 lys2-801 his3-Δ200 kar2-159*	
MS1380	*MATα ura3-52 kar2-159*	
MS1383	*MATa ura3-52 ade2-101 his4-539 kar2-159*	
MS1559	*MATα ura3-52 sec63-1* [pMR1642:*CEN URA3 KAR2-669*]	
MS1561	*MATα ura3-52 sec63-1* [pMR1643:*CEN URA3 KAR2-6143*]	
MS1563	*MATα ura3-52 sec63-1* [pMR1647:*CEN URA3 KAR2-609*]	
MS1565	*MATα ura3-52 sec63-1* [pMR1676:*CEN URA3 KAR2-6116*]	
Ms1567	*MATα ura3-52 sec63-1* [pMR1667:*CEN URA3 KAR2-6121*]	
MS1569	*MATα ura3-52 sec63-1* [pMR1678:*CEN URA3 KAR2-6139*]	
MS1571	*MATα ura3-52 sec63-1* [pMR1650:*CEN URA3 KAR2-6199*]	
MS1574	*MATa ura3-52 leu2-3 leu3-112 kar2-159* [pMR397:*CEN URA3 KAR2*]	
MS1722	*MATα leu2-3 leu2-112 trp1Δ1 kar2-159*	
MS1724	*MATa ade2-101 leu2-3 leu2-112 trp1Δ1 kar2-159*	
MS3079	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-133* [pMR397:*CEN URA3 KAR2*]	
MS3080	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-157* [pMR397:*CEN URA3 KAR2*]	
MS3086	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-191* [pMR397:*CEN URA3 KAR2*]	
MS3088	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-203* [pMR397:*CEN URA3 KAR2*]	
MS3089	*MATa ura3-52 ade2-101 leu2-3 leu2-112 kar2-1* [pMR397:*CEN URA3 KAR2*]	
MY2248	*MATα ura3-52 sec63-1*	
MY2632	*MATa ura3-52 leu2-3 leu2-112 kar2-159 sec63-1* [pMR397:*CEN URA3 KAR2*]	
MY2798	*MATα ura3-52 sec63-1* [pMR483:*CEN URA3*]	
MY2799	*MATα ura3-52 sec63-1* [pMR890:2μ *URA3 KAR2*]	
MY2802	*MATα trp1Δ1 sec63-1*	
MY2808	*MATa* trp1Δ1 *sec63-1*	
MY2824	*MATα ura3-52 sec63-1* [pMR397:*CEN URA3 KAR2*]	
MY3216	*MATα ura3-52 sec63-1* [pTK81:*CEN URA3 SEC63*]	
MY3251	*MATa ura3-52 leu2-3 leu2-112 his3Δ200 kar2-159 sec63-1* [pTK81:*CEN URA3 SEC63*]	
RDM15-5B	*MATα ura3-52 leu2-3 leu2-112 ade2 pep4-3 sec61-2*	R. Schekman
RDM50-94C	*MATα ura3-52 leu2-3 leu2-112 his4 sec62-1*	R. Schekman
PSY5	*MATa ura3-1 ade2-1 leu2-3 leu2-112 his3-11 his3-15 trp1 can1-100 cyt1::HIS3 sec63-101*	P. Silver
PSY30	*MATα ura3-1 ade2-1 leu2-3 leu2-112 his3-11 his3-15 trp1 can1-100 cyt1::HIS3 sec63-106*	P. Silver
C65	*MATα ura3-52 leu2-3 leu2-112 trp1-1 his4-401 HOL1-1 sec63-7*	R. Schekman
RSY457	*MATα ura3 ade2 trp1 leu2 his3 sec65-1*	R. Schekman
SEY2109	*MATa ura3-52 leu2-3 leu2-112 suc2-Δ9 prc1Δ::LEU2*	S. Emr

[a] Unless otherwise noted strains were obtained from laboratory collection of M. Rose.

methylsulfonyl fluoride [PMSF]). Cells were broken by vortexing for 5 × 1-min intervals with glass beads. The supernatant was removed, and the beads were washed in 0.2 ml of breaking buffer. The supernatant was boiled for 4 min and centrifuged (12 000 × g) for 10 min to remove the insoluble material. The supernatant equivalent of 0.5 OD units of cells was added to 700 μl of immunoprecipitation dilution buffer (1.25% Triton X-100, 190 mM NaCl, 6 mM EDTA, 60 mM Tris-HCl pH 7.4, and 1 mM PMSF), and 1% SDS was added to a final concentration of 0.3% SDS. Nonradioactive protein extract from a *prc1Δ* strain (SEY2109) was added to the carboxypeptidase Y (CPY) immunoprecipitations at a final concentration of 1 mg/ml to act as a competitor. Antibodies to Kar2p (0.5 μl/0.5 OD_{600}) and CPYp (0.3 μl/0.5 OD_{600}) were added and incubated overnight at 4°C, and immune complexes were precipitated for 1 h at room temperature with the addition of 20 μl of a 50% slurry of protein A Sepharose. Immune complexes were pelleted and washed sequentially in 1 ml each of urea buffer (1% Triton X-100, 0.2% SDS, 2 M urea, 150 mM NaCl, 5 mM EDTA, and 50 mM Tris-HCl pH 7.4), immunoprecipitation buffer (1% Triton X-100, 0.2% SDS, 150 mM NaCl, 5 mM EDTA, and 50 mM Tris-HCl pH 7.4), and low salt buffer (150 mM NaCl, 5 mM EDTA, and 50 mM Tris-HCl pH 7.4). Immunoprecipitated proteins were resuspended in Laemmli buffer, boiled for 4 min, and sep-

Table 2. *kar2-159 sec63-1* double mutants are inviable at 23°C

Cross			Germination temperature	Total tetrads	Segregation of viable spores (viable/inviable) 4/4	3/4	2/4	% Viable
1. *kar2-159* (MS1379)	×	WT (MS21)	23°C	17	16	1	0	98%
2. WT (MS21)	×	*sec63-1* (MY2248)	23°C	7	7	0	0	100%
3. *kar2-159* (MS1379)	×	*sec63-1* (MY2248)	23°C	35	6	22	7	74%
			13°C	10	9	1	0	98%
4. *kar2-159* pMR397 (MS1574)	×	*sec63-1* (MY2248)	23°C	31	16	12	3	86%
5. *kar2-159* (MS1380)	×	*sec63-101* (PSY5)	23°C	11	11	0	0	100%
6. *kar2-159* (MS1383)	×	*sec6-106* (PSY30)	23°C	11	11	0	0	100%
7. *kar2-159* (MS1379)	×	*sec61-2* (RDM15-5B)	23°C	10	10	0	0	100%
8. *kar2-159* (MS1379)	×	*sec62-1* (RDM50-94C)	23°C	10	8	2	0	95%

arated on a 7.5% SDS-polyacrylamide gel. Gels were fixed in 25% isopropanol and 10% acetic acid for 20 min, treated with AMPLIFY (Amersham, Arlington Heights, IL) for 20 min, dried, and then subjected to autoradiography at −70°C. CPY antibodies were generous gift of R. Schekman (University of California Berkeley).

