Characterization of SIS1, a *Saccharomyces cerevisiae* Homologue of Bacterial dnaJ Proteins

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Abstract. The *Saccharomyces cerevisiae SIS1* gene was identified as a high copy number suppressor of the slow growth phenotype of strains containing mutations in the *SIT4* gene, which encodes a predicted serine/threonine protein phosphatase. The SIS1 protein is similar to bacterial dnaJ proteins in the amino-terminal third and carboxyl-terminal third of the proteins. In contrast, the middle third of SIS1 is not similar to dnaJ proteins. This region of SIS1 contains a glycine/methionine-rich region which, along with more amino-terminal sequences, is required for SIS1 to associate with a protein of apparent molecular mass of 40 kD. The *SIS1* gene is essential. Strains limited for the SIS1 protein accumulate cells that appear blocked for migration of the nucleus from the mother cell into the daughter cell. In addition, many of the cells become very large and contain a large vacuole. The SIS1 protein is localized throughout the cell but is more concentrated at the nucleus. About one-fourth of the SIS1 protein is released from a nuclear fraction upon treatment with RNase. We also show that overexpression of YDJ1, another yeast protein with similarity to bacterial dnaJ proteins, can not substitute for SIS1.

THREE DNA binding proteins, GCN4, BAS1, and BAS2, are required for transcriptional activation of the *Saccharomyces cerevisiae HIS4* gene (Arndt et al., 1987; Tice-Baldwin et al., 1989). Strains containing deletions of *GCN4*, *BAS1*, and *BAS2* require histidine for growth due to the lack of sufficient transcription of the *HIS4* gene. The *trans*-acting *sit1* through *sit4* mutations were isolated by their ability to restore sufficient transcription at the wild-type *HIS4* initiation site so that a strain containing deletions of *GCN4*, *BAS1*, and *BAS2* could grow in the absence of histidine (Arndt et al., 1989). All of the *sit* mutations alter the transcription of many diverse genes in addition to *HIS4* and result in a slow growth phenotype. The *sit1* alleles are mutations in the gene encoding the largest subunit of RNA polymerase II and the *sit2* alleles are mutations in the gene encoding the second largest subunit of RNA polymerase II (Arndt et al., 1989).

The *sit4* alleles are mutations in a gene encoding a predicted protein that is 56% identical to mammalian type 2A and 43% identical to mammalian type 1 serine/threonine protein phosphatases (Arndt et al., 1989). Subsequent genetic analysis has shown that the SIT4 protein phosphatase is required for cell-cycle progression: SIT4 functions in late G1 for progression into S phase (Sutton et al., 1991). To gain insight into the role of SIT4 in transcription and cell cycle progression, we isolated wild-type genes that in high copy number suppress the slow growth phenotype of strains containing transcriptional suppressor mutations in *SIT4*. One of these genes, termed *SIS1*, encodes a protein that is similar to bacterial dnaJ proteins.

The *dnaJ* gene of *Escherichia coli* was initially identified as a host mutation that results in the inability to replicate bacteriophage λ DNA (Georgopoulos and Herskowitz, 1971). Subsequently, *dnaJ* was shown to be a member of the heat shock family of genes (reviewed in Lindquist and Craig, 1988). Strains containing mutations in *dnaJ* have many phenotypes in common with strains containing mutations in *dnaK*, another heat shock gene in *E. coli* that is similar to *HSP70* genes in eukaryotic organisms. Mutations in either *dnaJ* or *dnaK* result in slow growth at temperatures above 30°C, the inability to grow at temperatures of 43°C and higher, and the inability to replicate λ phage DNA at any temperature (Sell et al., 1990). Renaturation of denatured λ phage cI857 protein is slower in *dnaJ* and *dnaK* mutants (Gaitanaris et al., 1990). At the nonpermissive temperature, strains containing temperature-sensitive mutations in either *dnaJ* or *dnaK* have reduced phosphorylation of glutaminyl-tRNA synthetase and threonyl-tRNA synthetase (Itikawa et al., 1989) and are severely inhibited for both RNA and DNA synthesis (Wada et al., 1982). The inhibition of RNA synthesis may be due to induction of the stringent response since high levels of ppGpp accumulate at the nonpermissive temperature and the inhibition of RNA synthesis (but not DNA synthesis) is relieved by *relA* mutations (Itikawa et al., 1986).

The common defects of *dnaJ* and *dnaK* mutants and the finding that dnaJ can stimulate the ATPase activity of dnaK in vitro (Sell et al., 1990) suggest that dnaJ may function in the same biochemical pathway as dnaK. However, dnaJ also functions independently of dnaK. *dnaJ* mutants have a more severe defect in proteolysis than *dnaK* mutants (Straus et al.,

1988). Also, mutation of *dnaJ*, but not *dnaK*, causes defects in the synthesis of β-galactosidase and certain membrane proteins at the nonpermissive temperature (Ohki et al., 1987). For β-galactosidase, the defect in protein synthesis was shown to result from lowered levels of *lac* mRNA.

Despite these known defects due to mutations in *dnaJ*, the specific functions that dnaJ provides for bacterial cell growth are not currently known. The role of dnaJ in P1 and λ phage DNA replication is better understood from studies using in vitro replication systems. For in vitro P1 phage DNA replication, addition of dnaJ and dnaK stimulates DNA replication (Wickner, 1990). The dnaJ protein binds directly to the P1 encoded repA protein (Wickner, 1990). In a reaction that is dependent on both dnaJ and dnaK, the repA protein is altered so that it is more active for binding to the P1 replication origin (Wickner et al., 1991). For λ phage DNA replication (Zylicz et al., 1989; Liberek et al., 1990), dimers of λO protein bind to the λ origin of replication and locally unwind the DNA. The dnaB helicase, complexed with λP protein, binds to the λO/*ori*λ complex (at least partially via the λO–λP interaction). However, in this complex, the activity of dnaB is inhibited by λP protein. The dnaJ protein (which specifically interacts with dnaB) and the dnaK protein (which interacts with λO) are essential for dissociation of this complex (releasing λP) and for activation of the dnaB helicase. Therefore, the results from the in vitro phage DNA replication systems suggest that the mechanism by which *E. coli* dnaJ functions is by dissociation of specific protein/protein complexes. The sequence similarity of the yeast SIS1 protein to bacterial dnaJ proteins suggests that SIS1 may perform its cellular functions by a similar mode of action. In this report, we present the isolation and initial characterization of the yeast *SIS1* gene and its protein product.

Materials and Methods

Yeast Strains

The yeast strains used in this study are shown in Table I. YPD, YNB, and SC media are as described (Arndt et al., 1989). The carbon source was 2% glucose or 2% galactose except as indicated.

Isolation of SIS1

Two *sit4* strains (S/A225-23-5 = *sit4-36* and S/A225-26-3 = *sit4-37*) were transformed with a high copy number library containing yeast genomic DNA inserts (Carlson and Botstein, 1982). After 3 d at 30°C, the library plasmid was recovered from 84 fast growing *sit4* colonies out of a total of 50,000 transformants. About 50 of these plasmids contained the wild-type *SIT4* gene, but these plasmids were recovered from colonies that grew slightly better than the other fast growing colonies. In addition to *SIT4*, we obtained four different genes (*SIS1* through *SIS4*) that dramatically increase the growth rate of either of the two starting *sit4* strains. All four of these genes also increase the growth rate of strain S/A258 which contains the *sit4-258* allele. Each of the four genes (*SIS1* through *SIS4*) was independently isolated from at least three different fast growing transformants.

Sequencing

A 2-kb BstEII/BstEII restriction fragment containing the entire *SIS1* gene was placed into pUC118 and sequenced completely on both strands as described (Tice-Baldwin et al., 1989). To demonstrate that the major open reading frame on this DNA fragment encodes the SIS1 protein, shifts in the reading frame were introduced at various positions by digestion with different restriction enzymes, treating with Klenow enzyme (Boehringer Mannheim Diagnostics, Inc., Houston, TX) or T4 DNA polymerase (New England Biolabs, Beverly, MA), ligating, and transforming into a *sit4* strain to determine complementation. A frame shift either at nucleotide +209 (by cutting with BglII and treating with Klenow enzyme) or at nucleotide +682 (by cutting with KpnI and treating with T4 DNA polymerase) eliminates complementation.

Genetic Mapping of SIS1

The 2-kb BstEII/BstEII restriction fragment containing the *SIS1* gene was cloned into the yeast *URA3* integrating plasmid YIp5. This plasmid was digested with XhoI (cuts at nucleotide +205 of *SIS1*) and transformed into strain W303. Integration was shown to occur at the *SIS1* locus by Southern analysis. Meiotic mapping shows that the distance between *SIS1::URA3* and *pet8* is <1 cM (180 parental ditypes, 0 nonparental ditypes, and 0 tetratypes in 180 tetrads).