RNA Isolation and Northern Blot Hybridizations

Yeast cultures were pregrown at 23°C in YEPD. One-half the culture was shifted to 37°C for 2 h while the other half remained at 23°C. Approximately 2×10^8 cells were harvested from both the 23°C and 37°C cultures, and total RNA was isolated as described in Rose *et al.* (1990). Five micrograms of total RNA was denatured in formamide and formaldehyde and separated on a 1% agarose gel using 6% formaldehyde in the running buffer. RNAs were blotted to Gene Screen nylon membranes (Du Pont, Wilmington, DE) as described by the manufacturer. A 300-base pair (bp) *Xba* I-*Hin*dIII fragment containing the carboxy-terminal coding sequence of *KAR2* was obtained from plasmid pMR992 for use as a *KAR2*-specific probe. A 282-bp *Eco*RI-*Hin*dIII fragment containing internal coding sequence of the *ACT1* gene was isolated from the plasmid pYST122 (T. Som, Princeton University, NJ) and used as an internal control for normalization of RNA loaded. Probes were prepared using random hexanucleotides (Pharmacia, Piscataway, NJ) to prime DNA synthesis using the Klenow fragment of DNA polymerase I (New England Biolabs, Boston, MA). Hybridization was performed at 65°C in 5× PP_iESS (10× PP_iESS: 1.5 M NaCl, 10 mM $Na_4P_2O_7$, 10 mM Na_2EDTA, 200 mM $Na_2HPO_4 \cdot H_2O$, and 50 mM $NaH_2PO_4 \cdot H_2O$), 0.02% Ficoll, 0.02% polyvinylpyrrolidine, 0.02% bovine serum albumin, 1% SDS, and 100 μg/ml of denatured salmon sperm DNA. Filters were washed once in 0.2× PP_iESS, 0.5% SDS at room temperature for 20 min and once in 0.2× PP_iESS, 0.5% SDS at 65°C for 20 min. Filters were subjected to autoradiography at −70°C using preflashed film.

RESULTS

sec63-1 Displays Synthetic Phenotypes with Mutations in KAR2

A genetic interaction referred to as a "synthetic phenotype" occurs when the combination of two independent mutant loci in a haploid cell produces a more extreme phenotype than either mutant locus produces alone (reviewed in Huffaker *et al.*, 1987). In some cases, the double mutant is inviable under conditions where the single mutants are viable, a phenomenon referred to as "synthetic lethality." Several studies suggest that synthetic lethal interactions can arise when both genes affect a common complex process or act at the same stage of a single pathway (Salmien and Novick, 1987; Rothblatt *et al.*, 1989). Given Kar2p's localization to the ER lumen and its requirement for translocation, the possibility existed that Kar2p interacts with or acts upon the Sec61p, Sec62p, and Sec63p complex. Therefore genetic interactions between temperature-sensitive *KAR2* alleles and representative temperature sensitive *SEC61*, *SEC62*, and *SEC63* alleles were tested. Heterozygous diploids were formed, sporulated, and allowed to germinate at the permissive temperature. The phenotypes of the double mutants were than analyzed.

A synthetic lethal interaction was observed between the *kar2-159* (MS1379) and *sec63-1* alleles (MY2248). Normally, 23°C is the permissive temperature for each single mutant, and spore viability in crosses involving these strains is excellent (Table 2, crosses 1 and 2). In contrast, at 23°C, 26% of the spores from a cross between them were inviable (Table 2, cross 3). The segregation of temperature-sensitive alleles in the viable meiotic progeny suggested that the inviable spores included all of the *kar2-159 sec63-1* double mutants. First, all tetrads containing four viable spores consisted of four Ts^- spores, the parental ditype configuration. Second, all tetrads containing two viable spores consisted

of two Ts^+ spores, corresponding to the nonparental ditype class. Finally, the major class of tetrads consisted of three viable spores of which two were Ts^- and one was Ts^+. Assuming that inviability results from a synthetic lethal interaction, these would correspond to the tetratype asci. Complementation analysis confirmed that all viable spores were either wild-type or carried only one of the temperature-sensitive alleles (Table 3). Therefore, the double *kar2-159 sec63-1* spores were inviable.

Four tetrads appeared that did not fit the simple pattern described above; complementation analysis demonstrated that a few spores other than the double mutants were also inviable. The inviability in these tetrads is presumably because of either random spore death or gene conversion (Table 3).

When spores from the identical cross were germinated at 13°C instead of 23°C, 98% of spores were viable (Table 2, cross 3). Spores containing both *sec63-1* and *kar2-159* as determined by complementation analysis were viable only at 13°C and not at higher temperatures (Table 3). Thus, in this case, synthetic lethality is manifest as a greatly reduced permissive temperature.

Allele specificity of genetic interactions is often interpreted to be an indication of direct interaction. To determine whether the synthetic lethal interaction observed between the *kar2-159* allele and the *sec63-1* allele is allele specific, the *kar2-159* allele was crossed to several other independently isolated *sec63* temperature-sensitive alleles as shown in Table 2, crosses 5 and 6. Greater than 95% spore viability was seen in all these crosses, thereby demonstrating that strict allele specificity exists for this *kar2-159* genetic interaction with respect to *SEC63* alleles. To determine whether the synthetic lethality is also gene specific, double mutants were constructed between *kar2-159* and representative alleles of the other translocation defective mutants (*sec61* and *sec62*) (Table 2, crosses 7 and 8), as well as with other late acting secretory mutants: *sec1, sec7, sec13, sec16, sec17, sec18, sec20, sec21, sec22,* and *sec23*. In all cases, double mutants could be obtained at 23°C, and spore viability from the crosses was high. Additionally, all three tetrad types (PD, NPD, and TT) were obtained from tetrads with four viable spores. Therefore, the synthetic lethal interaction appears to be specific to *SEC63*.

To further explore the specificity of the synthetic lethal interaction between *SEC63* and *KAR2*, we performed crosses between several different temperature-sensitive *kar2* mutants and *sec63-1*. Several other *kar2* Ts^- alleles were found to be synthetically lethal in combination with the *sec63-1* allele at 23°C (Table 4). However, not all Ts^- alleles in *KAR2* were synthetically lethal with the *sec63-1* allele. Viable double mutants were obtained with the *kar2-113, kar2-165,* and *kar2-190* alleles at 23°C (Table 4, crosses 1, 2 and 3). Several, but not all, of the double mutant combinations could be obtained at 13°C.