Preparation of sis1-2

A 2-kb BstEII/BstEII restriction fragment containing the *SIS1* gene was placed into the Xba I site of pUC118, regenerating the BstEII sites at each end. An oligonucleotide-directed deletion (Kunkel, 1985) removed DNA sequences encoding SIS1 amino acids 1–350 (of 352 total) and replaced them with a BglII site. Then, a 1.8-kb BamHI/BamHI DNA fragment containing the *HIS3* gene was placed into this BglII site. The resulting plasmid was digested with BstEII and transformed into a W303 diploid, yielding strain CY406. Replacement of the *SIS1* open reading frame with *HIS3* at the *SIS1* locus was confirmed by Southern analysis. This null allele of *SIS1* is termed *sis1-2*. Phenotypes identical to strains containing *sis1-2* are obtained with strains containing a smaller deletion of *SIS1* (termed *sis1-1*). This smaller *SIS1* deletion, which removes SIS1 amino acids 70–189, was prepared by replacing the sequences between the XbaI and XhoI sites with the *HIS3* gene.

Epitope Tagging of SIS1

To epitope tag the SIS1 protein, an SpeI restriction site (5′-ACTAGT-3′) was inserted by oligonucleotide-directed mutagenesis (Kunkel, 1985) at positions corresponding to either the very amino terminus (between the codons for the first and second amino acids) or the very carboxyl terminus (between the codon for the last amino acid and the TAA stop codon) of SIS1. A duplex oligonucleotide encoding the hemagglutinin epitope was then inserted into these SpeI sites. The sequence of SIS1 was changed from MVKET . . . to MTSYPYDVPDYASSVKET . . . for the amino-terminal-tagged SIS1 protein. The sequence of SIS1 was changed from . . . DENF(stop) to . . . DENFTSYPYDVPDYASS(stop) for the carboxyl-terminal-tagged SIS1. Both epitope tagged SIS1 proteins were functional because expression of either one in cells containing a deletion of *SIS1* resulted in a wild-type growth rate.

Conditional Expression of SIS1

A BamHI restriction site was created at position −13 of *SIS1* (relative to the A of the *SIS1* ATG) by inserting the self complementary oligonucleotide 5′-CTAGTCATTATTAGTTGGATCCAACTAATAATGA-3′ (the BamHI site and *SIS1* ATG are underlined) into the amino-terminal SpeI site of *SIS1* that was created for insertion of the oligonucleotide encoding the epitope tag. The resulting plasmid was digested with BamHI and the fragment encoding *SIS1* was inserted downstream of the p*GAL* promoter in YCp50. The resulting p*GAL:SIS1* expression plasmid is pCB789. The SIS1 expressed from pCB789 has the sequence MSVKET . . . instead of MVKET . . . for wild-type SIS1. On galactose medium, strain CY719 (containing pCB789 and a chromosomal deletion of *SIS1*) has a growth rate that is indistinguishable from that of an isogenic wild-type strain.

Immunoprecipitation of SIS1

Immunoprecipitation of SIS1 from extracts prepared from ^{35}S-labeled cells grown on 3% glucose YNB medium was performed as described in Sutton et al. (1991) except for the buffers used to prepare the extracts. For SIS1, the cells were broken by vortexing for 1 min with glass beads in 0.3 ml of breaking buffer (100 mM Tris-HCl, pH 7.0 at 23°C, 200 mM NaCl, 1 mM EDTA, 5% glycerol, 0.5 mM DTT, plus proteinase inhibitors). Then, 0.3 ml of RIPA buffer (50 mM Tris-HCl, pH 7.0 at 23°C, 200 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, plus proteinase inhibitors) was added and the cells were vortexed an additional 10 s. The liquid was removed from the glass beads and centrifuged for 10 min. The resulting

Table 1. Yeast Strains

Strain	Background	Genotype	Source
L3110	L	*MATa gcn4-2 bas1-2 bas2-2 ura3-52*	Arndt et al., 1989
S/A225-23-5	L	*sit4-36*	Arndt et al., 1989
S/A225-26-3	L	*sit4-37*	Arndt et al., 1989
S/A258	L	*sit4-258*	Arndt et al., 1989
W303	W	*MATa ade2-1 his3-11,15 leu2-3,112 ura3-1 trp1-1 ssd1-d2 can1-100*	R. Rothstein*
CY406	W	*sis1-2/SIS1* W303 diploid	This study
CY439	W	sis1-2 {*SIS1* on YCp50}	This study
CY455	W	*sis1-2* {*SIS1* on *LEU2*/cen plasmid}	This study
CY457	W	*sis1-2* {NH_2-tagged *SIS1* on *LEU2*/cen plasmid}	This study
CY461	W	*sis1-2* {COOH-tagged *SIS1* on *LEU2*/cen plasmid}	This study
CY661	W	*sis1-2* {NH_2-tagged *SIS1* on YEp24}	This study
CY678	W	*sis1-2* {NH_2-tagged (2 copies) *SIS1* on *LEU2*/cen plas.}	This study
CY719	W	*sis1-2* {p*GAL:SIS1* on YCp50}	This study
CY699	W	CY439 {NH_2-tagged *sis1-79* on *LEU2*/cen plasmid}	This study
CY701	W	CY439 {NH_2-tagged *sis1-80* on *LEU2*/cen plasmid}	This study
CY703	W	CY439 {NH_2-tagged *sis1-81* on *LEU2*/cen plasmid}	This study
CY704	W	CY439 {NH_2-tagged *sis1-82* on *LEU2*/cen plasmid}	This study
CY705	W	CY439 {NH_2-tagged *sis1-84* on *LEU2*/cen plasmid}	This study
CY706	W	CY439 {NH_2-tagged *sis1-85* on *LEU2*/cen plasmid}	This study
CY707	W	CY439 {NH_2-tagged *sis1-86* on *LEU2*/cen plasmid}	This study
CY708	W	CY439 {NH_2-tagged *sis1-87* on *LEU2*/cen plasmid}	This study
CY709	W	CY439 {COOH-tagged *sis1-79* on *LEU2*/cen plasmid}	This study
CY711	W	CY439 {COOH-tagged *sis1-80* on *LEU2*/cen plasmid}	This study
CY712	W	CY439 {COOH-tagged *sis1-81* on *LEU2*/cen plasmid}	This study
CY713	W	CY439 {COOH-tagged *sis1-82* on *LEU2*/cen plasmid}	This study
CY714	W	CY439 {COOH-tagged *sis1-84* on *LEU2*/cen plasmid}	This study
CY715	W	CY439 {COOH-tagged *sis1-85* on *LEU2*/cen plasmid}	This study
CY716	W	CY439 {COOH-tagged *sis1-86* on *LEU2*/cen plasmid}	This study
CY717	W	CY439 {COOH-tagged *sis1-87* on *LEU2*/cen plasmid}	This study
CY886	W	*sis1-2 LEU2*::NH_2-tagged *sis1-81*	This study
CY889	W	*sis1-2 LEU2*::NH_2-tagged *sis1-82*	This study
CY891	W	*sis1-2 LEU2*::NH_2-tagged *sis1-85*	This study
CY893	W	*sis1-2 LEU2*::NH_2-tagged *sis1-86*	This study
BJ926		*MATa/α trp1/+ his1/+ prc1- 126/− pep4-3/− prb1- 1122/- can1/- gal2/-*	E. Jones‡ (via A. Caplan)§

L, all strains in this series are isogenic to strain L3110 except as indicated.
W, all strains in this series are isogenic to strain W303 *MATa* except as indicated.
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‡ Carnegie Mellon University, Pittsburgh, PA.
§ University of North Carolina, Chapel Hill, NC.

supernatant was used for immunoprecipitation. The proteinase inhibitors used in the buffers are described in Sutton et al. (1991).

Preparation of Mutations in SIS1

Oligonucleotide directed deletions in the *SIS1* gene that removed 22 amino acid regions of SIS1 (see Fig. 3) were prepared by the method of Kunkel (1985). Two separate deletion series were prepared: one series used the NH_2 epitope tagged *SIS1* and the other series used the COOH epitope tagged *SIS1* as the starting gene. For each *sis1* mutation, the deletion junction and at least 100 bases on both sides were sequenced to confirm the desired mutation. The results from the NH_2 epitope-tagged SIS1 series and the COOH epitope-tagged SIS1 series were identical except that strains containing NH_2 epitope-tagged SIS1-80 were inviable while strains containing COOH epitope-tagged SIS1-80 were viable. To demonstrate that the inviability of NH_2 epitope-tagged SIS1-80 strains was not due to an unidentified mutation outside the region sequenced, a new NH_2 epitope-tagged *sis1-80* gene was prepared by subcloning a XhoI/Xba I restriction fragment containing the *sis1-80* mutation from COOH epitope-tagged *sis1-80* into the unmutagenized NH_2 epitope-tagged *SIS1* gene. A strain containing the new NH_2 epitope-tagged *sis1-80* mutation was also inviable.

Immunofluorescence Microscopy

DAPI staining and immunological staining of formaldehyde fixed cells was carried out as described (Kilmartin and Adams, 1984). For tubulin staining, the primary antibody was anti-tubulin antibody YOL/34 (Sera Lab Limited, Crawley Down, Sussex, England) and the secondary antibody was goat anti-rat IgG-FITC (Boehringer Mannheim Diagnostics, Inc.). For SIS1 localization, the primary antibody was ascites of 12CA5 monoclonal antibody (Field et al., 1988) and the secondary antibody was goat anti-mouse IgG-FITC (Jackson Immunoresearch, Westgrove, PA). For SIS1 localization, the cells were blocked with 1% BSA and 2% normal goat serum to reduce nonspecific background staining. Cells were viewed with a Zeiss Axiophot microscope using a 100× objective. Kodak Tri-X pan 400 film was used for photography.

Subcellular Fractionation

Yeast nuclear fractions were prepared according to the method of Kalinich and Douglas (1989). The yeast nuclear fractions were treated with DNase I (Worthington Biochemical Corp., Freehold, NJ), RNase A (Sigma Chemical Co., St. Louis, MO), NaCl, or Triton X-100 (Bio-Rad Laboratories, Richmond, CA) according to the method of Allen and Douglas (1989). For

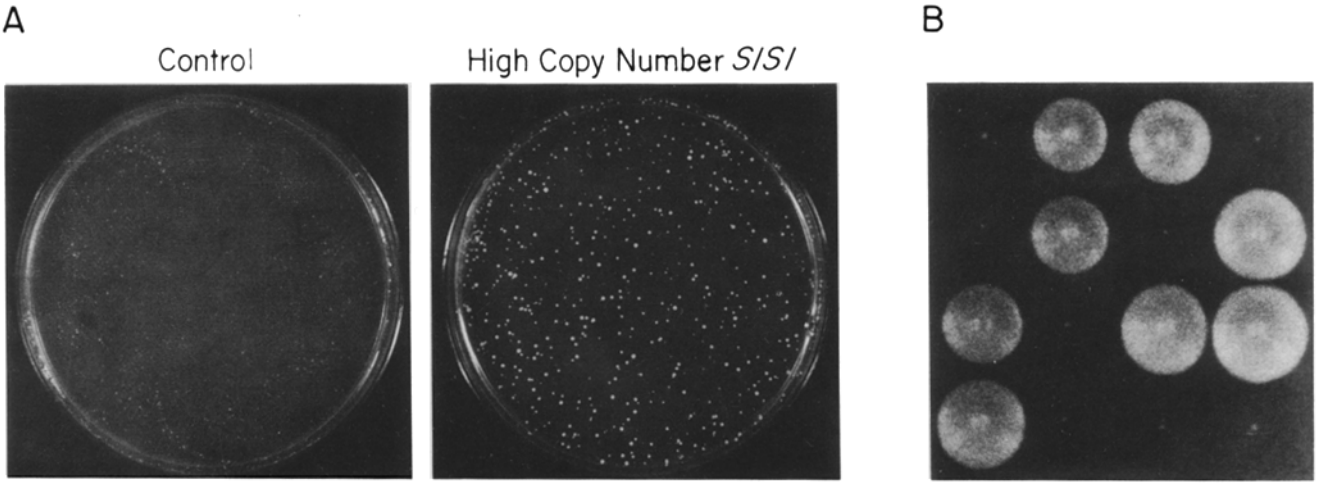

Figure 1. Suppression of the *sit4* Growth defect by high copy number *SIS1*. (*A*) Strain S/A225-26-3 (*sit4-37*) was transformed with the high copy number vector YEp24 (*Control*) or the 2-kb BstEII/BstEII fragment containing *SIS1* on YEp24. The plates were incubated for 2 d, 12 h at 30°C. An isogenic wild-type strain (L3110) gives colonies of similar size as the colonies in the right panel after 1 d, 18 h. (*B*) A *sis1-2/SIS1* diploid (CY406) was sporulated and the tetrads dissected. Each tetrad, as shown in a vertical column, contains two large His$^-$ *SIS1* colonies and two very small colonies each with ~500 inviable cells. About half of the *sis1-2* cells have a terminal phenotype as a very large sized cells. The growth is shown after 5 d at 30°C. *SIS1* is also essential for viability at lower temperatures because similar results are obtained when the plates are incubated at 23°C.

Western analysis of SIS1 levels, anti-SIS1 antiserum (No. 252) directed against the nonconserved central region of SIS1 was prepared by injecting rabbits with a trpE/SIS1 fusion protein containing amino acids 74–191 of SIS1. At the dilution (1:1,000) used for Western analysis of yeast extracts separated on SDS-polyacrylamide gels, this antiserum detected only SIS1 in the size range of 30–45 kD.

Results

Isolation of SIS1

Transcriptional suppressor mutations in the *SIT4* protein phosphatase alter the transcription of many diverse genes and cause a slow growth defect (doubling times in YPD medium of ~300 min compared to 90 min for an isogenic wild-type *SIT4* strain). To identify genes whose products: (*a*) regulate SIT4 activity; (*b*) can partially substitute, directly or indirectly, for SIT4; or (*c*) are substrates of SIT4 (or act downstream of SIT4 in the SIT4 pathway), we isolated wild-type genes that, when present in high copy number, can suppress the slow growth phenotype of *sit4* strains.

From this screen (see Materials and Methods), we obtained four genes that, when present on a high copy number 2μ plasmid, increase the growth rate of *sit4* strains. We term these four suppressor genes *SIS1* through *SIS4*, for *sit4* suppressor. The suppression of the *sit4* growth defect is not specific to a particular *sit4* allele because each of the *SIS* genes in high copy number can increase the growth rate of three different *sit4* mutants. The suppression of the growth defect of the *sit4-37* strain by high copy number *SIS1* is shown in Fig. 1 *A*.

The *SIS1* through *SIS4* genes in high copy number are not able to suppress the temperature sensitive phenotype of *sit4* strains. In addition, *SIS1* through *SIS4* in high copy number are not able to suppress the lethality (Sutton et al., 1991) due to deletion of the *SIT4* gene. Therefore, none of the *SIS* genes in high copy number can substitute for SIT4. Suppression by *SIS1*, *SIS2*, or *SIS4* is specific for *sit4* strains because none of these genes in high copy number can suppress the growth defect of *sit1*, *sit2*, or *sit3* strains. In addition, the *SIS* genes in high copy number do no increase the growth rate of wild-type strains (data not shown).

The suppression of the growth defect of *sit4* strains is greater with high copy number *SIS1* or *SIS2* than with high copy number *SIS3* or *SIS4* (data not shown). The *SIS4* gene (renamed *PPH2*α) encodes a predicted protein that is 80% identical to mammalian type 2A protein phosphatases (Sutton et al., 1991). Therefore, overexpression of a type 2A protein phosphatase can partially compensate for one or more of the defects due to mutation of the *SIT4* protein phosphatase. The analysis of *SIS2* and *SIS3* will be presented elsewhere. In this report, we present the initial characterization of the *SIS1* gene.

Genetic Mapping of SIS1

The minimal region of the high copy number *SIS1* plasmid necessary for suppression was determined by restriction mapping the yeast insert, followed by deletion analysis and/or subcloning and assaying for suppression of the growth defect of *sit4* strains. Full suppression of *sit4* strains for faster growth is obtained by using a 2.0-kb BstEII/BstEII subclone of the original high copy number *SIS1* containing plasmid. This *SIS1* containing DNA fragment was used by L. Riles and M. Olsen (Washington University, St. Louis, MO) to probe their overlapping set of yeast genomic inserts in λ phage. The 2.0-kb *SIS1*-containing DNA fragment hybridized to chromosome 14, very close to the centromere. The *SIS1* gene maps to a previously unidentified genetic locus, <1 cM from *pet8* (see Materials and Methods). One of the original *SIS1*-containing plasmids that contains a 15-kb yeast DNA insert complements the *pet8* mutation. However, *SIS1* is not *PET8* because the 2.0-kb BstEII/BstEII subclone that contains *SIS1* does not complement *pet8*.

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                                                    CATCCATCTGTTGTCCTGTTGAAAAGAAACGGAGGTAACGTT
TCCCCGATGACCCTCAAAGGATGCTACTGGATTGGGATTTGTCGTCCGAATGTCATAAAGCCGTACGTTTT -659
GATGGCCTGCTGTTGCCAACAACTTTTTATCGTTGGTGATTTCTAAGCGATTAACCTGTGAGTCGGAATGC
TGAATTGTTCTTGAACAAACCCCAGTAAGAGCCTCCCAAAATCTTATTGTGTGGTCATAGCCAGCAGATAC -517
TAAGATAACAGACATCAATCAGAACTCCTTTTTTATATGGATGACAAGACTGCCACGTTGATCGCTGCGCT
AATAATATATGGTTGTCGCACTCTTCTATAACATGATCAGTAATAGCTAGTCGTTACTTTTCTGAATCTTA -375
ACGTATTGTGTTACCCTCTTTGGTTTATATGAACGTTCCAGAAACTTCTGGAAAAAGAATGGGATATCCTT
TTACGGGTTTTTCAACCACTGTCAGCACAAACTCCTTGCCAAGGTAGTTCGAGATTTAATATATATTGTAT -233
AGATTTCTCGTCCCTTTACCGTATTGTTAATCCCTTTTTAAACTTATGCGCTTTCTTGTCAAAAATTCTAA
TTAATATTATTTAAGCTCTTACACGAATTCTTTTAACGACTTAGAATTGTATAACCATCCAGAAACTCTTC -91
AGAGGATAAAGGATAAGTTGTTTGCATTTTAAGATTTTTTTTTTAATACATTCACATCAATATAATAGAGT
ATAGTATACAGAACTAATA ATG GTC AAG GAG ACA AAA CTT TAT GAT TTA CTT GGA GTA +39
                    M   V   K   E   T   K   L   Y   D   L   L   G   V    13