Table 3. Segregation analysis of spores derived from *kar2-159* × *sec63-1* crosses

Cross	Tetrad class	Number of tetrads	Genotype[a]
kar2-159 × *sec63-1* 23°C	PD	6	2 *kar2-159 SEC63*, 2 *KAR2 sec63-1*
		2	2 *kar2-159 SEC63*, 1 *KAR2 sec63-1* 1 inviable (*KAR2 sec63-1*)[b]
	NPD	5	2 *KAR2 SEC63*, 2 inviable (*kar2-159 sec63-1*)[c]
	TT	20	1 *KAR2 SEC63*, 1 *kar2-159 sec63-1*, 1 *KAR2 sec63-1*, 1 inviable (*kar2-159 sec63-1*)[c]
		1	1 *KAR2 SEC63*, 1 inviable[b] (*kar2-159 SEC63*), 1 *KAR2 sec63-1*, 1 inviable (*kar2-159 sec63-1*)[c]
kar2-159 × *sec63-1* 13°C	PD	2	2 *kar2-159 SEC63*, 2 *KAR2 sec63-1*
		1	1 *kar2-159 SEC63*, 1 inviable (*kar2-159 SEC63*)[b], 2 *KAR2 sec63-1*
	NPD	0	2 *KAR2 SEC63*, 2 (*kar2-159 sec63-1*)
	TT	7	1 *KAR2 SEC63*, 1 *kar2-159 SEC63*, 1 *KAR2 sec63-1*, 1 *kar2-159 sec63-1*[d]
kar2-159 pMR397 × *sec63-1* 23°C	PD	5	2 *kar2-159 SEC63*, 2 *KAR2 sec63-1*
		1	2 *kar2-159 SEC63*, 2 inviable (*KAR2 sec63-1*)[b]
	NPD	3	2 *KAR2 SEC63*, 2 FOA[s] (*kar2-159 sec63-1*)
		2	2 *KAR2 SEC63*, 1 FOA[s] (*kar2-159 sec63-1*) 1 inviable (*kar2-159 sec63-1*)[c]
	TT	10	1 *KAR2 SEC63*, 1 *kar2-159 SEC63*, 1 *KAR2 sec63-1*, 1 inviable (*kar2-159 sec63-1*)[c]
		10	1 *KAR2 SEC63*, 1 *kar2-159 SEC63*, 1 *KAR2 sec63-1*, 1 FOA[s] (*kar2-159 sec63-1*)
		1	1 *KAR2 SEC63*, 1 inviable (*kar2-159 SEC63*)[b], 1 *KAR2 sec63-1*, 1 FOA[s] (*kar2-159 sec63-1*)

PD, parental ditype asci; NPD, tetratype asci; TT, tetratype asci.

[a] Genotype of spores determined by complementation analysis with both *kar2-159* (MS1722 and MS1724) and *sec63-1* strains (MY2802 and MY2808) at 37°C.

[b] Inviability because of random spore death or gene conversion.

[c] Inviability because of synthetic lethal combination of *kar2-159* and *sec63-1*.

[d] *kar2-159 sec63-1* spores viable only at 13°C.

Table 4. Allele specificity of *sec63-1* synthetic lethality with *kar2* mutations

Cross			Germination temperature	Total tetrads	Segregation of viable spores (viable/inviable) 4/4	3/4	2/4	% Viable
1. *kar2-113* (MS192)	×	*sec63-1* (MY2248)	23°C	10	9	1	0	98%
2. *kar2-165* (MS965)	×	*sec63-1* (MY2248)	23°C	9	9	0	0	100%
3. *kar2-190* (MS197)	×	*sec63-1* (MY2248)	23°C	8	8	0	0	100%
4. *kar2-1* (MS1111)	×	*sec63-1* (MY2248)	23°C	21	2	14	5	71%
			13°C	11	10	1	0	96%
5. *kar2-1* pMR397 (MS3089)	×	*sec63-1* (MY2248)	23°C	11	10	1	0	96%[a]
6. *kar2-133* (MS193)	×	*sec63-1* (MY2248)	23°C	23	3	15	5	73%
			13°C	9	6	3	0	92%
7. *kar2-133* pMR397 (MS3079)	×	*sec63-1* (MY2248)	23°C	10	6	3	1	90%[a]
8. *kar2-203* (MS1028)	×	*sec63-1* (MY2248)	23°C	21	5	12	4	76%
			13°C	11	8	3	0	93%
9. *kar2-203* pMR397 (MS3088)	×	*sec63-1* (MY2248)	23°C	10	5	4	1	85%[a]
10. *kar2-157* (MS195)	×	*sec63-1* (MY2248)	23°C	19	2	16	1	76%
			13°C	11	1	6	4	68%
11. *kar2-157* pMR397 (MS3080)	×	*sec63-1* (MY2248)	23°C	9	4	5	0	86%[a]
12. *kar2-191* (MS958)	×	*sec63-1* (MY2248)	23°C	30	6	20	4	76%
			13°C	11	1	9	1	75%
13. *kar2-191* pMR397 (MS3086)	×	*sec63-1* (MY2248)	23°C	26	6	17	3	78%[b]
14. *kar2-191* (MS958)	×	*sec63-1* pMR2454 (MY3216)	23°C	10	1	7	2	73%[b]

[a] 5FOA sensitive spores obtained.

[b] No 5FOA sensitive spores obtained.

Some double mutant combinations with *kar2-157* and *kar2-191* were not viable at 23°C or 13°C (Table 4, crosses 10 and 12). This suggests that in these strains a more severe defect was caused by the combination of these particular mutant alleles. Thus, although "strict" allele specificity was not observed, considerable phenotypic variation was evident with the different *KAR2* alleles. In addition, none of the *kar2* Ts^- alleles tested were synthetically lethal with *sec61-1, sec62-1, sec65-1, sec63-101,* nor *sec63-106,* further demonstrating the gene specificity of the interaction.

Synthetic lethality can arise when a complex formed between two proteins is compromised by each of the two mutations or if the complex formed by the double mutant proteins is in some way toxic to the cell. To determine the nature of the synthetic lethality, the recessivity or dominance of the interaction was tested. Strains were constructed that carried a *kar2* temperature-sensitive allele in the chromosome but were covered by a *URA3*-marked centromere plasmid carrying the wild-type *KAR2* gene (pMR397). These strains were then crossed to MY2248 (*sec63-1*), sporulated, and allowed to germinate at 23°C. For all but one allele (*kar2-191*) (Table 4, cross 13), spore viability was increased when the wild-type *KAR2* plasmid was present (Table 2, cross 4; Figure 1, and Table 4, crosses 5, 7, 9, and 11). To