TCT CCA AGT GCT AAT GAG CAA GAA CTG AAA AAG GGT TAT AGA AAA GCA GCT CTA
S   P   S   A   N   E   Q   E   L   K   K   G   Y   R   K   A   A   L

AAA TAT CAT CCA GAT AAG CCA ACA GGT GAC ACA GAA AAG TTT AAG GAG ATA TCA 147
K   Y   H   P   D   K   P   T   G   D   T   E   K   F   K   E   I   S    49

GAG GCC TTT GAA ATT TTA AAT GAT CCT CAA AAA AGG GAA ATA TAT GAT CAA TAC
E   A   F   E   I   L   N   D   P   Q   K   R   E   I   Y   D   Q   Y

GGT CTC GAG GCT GCT AGA TCT GGT GGT CCA AGC TTT GGT CCT GGT GGT CCT GGC 255
G   L   E   A   A   R   S   G   G   P   S   F   G   P   G   G   P   G    85

GGT GCT GGA GGT GCT GGA GGC TTC CCT GGC GGT GCG GGC GGA TTC TCC GGA GGA
G   A   G   G   A   G   G   F   P   G   G   A   G   G   F   S   G   G

CAT GCG TTC AGT AAT GAG GAT GCT TTC AAT ATT TTT TCA CAA TTC TTT GGC GGC 363
H   A   F   S   N   E   D   A   F   N   I   F   S   Q   F   F   G   G   121

AGT TCC CCA TTC GGT GGT GCT GAT GAC AGT GGC TTC AGT TTC TCT AGT TAT CCA
S   S   P   F   G   G   A   D   D   S   G   F   S   F   S   S   Y   P

TCT GGC GGC GGT GCT GGT ATG GGA GGT ATG CCT GGA GGA ATG GGA GGA ATG CAT 471
S   G   G   G   A   G   M   G   G   M   P   G   G   M   G   G   M   H   157

GGC GGC ATG GGA GGT ATG CCT GGC GGC TTT AGA TCA GCA TCA AGC TCT CCC ACG
G   G   M   G   G   M   P   G   G   F   R   S   A   S   S   S   P   T

TAT CCA GAG GAA GAA ACA GTT CAA GTT AAT TTA CCA GTT AGT CTA GAA GAT TTG 579
Y   P   E   E   E   T   V   Q   V   N   L   P   V   S   L   E   D   L   193

TTT GTT GGT AAA AAG AAG TCA TTT AAA ATT GGA AGA AAG GGC CCA CAT GGG GCC
F   V   G   K   K   K   S   F   K   I   G   R   K   G   P   H   G   A

TCT GAA AAG ACA CAA ATT GAC ATT CAA TTA AAA CCG GGT TGG AAA GCT GGT ACC 687
S   E   K   T   Q   I   D   I   Q   L   K   P   G   W   K   A   G   T   229

AAA ATA ACA TAC AAG AAC CAG GGT GAT TAC AAT CCT CAA ACG GGC CGT AGA AAG
K   I   T   Y   K   N   Q   G   D   Y   N   P   Q   T   G   R   R   K

ACT TTG CAG TTT GTC ATC CAG GAA AAG AGC CAT CCA AAC TTT AAA AGA GAC GGT 795
T   L   Q   F   V   I   Q   E   K   S   H   P   N   F   K   R   D   G   265

GAT GAC CTA ATT TAC ACT CTG CCA CTA TCT TTC AAG GAA TCA TTG TTA GGT TTT
D   D   L   I   Y   T   L   P   L   S   F   K   E   S   L   L   G   F

TCA AAA ACT ATC CAA ACA ATT GAT GGC AGA ACC TTA CCT TTG TCG AGA GTA CAG 903
S   K   T   I   Q   T   I   D   G   R   T   L   P   L   S   R   V   Q   301

CCT GTC CAA CCC TCA CAA ACT TCT ACT TAT CCT GGT CAA GGT ATG CCA ACT CCA
P   V   Q   P   S   Q   T   S   T   Y   P   G   Q   G   M   P   T   P

AAG AAC CCA TCT CAG AGA GGT AAT TTG ATT GTA AAA TAT AAA GTG GAC TAT CCA 1011
K   N   P   S   Q   R   G   N   L   I   V   K   Y   K   V   D   Y   P   337

ATA TCA CTA AAC GAC GCT CAA AAA CGT GCT ATA GAT GAA AAT TTT TAA TAGTAAT 1066
I   S   L   N   D   A   Q   K   R   A   I   D   E   N   F   Stop        352

TCCTAAGCAAATATAATTATAAACTCAAATAAATTTATCACCACAGAAATCATATAATATACATCATTAAT
TTTGACTCAATAATCTTAAATTGCGCGGTGCGGTGCGCGGTAAATTAAAGTTTTTGTATGTCTGCATTACC 1208
CTGCCCTCTGGCATATAAATGATGTATGTAAGGAATGGTTGACTTGACAGAAGGTCACC
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Figure 2. DNA and predicted protein sequence of *SIS1*. These sequence data are available from EMBL/GenBank/DDBJ under accession number X58460.

SIS1 Encodes a dnaJ Homologue

The DNA sequence of the *SIS1* gene predicts a protein of 352 amino acids with a molecular mass of 37,592 D (Fig. 2). Searches of the databases reveal that SIS1 is similar (~28% identical overall) to *E. coli* and *M. tuberculosis* dnaJ proteins (Fig. 3). SIS1 is most similar to bacterial dnaJ proteins in the amino-terminal third of the proteins (50% identity for SIS1 and *E. coli* dnaJ for the first 104 amino acids). Amino acids 75–104 of SIS1 are very glycine rich, as are both bacterial dnaJ proteins. The carboxyl-terminal third of SIS1 is also similar to dnaJ proteins. However, the middle third of SIS1 (residues 100–220) has very little similarity to the dnaJ proteins. The middle third of dnaJ proteins contains four cysteine-rich direct repeats whose common feature is Cxx CxGxG (indicated by *arrows* in Fig. 3). SIS1 does not contain these cysteine-rich repeats. In place of two of the cysteine-rich repeats, the SIS1 protein has a striking glycine/methionine rich sequence; GMGGMPGGMGGMHGGMG-