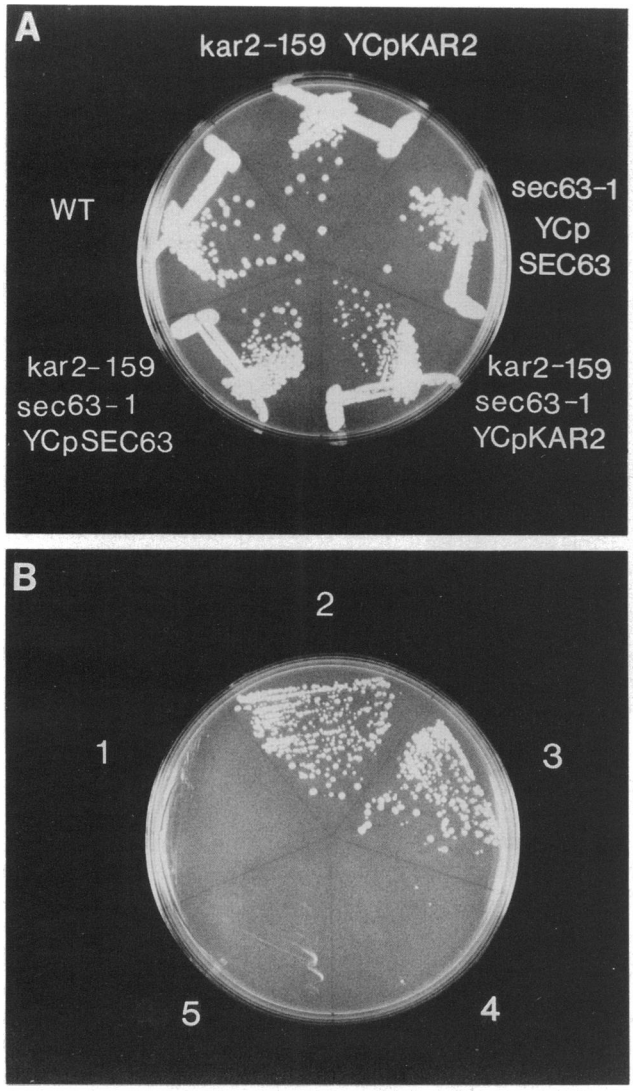

Figure 1. A single copy of *KAR2* or *SEC63* rescues the inability of the *kar2-159 sec63-1* double mutant to grow at 23°C. Growth phenotypes of strains grown at 23°C on (A) YEPD plates or (B) 5FOA plates. (1) A *URA3*$^+$ wild-type strain (MS4) is shown as a 5FOA^S control. Both (2) *kar2-159* transformed with YCp*KAR2* (pMR397, strain MS1554) and (3) *sec63-1* transformed with YCp*SEC63* (pTK81, strain MY3216) can segregate their plasmids and therefore appear to be 5FOA^R. (4) YCp*KAR2* (pMR397, strain MY2632) and (5) YCp*SEC63* (pTK81, strain MY3251) suppress the inviability of the *kar2-159 sec63-1* double mutant, and the viability of the double mutant is dependent on the plasmid because both strains are 5FOA^S.

prove that the increase in viability was because of the ability of the wild-type *KAR2* to rescue the lethality of the *kar2 sec63-1* double mutant, we demonstrated the appearance of a novel class of spores in these crosses. This class consisted of cells sensitive to 5-fluoro-orotic acid (5FOA), which selects against Ura3$^+$ cells (Boeke *et al.*, 1984). In this case, 5FOA sensitivity shows that the strains are unable to segregate the wild-type *KAR2* plasmid. A typical segregation analysis of spores from one cross (*kar2-159* pMR397 and *sec63-1*), shown in Table 3, demonstrated that all of the 5FOA^s spores are the *kar2-159 sec63-1* double mutant covered by pMR397. The recovery of viable double mutants when covered by a wild-type copy of *KAR2* demonstrated that in most cases, the interaction is recessive. The sensitivity to 5FOA also provided an independent test of the synthetic lethal interaction and demonstrated that the double mutants were not simply defective for spore germination.

For one pair, *kar2-159* and *sec63-1*, we demonstrated that the synthetic lethal interaction was also recessive to *SEC63* because a single copy of *SEC63*, on plasmid pTK81, could rescue the inability of the double mutant to grow at 23°C (Figure 1). Therefore, the synthetic lethality involving this pair of *KAR2 SEC63* alleles arises from the interaction between two partially functional proteins leading to a further loss of function.

Interestingly, in one case (*kar2-191* with *sec63-1*) spore viability was not increased when an extra copy of either *KAR2* (pMR397) or *SEC63* (pTK81) was introduced (Table 4, crosses 13 and 14). Furthermore, no 5FOA-sensitive spores were recovered from this cross. Thus, these data suggest that this specific combination is partially dominant. Several models may explain the partial dominance. One possibility is that this combination leads to a more severe defect than the other combinations. Alternatively, the dominant interaction may imply that the lethality arises from an unproductive interaction between the two mutant proteins that is toxic. A third possibility is that the double mutant complex is unresponsive to the presence of the additional wild-type protein, as may be the case if the two proteins were bound in a stable complex. The observation that the double mutant combination does not grow at 13°C is consistent with all of the above.

In sum, the defect seen for each mutant alone is exacerbated when the other gene is also mutationally compromised. These data suggest that the two genes are functionally dependent on one another, possibly through a physical interaction between Kar2p and Sec63p.

Dominant KAR2 Suppressors of sec63-1

A second method of demonstrating a genetic interaction is by the isolation of dominant mutations in one gene that suppresses mutations in another gene. If Kar2p and Sec63p interact, then we should be able to identify dominant, gain of function, mutations in one of the genes that suppress defects in the other. A genetic screen was designed to identify dominant mutations in *KAR2* that could suppress the temperature-sensitive growth defect of the *sec63-1* strain, MY2248. As described below, the screen identifies only dominant mutations in *KAR2* because it mandates that a mutagenized copy of *KAR2* suppress the *sec63-1* chromosomal defect in the presence of a wild-type copy of *KAR2* on the chromo-