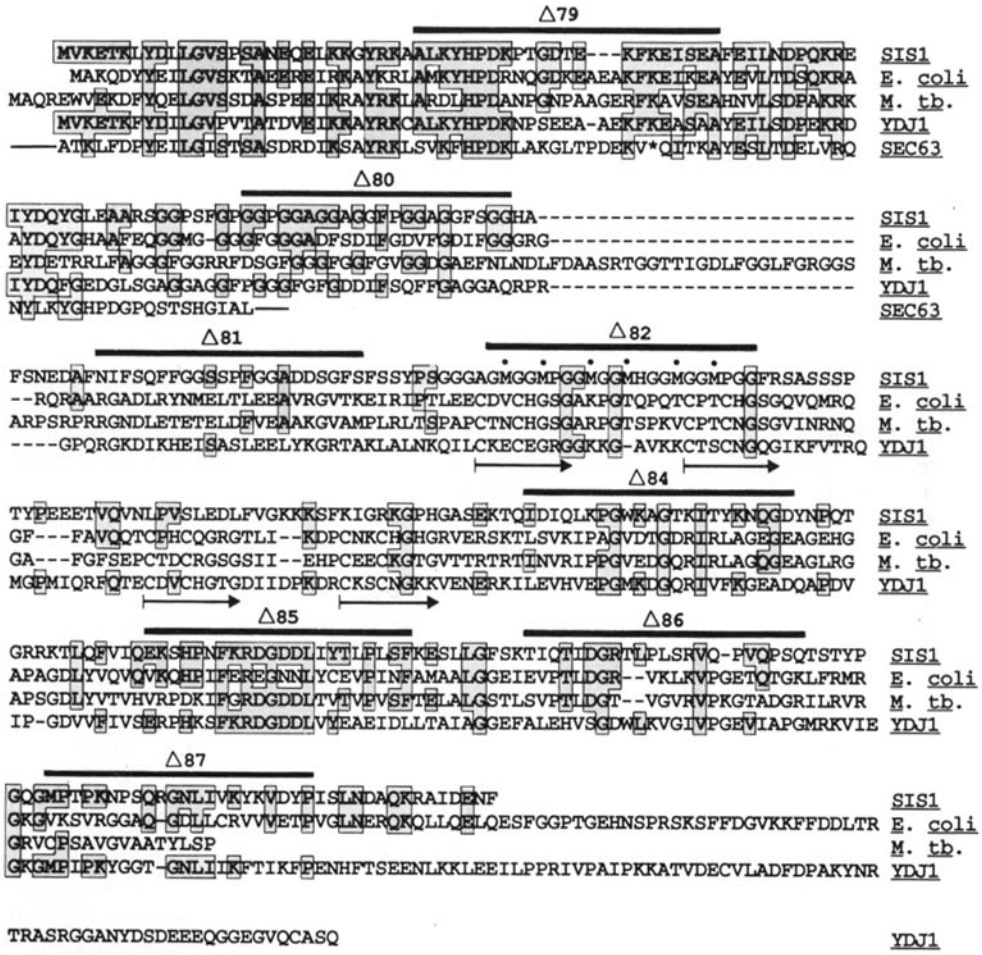

Figure 3. SIS1 is similar to dnaJ proteins. The predicted SIS1 protein is compared to *E. coli* dnaJ (Bardwell et al., 1986), *M. tub.* dnaJ (Lathigra et al., 1988), the yeast YDJ1 protein (Caplan and Douglas, 1991), and a region of the SEC63 protein (Sadler et al., 1989). Only identical amino acids shared between SIS1 and the other proteins are boxed and shaded. The asterisk for the SEC63 sequence indicates an insertion of the amino acids SVMEETY. The first amino acid shown for SEC63 is at position 120 of 663 total amino acids. The four arrows indicate the four cysteine rich repeats that are absent in SIS1. The solid bars above the SIS1 sequence indicate the 22-amino acid region deleted for each altered SIS1 protein. Two attempts to prepare a *SIS1-83* mutation did not give the desired mutation. The dots under the SIS1-82 bar indicate the regularly spaced methionines in this region of SIS1.

GMPGG, which can be viewed either as a $(GGMGGMP)_n$ motif or as containing two direct repeats of the sequences GMGGMPGG (Fig. 3). Within this region of SIS1, the six methionines are positioned $M_{xx}M_{xxx}$ and would lie along the same surface if this region formed an α helix. However, due to the high glycine content and the two proline residues, this region is predicted not to be α helical. The SIS1 glycine/methionine-rich region is reminiscent of the glycine/methionine-rich carboxyl terminus of the *E. coli* groEL protein and eukaryotic HSP60 proteins. The carboxyl terminus of the groEL protein is $(GGM)_4M$ (Hemmingsen et al., 1988) while that of the *S. cerevisiae* HSP60 protein is $(GGMP)_2GMP$-GMM (Johnson et al., 1989). The function of the carboxyl-terminal regions of these proteins is not known.

SIS1 is also similar to dnaJ homologues identified in yeast. The SEC63 protein, which is required for import of proteins into the endoplasmic reticulum, contains a 70-amino acid region that is similar to the amino-terminal 70 amino acids of SIS1 or bacterial dnaJ proteins (Rothblatt et al., 1989; Sadler et al., 1989; Fig. 3). SEC63 has no similarity to dnaJ proteins outside this 70-amino acid region. SIS1 is also similar to the yeast YDJ1 protein, identified by Caplan and Douglas (1991) by using antibodies directed against matrix lamina pore complexes, and to the SCJ1 protein, identified by its ability, when overexpressed, to possibly cause alterations in protein sorting (Blumberg and Silver, 1991). The amino-terminal third and carboxyl-terminal third of SIS1 are both similar to the corresponding regions of YDJ1 (Fig. 3) and SCJ1. However, the middle third of SIS1 has very little similarity to the corresponding region of YDJ1 or SCJ1. The central third region of both YDJ1 and SCJ1 has four cysteine-rich repeats that are very similar to the four cysteine-rich repeats in the two bacterial dnaJ proteins.

SIS1 Is an Essential Gene

A deletion allele of *SIS1*, termed *sis1-2*, was prepared that replaces DNA sequences encoding amino acids 1–350 of SIS1 (of 352 total amino acids) with a 1.8-kb DNA fragment that contains the *HIS3* gene (see Materials and Methods). When a *sis1-2/SIS1* diploid strain (CY406) is sporulated and the tetrads dissected, each tetrad gives rise to two normal-sized His^- *SIS1* colonies and two very small colonies, Fig. 1 *B*. After 6 d of incubation at 23 or 30°C, each small colony contains ~500 inviable cells, about one-half of which are very large in size. This inviability does not result from a defect acquired only during spore germination. When a *sis1-2/SIS1* diploid containing the wild-type *SIS1* gene on the *URA3* centromere plasmid YCp50 is sporulated and the tetrads dissected, haploid *sis1-2* (His^+) progeny containing the wild-type *SIS1* gene on YCp50 are obtained. When this strain is grown on 5-fluoro-orotic acid medium, which al-

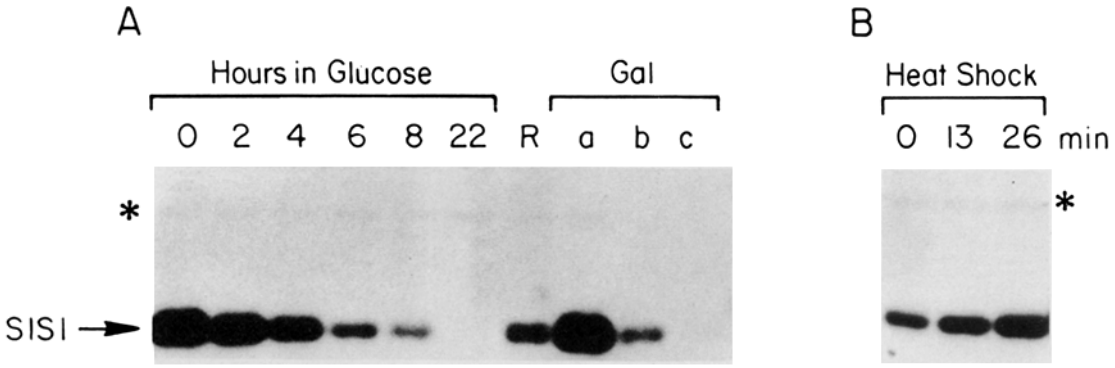

Figure 4. Levels of SIS1 protein after conditional expression or heat shock. (*A*) An exponentially growing culture of strain CY719 in SC galactose medium was centrifuged, washed once in SC glucose medium, resuspended in SC glucose medium, and grown at 30°C. At various times cells were collected from a portion of the culture, frozen in dry ice/ethanol, and stored at −70°C. A parallel culture was also grown for 30 h on SC raffinose medium (indicated by *R*). At all times, the OD_{600} of the cultures was maintained between 0.1 and 0.5 by appropriate dilution. Extracts were prepared from the cells and 75 μg of total protein for each extract was loaded onto a SDS/10% polyacrylamide gel. The separated proteins were transferred to nitrocellulose membrane and probed with anti-SIS1 antiserum No. 252 which was raised against a trpE-SIS1 fusion protein containing amino acids 74–191 of SIS1. Undiluted (*a*), 1:10 dilution (*b*), and a 1:100 dilution (*c*) of an extract prepared from cells grown on SC galactose medium (*Gal*) were loaded as standards. (*B*) Levels of SIS1 protein after heat shock. An exponentially growing culture of strain W303 in YPD medium at 23°C was divided into two portions and centrifuged. One of the cell pellets was resuspended in 23°C YPD medium and grown at 23°C. The other cell pellet was resuspended in 39°C YPD medium and grown at 39°C. These cells were harvested at 13 or 26 min after transfer to 39°C. Extracts were prepared and 40 μg of protein for each extract was loaded onto a SDS/10% polyacrylamide gel. The levels of SIS1 were determined by Western analysis using No. 252 anti-SIS1 antiserum. For both *A* and *B*, the asterisk indicates a nonspecific band that cross-reacts with the No. 252 antiserum and can be used as control for loading similar amounts of cell extract.

lows only Ura⁻ segregants to grow (Boeke et al., 1984), the Ura⁻ *sis1-2* segregants give rise to very small colonies of ~500 inviable cells.

Therefore, *S. cerevisiae* cells grow, on the average, for about nine generations (~500 cells total) in the absence of the *SIS1* gene. There are two possibilities for such a delayed lethality. One possibility is that the cells cannot divide at all in the absence of the SIS1 protein and that the cells take nine generations to dilute the SIS1 protein below some critical level, at which point the cells are inviable. The second possibility is that the cells run out of SIS1 very rapidly but can divide in the absence of SIS1. Here, each division in the absence of sufficient SIS1 protein either results in some probability of cell death or can result in a specific cellular defect. If enough defects are accumulated for a given cell, the cell becomes inviable.