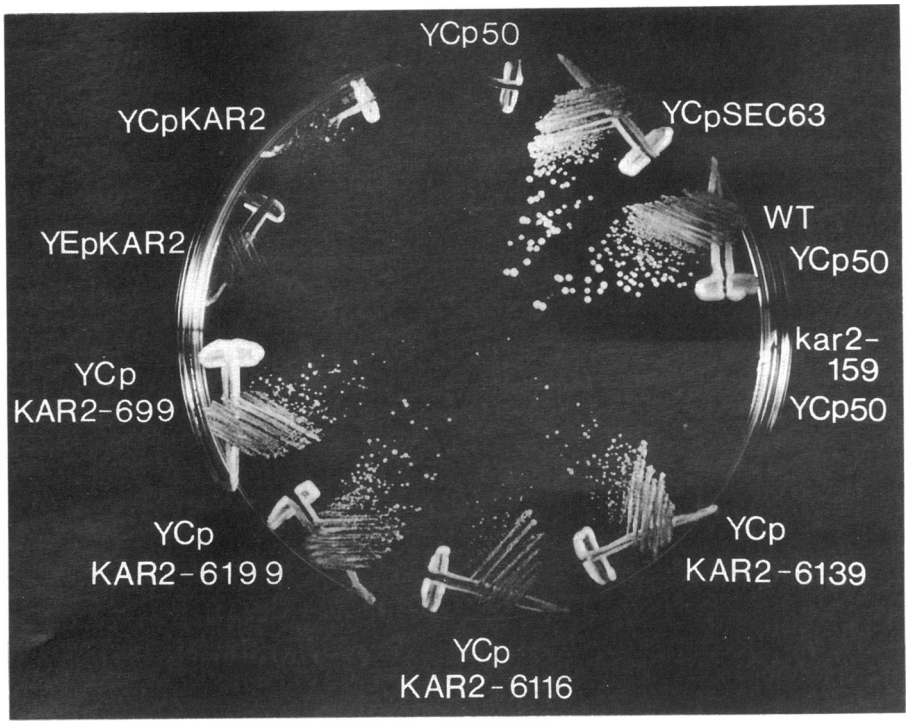

Figure 2. Dominant *KAR2* mutations suppress the temperature sensitivity of *sec63-1*. Shown are the growth phenotypes of strains on YEPD at 37°C. Except where noted, each strain carries the *sec63-1* mutation in the chromosome. For controls a *sec63-1* mutant, MY2248, was transformed with YEp*KAR2* (pMR890, strain MY2799), YCp*KAR2* (pMR397, strain MY2824), or YCp50 (pMR482, strain MY2798). All three control strains remained temperature sensitive. YCp*SEC63* (pTK81, strain MY3216) restored growth to wild-type levels. (Compare to the wild-type strain, MS961). The *KAR2* dominant suppressor plasmids YCp*KAR2-6139* (pMR1678, strain MS1569), YCp*KAR2-6116* (pMR1676, strain MS1565), YCp*KAR2-6199* (pMR1650, strain MS1571), and YCp*KAR2-699* (pMR1624, strain MS1561) all partially alleviated the temperature sensitivity of *sec63-1*. A temperature-sensitive *kar2-159* strain transformed with YCp50, MS945, is shown for comparison.

some. A *URA3*-marked centromere plasmid carrying wild-type *KAR2* gene (pMR397) was mutagenized in vitro with hydroxylamine and transformed into a *sec63-1* strain, MY2248. Transformants were selected at the permissive temperature, 23°C, and then replica plated to the restrictive temperature, 37°C, for 2–3 d. Examination of 15 000 transformants identified 17 Tr^+ colonies. In all cases only partial suppression was observed because growth of the suppressor-containing strains was intermediate between wild-type growth and *sec63-1* mutant growth at 37°C. This demonstrates that they were not true revertants. To determine whether the temperature-resistant phenotype was conferred by a mutation in the plasmid-borne copy of *KAR2* or was because of a chromosomal mutation, we tested whether the temperature-resistant phenotype was dependent on the plasmid. Transformants were incubated in 5FOA to select for cells that had lost the *URA3*-based plasmid. The 5FOA-resistant colonies were then tested for the ability to grow at the restrictive temperature. Seven of the transformants reverted to the temperature-sensitive phenotype of the parental *sec63-1* strain after growth of 5FOA, thereby demonstrating the dependence on the plasmid. The remaining candidate suppressors still grew at 37°C after growth on 5FOA showing that these strains contained chromosomal suppressor mutations that permitted growth at the high temperature. These were not studied further. The seven suppressor plasmids were recovered in *E. coli* and subsequently retransformed into the mutant *sec63-1* strain (MY2248). The retransformed strains were now temperature resistant, confirming that the *KAR2* mutagenized plasmid conferred the ability to grow at 37°C (Figure 2).

Suppression of the *sec63-1* defect was because of mutations in the *KAR2* gene. An internal 1.2-kilobase (kb) *Bst*EII-*Bst*EII fragment was subcloned into an unmutagenized copy of pMR397, thereby replacing the wild-type region with the identical region of *KAR2* derived from each of the mutagenized plasmids. Transformation with the subcloned plasmids showed that for all seven mutant alleles, the region required for suppression was contained in the 1.2-kb *Bst*EII-*Bst*EII fragment, because all transformants acquired the ability to grow at 37°C. Using internal primers specific to *KAR2*, the DNA sequence of the *Bst*EII-*Bst*EII fragment of the seven suppressor genes was determined. Single mutations were found in this region for all seven suppressors. Three alleles (*KAR2-699, KAR2-6143,* and *KAR2-609*) contain the identical amino acid change: $Thr^{449} \rightarrow$ Ile. Two other alleles (*KAR2-6199* and *KAR2-6121*) also contain an identical amino acid change: $Asp^{476} \rightarrow$ Asn. The two remaining suppressor alleles were caused by unique amino acid changes: *KAR2-6116* contained $Gly^{527} \rightarrow$ Ser, whereas *KAR2-6139* contained $Ser^{234} \rightarrow$ Phe. Thus three of the suppressors, isolated six times, clustered in the carboxyl terminal domain referred to as the substrate binding domain (Chappell *et al.*, 1987), whereas the fourth mapped to the amino terminal fragment or ATPase domain (Chappell *et al.*, 1987).

Allele Specificity of KAR2 Suppressors

To determine the nature of the suppression, the gene and allele specificity of the suppression was addressed.

The *KAR2* suppressor alleles carried on *URA3*-based centromere plasmids were transformed into various translocation defective mutant strains shown in Table 5. None of the other *sec63* alleles were suppressed by the dominant *KAR2* mutants (Table 5). Therefore, suppression is allele specific with respect to *SEC63* because only one allele, *sec63-1*, was suppressed. In addition, none of the *KAR2* suppressor mutations suppressed the temperature sensitivity of representative alleles of *SEC61* or *SEC65* (Table 5). Three alleles, *KAR2-6139*, *KAR2-6116*, and *KAR2-699*, show strict allele specificity, because they can compensate only for the *sec63-1* defect. However, with *KAR2-6199*, slight suppression of *sec62-1* was also seen. In this case the apparent lack of allele specificity suggests a broader interaction or an interaction with the translocation complex as a whole.

Overexpression of wild-type Kar2p is one possible mechanism that might account for the dominant suppression. Phillips and Silhavy (1990) have shown that increased levels of the *E. coli* heat shock proteins DnaK and GroEL can facilitate the export of LacZ hybrid proteins that lead to a lethal jamming event under normal conditions. To determine whether suppression was occurring by overproduction of Kar2p, a strain (MY2799) was constructed that carried the *sec63-1* mutant allele and *KAR2* carried on a multi-copy 2 μ-based plasmid (pMR890). Previous work has shown that Kar2p is overexpressed on this plasmid (Rose *et al.*, 1989). Because the temperature sensitivity was not suppressed by the presence of this plasmid (Figure 1), increased levels of wild-type Kar2p cannot be responsible for suppression.