Two lines of evidence support the second model for the delayed lethality. First, pedigree analysis of haploid *sis1-2* progeny resulting from a *sis1-2/SIS1* diploid shows that not every *sis1-2* cell goes through nine generations. Some individual cells are inviable after only three generations from the original *sis1-2* spore while other cells (only a few lineages) are viable after 13 generations from the original *sis1-2* spore. The second line of evidence comes from the analysis of strain CY719 that conditionally expresses SIS1 protein. Strain CY719 contains a deletion of *SIS1* at the chromosomal *SIS1* locus and a low copy number *URA3*/centromere plasmid containing the *SIS1* coding sequences under control of the *GAL1* promoter. CY719 is viable when grown on galactose medium (*GAL1* promoter induced, *SIS1* expressed) but inviable on glucose medium (*GAL1* promoter repressed, *SIS1* not expressed). When CY719 is transferred from galactose to glucose medium, the levels of SIS1 protein decrease (Fig. 4 *A*). After 8 h, the levels of SIS1 have decreased to about one-tenth the starting levels. At this time, the culture grows with a wild-type doubling time (75 min) and >95% of the cells are viable. After 22 h, the levels of SIS1 have decreased to ~1/100 of the starting levels. At this time, the culture continues to grow (but with a doubling time of 180 min) and about 50% of the cells are viable. When strain CY719 is grown on raffinose medium for 30 h (raffinose gives low level induction of the *GAL1* promoter), the cells have about the same amount of SIS1 as do cells after 8 h on glucose medium (Fig. 4 *A*). In contrast to strain CY719 grown on glucose for 8 h where >95% of the cells are viable, strain CY719 grown on raffinose for 30 h has <20% viable cells. Continued growth on raffinose medium results in complete loss of viability. These results suggest that yeast cells can grow and divide when the levels of SIS1 are below some critical level but that cell division under these conditions eventually results in inviability.

Phenotypic Defects Due to Limiting Levels of SIS1

When a *sis1-2* strain expressing *SIS1* under control of the *GAL1* promoter (CY719) is transferred from galactose to glucose medium, the levels of *SIS1* decrease over time (Fig. 4). After 31 h in glucose medium, the culture has an overall doubling time of 200 min. At this time, many of the CY719 cells have become very large (50% of cells) and many of the cells appear blocked for migration of the nucleus from the mother cell into the daughter cell (30% of cells) (Fig. 5). In some of the cells that appear blocked for nuclear migration, the nucleus partially migrates into the neck between the two cells. Much of the volume of most of the large cells is filled with a large structure that is seen as a rim just inside the cell

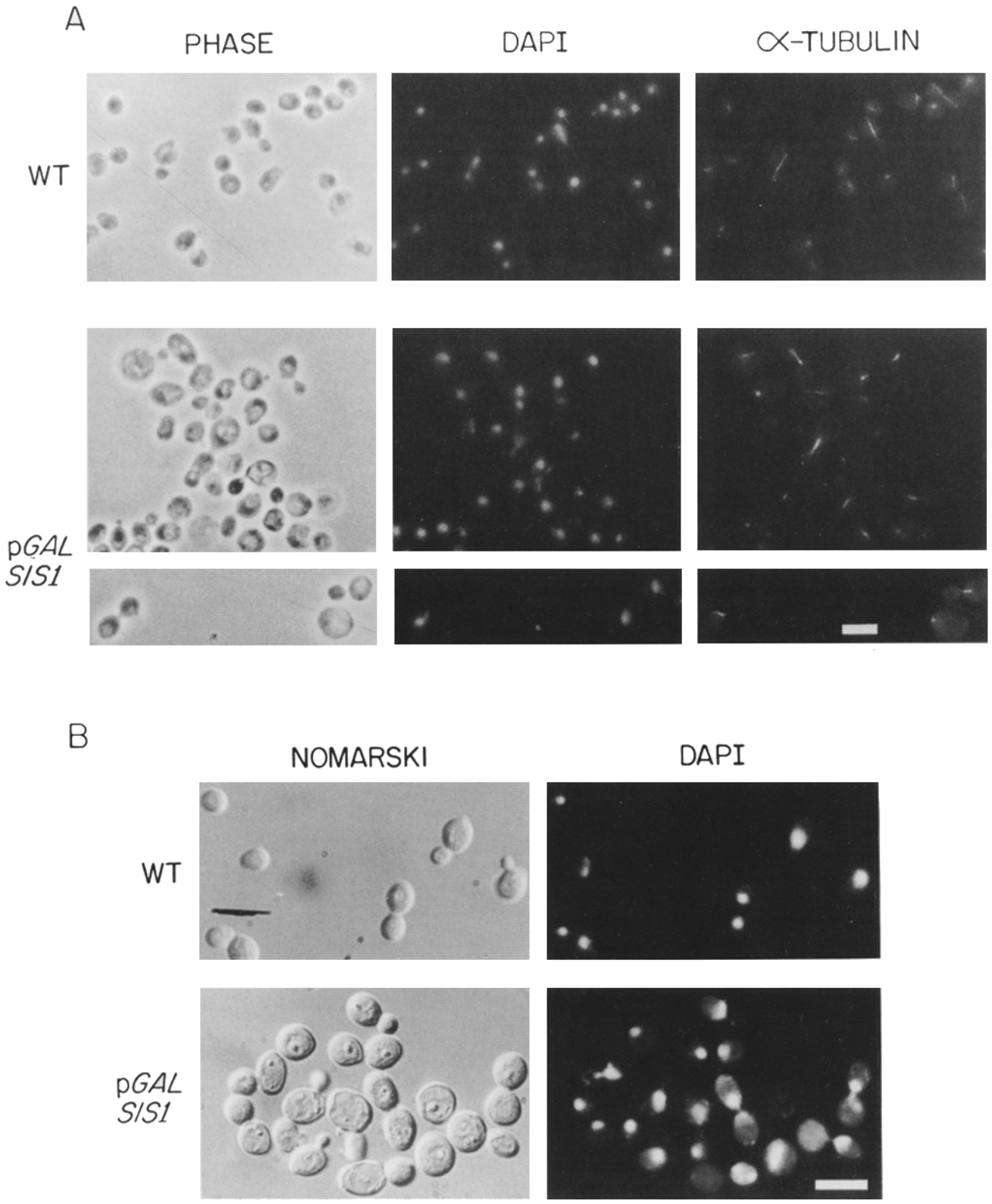

Figure 5. Phenotypes of cells limited for SIS1. (*A*) The panels show either phase contrast, staining for α-tubulin by indirect immunofluorescence, or DAPI staining of DNA by fluorescence. Exponentially growing cultures of strain CY719 (p*GAL:SIS1* on YCp50) on SC-ura galactose medium or W303 (wild-type *SIS1*) on SC galactose medium were centrifuged and resuspended in SC-ura or SC glucose media. The cells were collected after 31 h in glucose medium (maintaining the cultures between OD_{600} of 0.1 and 0.75 by dilution). The cells were fixed with formaldehyde and treated with zymolyase to remove the cell wall before incubation with the anti-α-tubulin antibody. (*B*) Nomarski optics and DAPI straining of DNA for CY719 cells after 31 h in glucose medium as in *A*. The cells were collected and fixed in 70% ethanol before staining of DNA with DAPI. Bars, 10 μm.

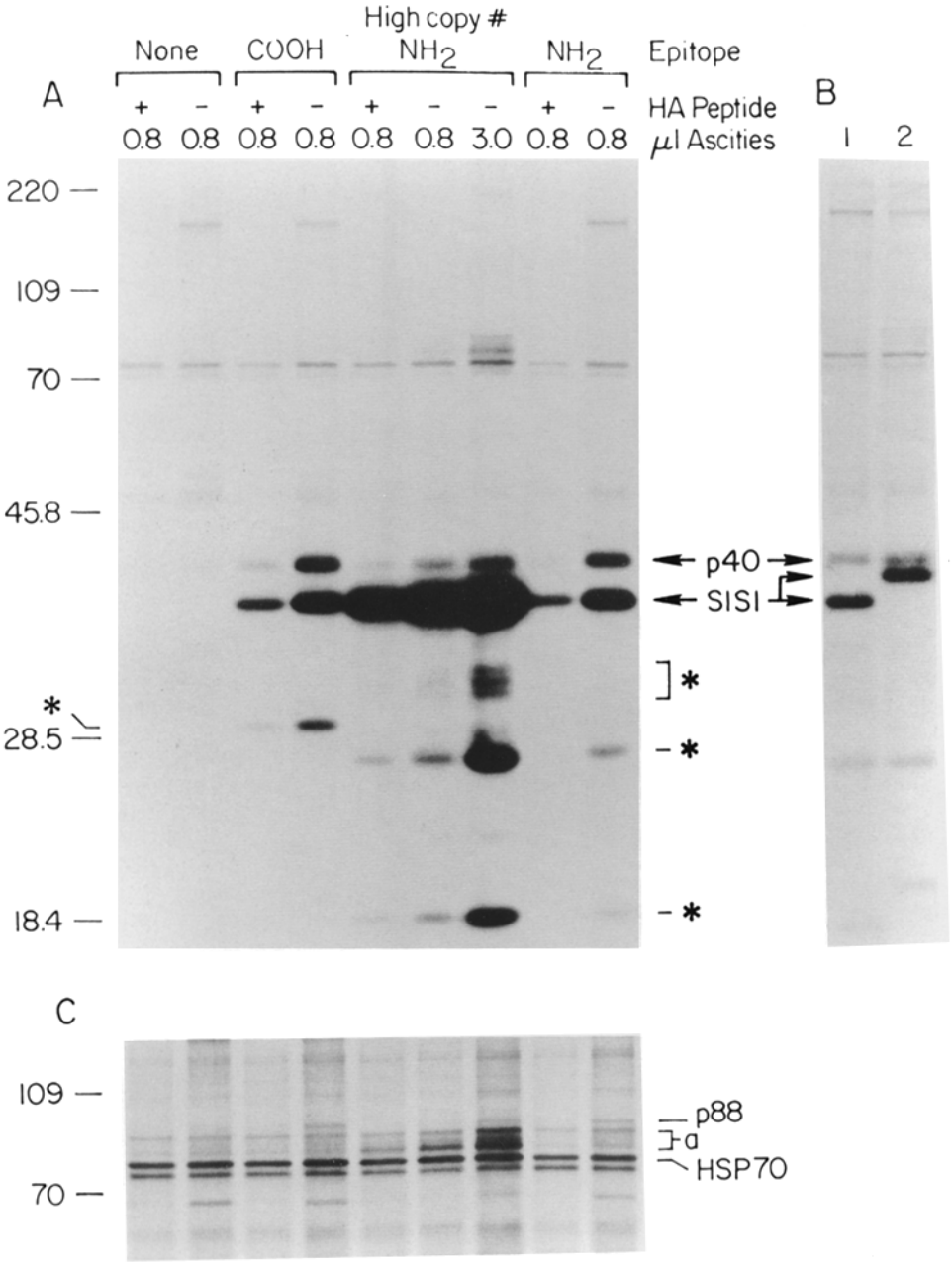

Figure 6. SIS1 associates with p40 and p88. (*A*) The epitope-tagged SIS1 in extracts (100 μl; all 1.6 mg protein/ml) prepared from ^{35}S-labeled cells was immunoprecipitated using the indicated amounts of ascites of 12CA5 monoclonal antibody. The strains used were: CY455 (wild-type *SIS1*) for the *None* lanes, CY461 (COOH epitope-tagged *SIS1*) for the *COOH* lanes, CY661 (high copy number NH_2 epitope-tagged *SIS1*) for the *High copy # NH_2* lanes, or CY457 (NH_2 epitope-tagged *SIS1*) for the *NH_2* lanes. Where indicated (+), the ascites was preincubated for 30 min with 10 μg of competing hemagglutinin epitope peptide (YPYDVPDYA) before addition to the extracts. The asterisks indicate SIS1 degradation products as determined by Western analysis. (*B*) Immunoprecipitation of SIS1 tagged at the amino-terminal end with one (lane *1*, strain CY457) or two (lane *2*, strain CY678) copies of the hemagglutinin epitope. (*C*) Longer exposure of a region of the gel shown in *A*. Western analysis of a parallel gel shows that the band labeled HSP70 reacts with antiserum raised using a peptide corresponding to a conserved amino-terminal region of yeast HSP70 proteins (gift from E. Craig). The *a* bracket indicates two bands that increase in intensity when SIS1 is overexpressed.

surface (Fig. 5 *B*) (Nomarski). This structure was shown to be a vacuole as determined by vital staining (Pringle et al., 1989) for the red dye that accumulates in the vacuole in *ade2* strains grown on limiting adenine (data not shown). In most of the cells with a large vacuole, the nucleus (located by DAPI staining) appears pressed against the side of the cell (Fig. 5 *B*). The phenotypic defects shown for strain CY719 grown on glucose medium for 31 h are also visible after 22 h on glucose medium but in a smaller percentage of the cells. These phenotypes are seldom seen for wild-type cells grown under identical conditions (Fig. 5).

The Levels of SIS1 Increase About Two-Fold After Heat Shock

The *E. coli* dnaJ protein is a typical heat shock protein: the *rate* of synthesis of *E. coli* dnaJ protein increases 10-fold after a heat shock from 30 to 43°C (Bardwell et al., 1986). As determined by Western analysis, the overall level of the SIS1 protein increases about twofold after a heat shock from 23 to 39°C (Fig. 4 *B*). Under these conditions of heat shock, the overall levels of HSP70 proteins also increase about twofold as determined by probing the same Western blot with antiserum (gift from E. Craig) directed against a peptide representing a highly conserved amino-terminal region of yeast HSP70 proteins (data not shown).

The finding that the level of the SIS1 protein increases upon heat shock is consistent with the presence of the sequence GAACGTTCCAGAAACTTCTGGAA (the first G is at −344 relative to the A of the *SIS1* ATG; Fig. 2) in the *SIS1* promoter. This sequence is similar to the heat shock consensus element, which is composed of 5 bp sequences of xGAAx alternating with xTTCx (Slater and Craig, 1987; Amin et al., 1988; Xiao and Lis, 1988).

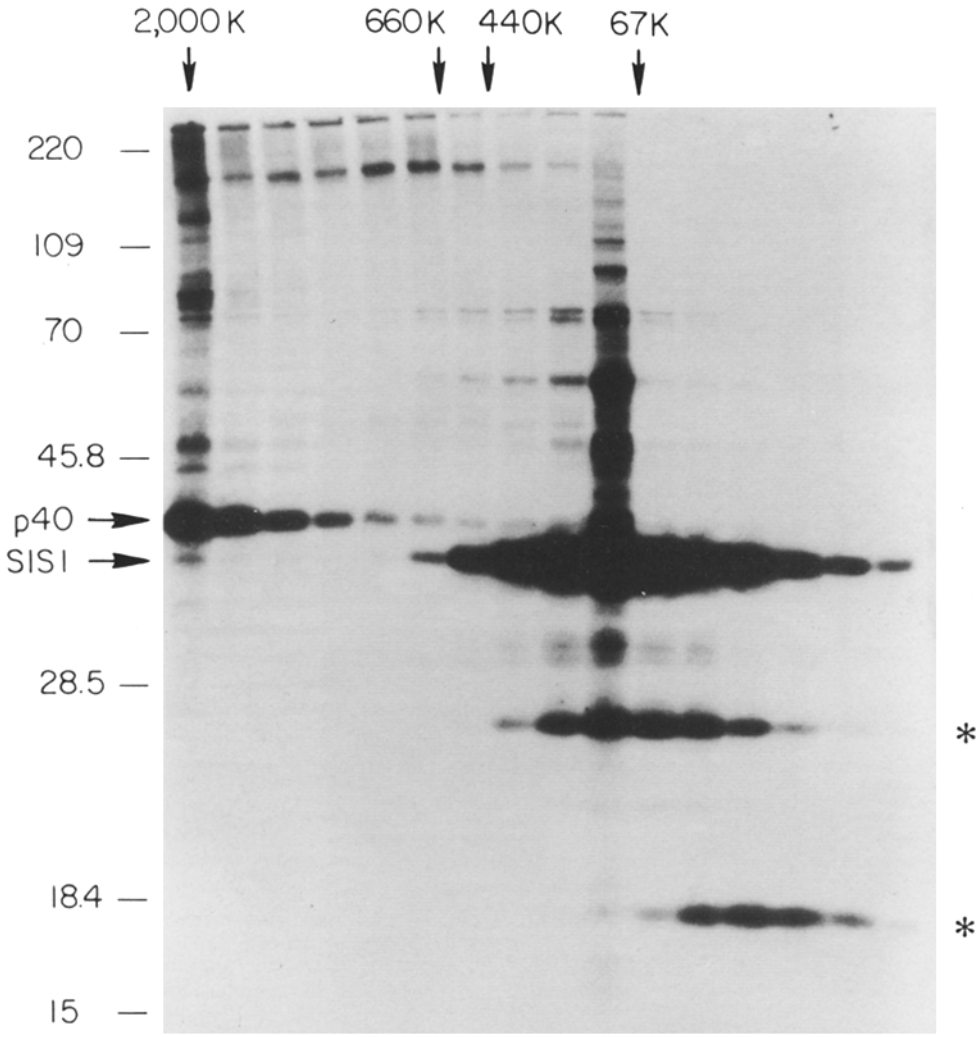

Figure 7. p40/SIS1 elutes as a very large complex. 0.5 ml of an extract (5 mg total protein/ml; in 50% RIPA/50% breaking buffer) prepared from ^{35}S-labeled cells containing NH_2-tagged SIS1 (strain CY457) was loaded onto a 1.0 × 40-cm Sepharose CL-6B column equilibrated in 50% RIPA/50% breaking buffer. Immunoprecipitates of alternate fractions were loaded onto a SDS/10% polyacrylamide gel. For this figure the gel was overexposed to show that the SIS1 protein is present in the high molecular mass p40/SIS1 complex. The asterisks indicate SIS1 degradation products. The column standards were blue dextran (2,000K), thyroglobulin (660K), ferritin (440K), and bovine serum albumin (67K).