KAR2 Mutations Suppress the Translocation Defect of sec63-1

The dominant *KAR2* suppressors were isolated by suppression of the temperature sensitive growth defect of the *sec63-1* mutant strain. To determine whether the suppressor mutations also alleviated the translocation block caused by the *sec63-1* mutation, we examined the export of two secretory proteins, CPY and Kar2p itself.

CPY is a vacuolar protein that is targeted to the vacuole via the SEC-dependent pathway and depends on wild-type Sec63p for translocation across the ER membrane (Stevens *et al.*, 1982). In the ER signal sequence, cleavage and core-glycosylation occur to generate the ER form of CPY, referred to as p1. CPY is further modified upon transit through the Golgi apparatus giving rise to the p2 form. The p2 form is then targeted to the vacuole where p2 is cleaved by the *PEP4* gene product (Hemmings *et al.*, 1981) to produce mature CPY. To examine the extent of CPY maturation in the *KAR2* suppressor containing strains, cells were grown at the permissive temperature (23°C), shifted to the restrictive temperature (37°C) for 2 h, and then pulse-labeled at 37°C for 5 min with ^{35}S-Translabel. Cell extracts were prepared and immunoprecipitated with antibody specific to CPY. The transit time of CPY is such that in wild-type cells, during a 5-min pulse, CPY is not cleaved to its final form and accumulates predominantly as p2 (Figure 3, lane 2). For comparison, Figure 3, lane 1 shows a *kar2-159* mutant that accumulates CPY precursor with the signal sequence intact because of the strong translocation block. The *sec63-1* mutant strain accumulates a precursor of identical mobility to the protein seen in the *kar2-159* (Figure 3, lane 3). The presence of a single extra copy of wild-type *KAR2* (Figure 3, lane 4) or multiple copies of wild-type *KAR2* (Figure 3, lane 5) did not alleviate the translocation block as evidenced by the accumulation of the precursor in the plasmid-containing strains. However, the presence of each of the *KAR2* suppressor plasmids partially alleviated the translocation block because all three forms of CPY, ppCPY, and

Table 5. Allele specificity of dominant *KAR2* suppressors as judged by growth at 37°C[a]

	Plasmid						
Strain	YCp50	p*KAR2*	2μ*KAR2*	p*KAR2-6139*	p*KAR2-6116*	p*KAR2-699*	p*KAR2-6199*
sec63-1 (MY2248)	−	−	−	+	+	+	+
sec63-7 (C65)	−	−	−	−	−	−	−
sec63-101 (PSY5)	−	−	−	−	−	−	−
sec63-106 (PYS30)	−	−	−	−	−	−	−
sec62-1 (RDM50-94C)	−	−	−	−	−	−	+/−
sec61-2 (RDM15-5B)	−	−	−	−	−	−	−
sec65-1 (RSY457)	−	−	−	−	−	−	−

[a] Strains were transformed with the indicated plasmids at 23°C and screened for growth at 37°C. +, indicates growth; +/−, partial growth; −, no growth at 37°C.

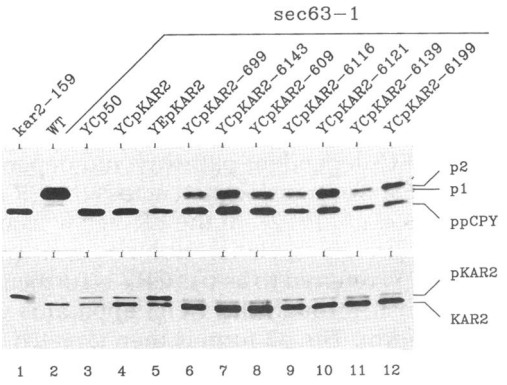

Figure 3. Dominant *KAR2* mutations suppress the translocation defect caused by *sec63-1*. Immunoprecipitation of CPY and Kar2p from cells pulse-labeled for 5 min with 35S-Translabel after a 2-h shift at 37°C. Lane 1 shows a *kar2-159* strain (MS945) in which precursor forms of CPY (ppCPY) and Kar2p (preKar2p) accumulate. Lane 2 shows an intermediate form of CPY and mature Kar2p in a wild-type strain, MS961. CPY and Kar2p synthesized in *sec63-1* strains transformed with plasmids bearing wild-type and mutant forms of *KAR2* are shown in lanes 3–12. Lane 3, YCp50 (strain MY2798); lane 4, YCp*KAR2* (pMR397, strain MY2824); lane 5, YEp*KAR2* (pMR890, strain MY2799); lane 6, YCp*KAR2-699* (pMR1642, strain MS1559); lane 7, YCp*KAR2-6143* (pMR1643, strain MS1561); lane 8, YCp*KAR2-609* (pMR1647, strain MS1563); lane 9, YCp*KAR2-6116* (pMR1676, strain MS1565); lane 10, YCp*KAR2-6121* (pMR1667, strain MS1567); lane 11, YCp*KAR2-6139* (pMR1678, strain MS1569); and lane 12, YCp*KAR2-6199* (pMR1650, strain MS1571). *KAR2-699, KAR2-6143,* and *KAR2-609* contain the same amino acid change. *KAR2-6116* and *KAR2-6139* contain the same amino acid change. *sec63-1* is more restrictive for CPY (only ppCPY accumulates) than for Kar2p (both preKar2p and Kar2p accumulate).

p1 and p2, were seen in these strains (Figure 3, lanes 6–12). Furthermore, partial suppression of the translocation block is consistent with the partial suppression of the temperature-sensitive growth defect.

To show that suppression of the *sec63-1* translocation defect is a general phenomenon and not specific to CPY, export of the Kar2 protein itself was examined. Kar2p is synthesized as a cytoplasmic precursor and upon translocation across the ER membrane, its signal sequence is cleaved producing the mature protein (Vogel *et al.*, 1990). Kar2p was immunoprecipitated from the same extracts as was CPY in the experiment shown above. Maturation of Kar2p was dependent on the presence of wild-type *KAR2* as shown by the precursor accumulation in the *kar2-159* strain at 37°C (Figure 3, lane 1). Kar2 translocation was also dependent on wild-type *SEC63*. The *sec63-1* allele is only partially defective for the Kar2p translocation as seen by the appearance of both forms of Kar2p, pre-Kar2p, and mature Kar2p (Figure 3, lane 3). As observed with CPY, the strains containing the *KAR2* suppressor plasmids are suppressed for the translocation defect, because the majority of Kar2 accumulated as mature protein (Figure 4, lanes 6–12). Thus, both phenotypes caused by *sec63-1*, temperature sensitive growth, and the translocation block are suppressed by the dominant *KAR2* mutants.

KAR2 RNA Is Induced by a Mutation in SEC63

Increased levels of *KAR2* mRNA are seen in all temperature-sensitive *kar2* mutants examined (Figure 4, lanes 3 and 4) (Misra and Rose, unpublished data). In addition, overexpression of Kar2p has been shown to block the induction of *KAR2* by the accumulation of misfolded proteins (Kohno *et al.*, 1993). Thus, at one level, Kar2p function ultimately feeds back on its level of expression. Therefore, regulation of *KAR2* mRNA levels appears to be a sensitive probe of Kar2p function and possibly of its interaction with other proteins. We reasoned therefore that an interaction between *KAR2* and *SEC63* might be detected by an effect of mutations in *SEC63* on the expression or regulation of *KAR2*.

The level of *KAR2* transcript in different mutant strains at the permissive and restrictive temperatures was examined. Yeast strains were pregrown in YEPD at 23°C. Cultures were divided in half with one-half remaining at 23°C and the other half shifted to 37°C for 2 h. Total RNA from both cultures was separated on an agarose gel, blotted, and probed with a *KAR2*-specific probe. The identical blot was also probed with an actin-specific probe to normalize each lane for the total amount of RNA loaded. As previously observed, after 2 h at 37°C, heat shock induction subsided, and equivalent levels of *KAR2* transcript were seen at both 23°C and 37°C in wild-type cells (Figure 4, lanes 1 and 2). In contrast to wild-type, *KAR2* mRNA levels were greatly induced in the *kar2-159* mutant at elevated temperatures (Figure 4, lanes 3 and 4) (unpublished data). One *sec63* mutant, *sec63-1*, also exhibited increased levels of *KAR2* mRNA after growth at 37°C (Figure 4, lanes 9 and 10). The induction showed both a gene- and allele-specific pattern; other alleles of *sec63* did not show the induction (Figure 4, lanes 11–14). As previously shown (Rose *et al.*, 1989), other mutations that block translocation, *sec61-2* (Figure 4, lanes 5 and 6) and *sec62-1* (Figure 4, lanes 7 and 8) did not cause induction of *KAR2*. These results imply that the induction of *KAR2*

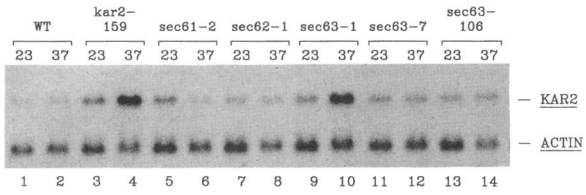

Figure 4. *KAR2* mRNA is specifically induced by *sec63-1* after a shift to the restrictive temperature, 37°C. Total RNA from cells grown at either 23°C or at 37°C after a 2-h shift was probed with both a DNA probe containing the carboxy-terminal region of *KAR2* and a DNA probe specific to *ACT1*. Lanes 1 and 2, wild-type strain MS17 at indicated temperatures; lanes 3 and 4, induction of *KAR2* mRNA caused by *kar2-159* strain MS177; lanes 5 and 6, *sec61-1* strain RDM15-5B; lanes 7 and 8, *sec62-1* strain RDM50-94C; lanes 9 and 10, *sec63-1* strain MY2248; lanes 11 and 12, *sec63-7* strain C65; lanes 13 and 14, *sec63-106* strain PYS30. Induction of *KAR2* mRNA caused by heat-shock regulation decays within 1 h.

caused by the *sec63-1* mutation is neither because of a translocation defect, nor is it simply because of the presence of a mutant form of *SEC63*. The induction caused by *sec63-1* is likely to be because of an intrinsic property of the specific mutation.

Two models may account for the specific induction by *sec63-1*. As discussed above, one possibility is that the activity of Kar2p is reduced by a defective interaction with Sec63-1p. An alternate possibility is that the cell senses the presence of the misfolded ER lumenal domain of Sec63-1p and responds by inducing Kar2p. We think the latter possibility is less likely because mutations in other ER membrane proteins required for translocation, as well as other alleles of *sec63*, did not cause the high level induction of *KAR2*. We presume that at least a few of the other mutant proteins would have also presented partially misfolded protein sequences to the ER lumen and induced *KAR2*. The *KAR2* suppressor strains appear to have been induced by *sec63-1* (Figure 3), but this would be consistent with the partial suppression by the mutations. Although we cannot yet present definitive evidence to distinguish between the two models, the observation that *KAR2* induction is strongly correlated with a mutation in the DnaJ domain of Sec63p remains intriguing because of the substantial genetic evidence for the interaction of this domain with Kar2p.

DISCUSSION

Genetic and biochemical data suggest that the ER membrane proteins, Sec61p, Sec62p, and Sec63p act together to facilitate protein translocation. In this paper, we present genetic evidence that Kar2p interacts with Sec63p in the lumen of the ER. Interaction between Kar2p and Sec63p provides a second example in *S. cerevisiae* of a compartmentalized DnaK/DnaJ-like interaction.

Synthetic Lethal Interactions

Certain double mutant combinations of recessive temperature-sensitive mutations in *KAR2* and *SEC63* are either inviable or viable only at a greatly reduced temperature. Synthetic lethality may result from several scenarios. In one, synthetic lethality arises from loss of function mutations in genes acting in independent but parallel pathways. Loss of function and inactivation of one pathway would cause little or no defect because activity of the other pathway can compensate; knocking out or reducing the efficiency of both pathways would create a deficiency and hence a growth defect. Because both *KAR2* and *SEC63* are essential genes, we think this explanation is unlikely.

Alternatively, decreased cell viability may be caused by the combination of two partially functioning genes acting at the same step in a common essential pathway, possibly as part of a single complex. Several aspects of the specificity of the synthetic lethality between *kar2* and *sec63* mutations suggest this hypothesis is more likely. First, mutations in other translocation genes (*SEC61* and *SEC62*) do not show synthetic lethality with mutations in *KAR2*, although they are synthetically lethal with *sec63* mutations (Rothblatt *et al.*, 1989). Second, not all translocation defective alleles of *SEC63* are synthetically lethal with mutations in *KAR2*. Third, not all translocation defective alleles of *KAR2* are synthetically lethal with mutations in *SEC63*. If Kar2p and Sec63p were simply acting at the same step in translocation pathway, performing the same function, then all defective mutations should show some similar synthetic phenotypes. Only one allele of *sec63* and only a few mutations in *kar2* caused the synthetic lethal phenotype. The *kar2* alleles that display synthetic lethality exhibit phenotypic differences, and the severity of the synthetic phenotypes did not correlate with either the severity of the temperature sensitivity or the severity of the karyogamy defect of the *KAR2* allele (Misra and Rose, unpublished data). Therefore, the synthetic lethal phenotype does not arise from simple loss of Kar2p function. Finally, one *kar2* allele showed a dominant synthetic lethality arguing strongly for an interaction between these two gene products.

Dominant Suppressors

We have isolated dominant mutations in *KAR2* that suppress both the temperature sensitivity and the translocation defect of *sec63-1*. Dominant allele-specific suppressors have often been found in proteins that are known to physically interact, such as Sac6p and actin (Adams *et al.*, 1989). In support of the model of a direct interaction between Sec63p and Kar2p, most of the suppressors are both gene and allele specific. Presumably these mutations suppress the specific defect caused by this unique *sec63* allele possibly by making a compensatory interaction with the mutant Sec63-1 protein.

One of the *sec63-1* suppressors also suppressed a *sec62* mutation. Because Sec62p and Sec63p coexist in a stable physical complex (Deshaies *et al.*, 1991), this suggests several explanations for this cross-suppression. For example, Kar2p might interact with both Sec62p and Sec63p. Alternatively, Kar2p might indirectly influence the affinity or stability of Sec63p's interaction with Sec62p.

What Is the Nature of the Interaction Between KAR2 and SEC63?

As to the nature of *KAR2* and *SEC63*'s interaction, one clue comes from the location of the mutations in each of the genes. In the dominant *KAR2* suppressors, three of the four suppressor alleles *KAR2-6116*, *KAR2-6199*, and *KAR2-699* cluster between amino acid 449 and 527. These mutations map to the carboxyl-terminal domain of Kar2p, which has been referred to as the

"substrate binding domain" (reviewed in Gething and Sambrook, 1992). Interestingly, secondary prediction studies (Flajnik *et al.*, 1991; Rippman *et al.*, 1991) have suggested that this carboxy-terminal domain of HSC70s is structurally similar to the peptide-binding domain of the human major histocompatibility complex class I antigen HLA proteins. This is consistent with the proposal that the carboxy-terminal domain of Kar2p is the peptide- or substrate-binding domain. Additionally, interaction of proteins or peptides through this domain might be involved in the regulation of the ATPase activity as proposed by Chappell *et al.*, 1987.

sec63-1 was the only allele that interacted with mutations in *KAR2*. The mutant phenotype of *sec63-1* is caused by a single amino acid change of an invariant alanine in the DnaJ domain of Sec63p (Nelson *et al.*, 1993). In contrast, *SEC63* alleles that did not genetically interact with mutations in *KAR2* map to the cytoplasmic domain of Sec63p (Nelson *et al.*, 1993). Therefore, we propose that Kar2p interacts with Sec63p via the lumenal DnaJ domain.

We can imagine three ways that Kar2p and Sec63p might interact. In the first, Sec63p would be a specific substrate of Kar2p that is required in the course of Sec63p's folding or assembly with other proteins. In the second, Sec63p and Kar2p specifically interact to enhance Kar2p's function. By analogy to DnaJ/DnaK, Sec63p may stimulate Kar2p's ATPase activity. Possibly, Sec63p may also serve to localize Kar2p to the translocation machinery to allow rapid interaction with the translocating peptides. In the third, we imagine that the interaction between Sec63-1 and Kar2p arises solely as a result of the mutation in the Sec63-1 protein. As discussed below, we believe the second hypothesis most likely.

Two observations support the idea that wild-type Kar2p and Sec63p interact in vivo. First, Sec63p and Kar2p copurify in a complex, and Kar2p fails to copurify with the Sec63-1 protein (Brodsky and Schekman, personal communication). Possibly the dominant *KAR2* suppressors compensate for the reduced binding between Kar2p and Sec63-1p. Second, HSP70s have a low intrinsic ATPase activity in vitro (Zylicz *et al.*, 1983; Welch and Feramisco, 1985) that can be modulated in vitro. DnaK is stimulated both by the DnaJ and GrpE (Liberek *et al.*, 1991), and Ssa1p is stimulated by Ydj1p (Cyr *et al.*, 1992). In support of a Kar2p-Sec63p interaction, a purified Sec63-Ma1E fusion protein stimulates the intrinsic ATPase activity of purified Kar2p in vitro (Vogel *et al.*, unpublished data). Therefore, Sec63-1p might be defective for the regulation of this Kar2p ATPase activity.

Role in Translocation

An understanding of the translocation of proteins across the ER membrane is still incomplete. Although numerous components involved in this process have been identified, the exact mechanism is still not understood. The current model suggests that polypeptides are targeted to the ER either by the cytoplasmic HSP70s or an SRP-like particle. Translocation subsequently occurs either cotranslationally or posttranslationally through proteinaceous pores (reviewed in Cleves and Bankaitis, 1992; Rapoport, 1992; Sanders and Schekman, 1992). From in vitro data, the translocating polypeptide interacts transiently with Sec62p and then more stably with Sec61p (Müsch *et al.*, 1992; Sanders *et al.*, 1992). In addition, the translocating polypeptide also interacts with Kar2p (Sanders *et al.*, 1992). Based on the in vitro data, it has been proposed that Kar2p functions at two different steps during the translocation process. First, Kar2p is required for polypeptide interaction with Sec61p, and second, Kar2p is required after the translocating polypeptide interaction with Sec61p but before translocation of peptide into the lumen of the ER (Sanders *et al.*, 1992). But what is the mechanism of Kar2p's role in this process and how can Sec63p influence this activity?

One model proposed for Kar2p's function in translocation was that it was only indirectly required for the maintenance of the translocation machinery (Vogel *et al.*, 1990). Because HSP70s have been shown to be required for processes that involve protein-protein assembly and disassembly (reviewed in Gething and Sambrook, 1992), one possible role for Kar2p might involve the assembly or disassembly of the translocation complex. Alternatively, Kar2p might facilitate the transfer of translocating peptide from Sec62p to Sec61p. Such a role would be consistent with the requirement of Kar2p for polypeptide interaction with Sec61p. However, although Kar2p might be involved in maintenance of the translocation machinery, Kar2p's interaction with the translocating peptide argues against this being Kar2p's only role.

Several more direct roles for Kar2p include binding to the nascent chain to prevent premature or unproductive interactions between the translocating polypeptides and maintain them in an unfolded translocation competent state. ATP is known to be required for translocation in vitro (Hansen *et al.*, 1986; Rothblatt and Meyer, 1986; Waters and Blobel, 1986), and so far, Kar2p is the only ATPase (Tokunaga and Kohno, 1992) known to be required. Therefore, through a cycle of binding and releasing, the energy derived from Kar2p's ATPase activity might be the driving force for polypeptide translocation. Upon completion of translocation, the release of Kar2p might aid in the polypeptides refolding in the lumen of the ER.

The role of Sec63p in translocation may serve only to regulate and modulate Kar2p's ATPase activity that might be required for any of the above functions. Lastly, because Kar2p is a soluble lumenal protein, there must exist a mechanism to concentrate Kar2p at the trans-

location site in physical proximity to the translocating peptide. Sec63p may act as a bridge, to bind and localize Kar2p to the active sites of translocation.

In conclusion we have shown genetic interactions that support the conclusion that Kar2p and Sec63p interact in vivo. Sec63p's interaction with Kar2p might either regulate Kar2p's activity or serve to localize Kar2p. Further experimentation will clarify whether the interaction with Kar2p is the only function for Sec63p in translocation.

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