SIS1 Associates with p40 and p88

Immunoprecipitation of SIS1 was used to determine if SIS1 associates with other proteins. The SIS1 protein was tagged with a 9-amino acid epitope for which a high affinity monoclonal antibody is available (12CA5; Field et al., 1988). The 12CA5 monoclonal antibody has low cross-reactivity to endogenous yeast proteins. To epitope tag the SIS1 protein, a DNA sequence encoding a 9-amino acid influenza hemagglutinin epitope was inserted into the *SIS1* gene at a position corresponding to either the very amino terminus or the very carboxyl terminus of the protein (see Materials and Methods). An isogenic set of three strains was prepared differing only in the source of the SIS1 protein: strain CY455 contains wild-type SIS1 without the epitope tag, strain CY457 contains only amino-terminal-tagged SIS1, and strain CY461 contains only carboxyl-terminal-tagged SIS1. The epitope tagged versions of SIS1 are functional since the strains containing only the epitope-tagged forms of SIS1 have phenotypes and doubling times indistinguishable from those of isogenic wild-type strains.

When the epitope-tagged SIS1 protein is immunoprecipitated from extracts prepared from ^{35}S-labeled cells, SIS1 specifically coimmunoprecipitates with a protein of apparent molecular mass of 40 kD, which we term p40 (Fig. 6 *A*). The p40 protein is not a slower migrating form of SIS1 by two criteria. First, p40 is not detected by Western analysis of SIS1 immunoprecipitates using either of two antisera directed against different regions of SIS1 (data not shown). These sera readily detect SIS1 in this analysis. The second criterion that p40 is not a form of SIS1 is that altering the mobility of SIS1 by addition of a second nine-amino acid hemagglutinin epitope does not change the apparent mobility of p40 (Fig. 6 *B*). This experiment also shows that SIS1 does not coimmunoprecipitate with a protein of the same mobility as the single epitope-tagged SIS1 protein.

Overexpression of SIS1 increases the amount of SIS1 in the immunoprecipitates (Fig. 6 *A*). In contrast, the amount of p40 in these immunoprecipitates does not increase. Therefore, there may be a limited amount of p40 that can associate with SIS1. In addition, the amount of p40 present in the immunoprecipitates depends on the buffer used to prepare the extract. RIPA buffer contains detergents while breaking buffer does not (see Materials and Methods). If the extracts are prepared using only breaking buffer, about one-third the amount of p40 is present in the SIS1 immunoprecipitates (data not shown) as compared to when the extracts are pre-

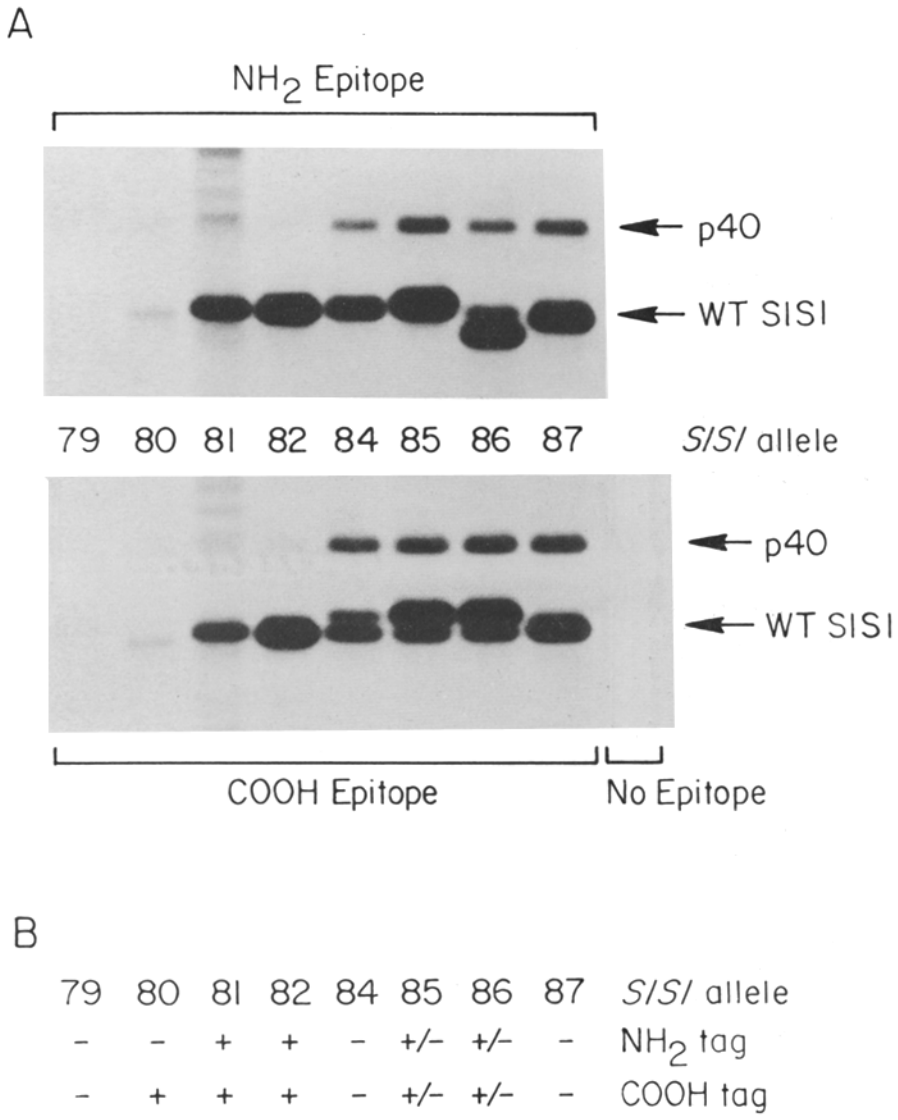

Figure 8. Regions of SIS1 required for association with p40 and p88. (*A*) Extracts were prepared from ^{35}S-labeled cells that contained the indicated epitope-tagged *SIS1* deletion or the wild-type *SIS1* gene (*No Epitope* lane) on a *LEU2*/cen plasmid. Each strain also contained wild-type *SIS1* gene on YCp50. The strains used are listed in Table I. The epitope-tagged SIS1 derivatives were immunoprecipitated from 100 μl of extract (all diluted to 1.6 mg protein/ml) using 0.8 μl of ascites of 12CA5 monoclonal antibody. The WT SIS1 arrow indicates the mobility of wild-type SIS1 that coimmunoprecipitates with the epitope-tagged SIS1 deletion derivatives. The levels of the p40 protein in the immunoprecipitates of epitope-tagged SIS1-84, SIS1-85, SIS1-86, and SIS1-87 are very similar to the levels in the immunoprecipitates of NH_2 or COOH epitope-tagged full-length SIS1 (data not shown). Therefore, the majority of the p40 protein in the epitope-tagged SIS1-84, SIS1-85, SIS1-86, and SIS1-87 immunoprecipitates is not present indirectly by association of p40 with the smaller amount of wild-type SIS1 present in the immunoprecipitates. (*B*) Viability of strains containing the indicated epitope-tagged SIS1 deletion derivative as the only source of SIS1. +, cells that have almost a wild-type growth rate and a Ts^+ phenotype, +/−, cells that have a slow growth phenotype and a Ts^- phenotype, and −, cells that are inviable.

pared using 50% RIPA/50% breaking buffer (Fig. 6). The use of 100% RIPA buffer does not give any increase in the amounts of p40 relative to the amounts seen using 50% RIPA/50% breaking buffer. In all cases, very similar amounts of SIS1 are present in the immunoprecipitates (only a very small fraction of the SIS1 protein is associated with p40; see next section). Therefore, while the solubility of SIS1 is similar in native buffers compared to detergent containing buffers, the p40/SIS1 complex is much more soluble in buffers containing detergents.

SIS1 also associates with another protein. Longer exposure of the gel shows that SIS1 specifically coimmunoprecipitates with a protein of apparent molecular mass of 88 kD (Fig. 6 *C*). In addition, immunoprecipitation of SIS1 from a strain containing the amino-terminal epitope-tagged *SIS1* gene on a high copy number vector causes two proteins, of apparent molecular masses of 78 and 85 kD, to increase in amounts in the immunoprecipitates (indicated by bracket labeled *a* in Fig. 6 *C*). However, the specificity of the interaction of these proteins with SIS1 remains to be determined because the same proteins or proteins of the same mobility also precipitate nonspecifically in the absence of SIS1 (first two lanes of Fig. 6 *C*). Neither p40, p88, nor the two proteins that increase in amounts in the immunoprecipitates when SIS1 is overexpressed are detected by antiserum (gift from E. Craig) directed against a peptide representing a highly conserved amino terminal region of yeast HSP70 proteins (data not shown). However, the band labeled HSP70 in Fig. 6 *C* does react with the anti-HSP70 antiserum.

The p40/SIS1 Complex Has a Very Large Size

To determine the approximate size of native SIS1 and the p40/SIS1 complexes, the cellular extract was fractionated on a Sepharose CL-6B (Pharmacia Inc.) column before immunoprecipitation. The great majority of SIS1 elutes at ~80 kD (Fig. 7). Therefore, the majority of SIS1 (predicted monomer molecular mass of 37.6 kD) probably exists as dimers. The *E. coli* dnaJ protein is also believed to exist as dimers (Zylicz et al., 1985). In contrast to the majority of SIS1, the p40/SIS1 complex elutes at the exclusion limit of this column, which is ~2–4 × 10^6 kD (Fig. 7). We can not ex-

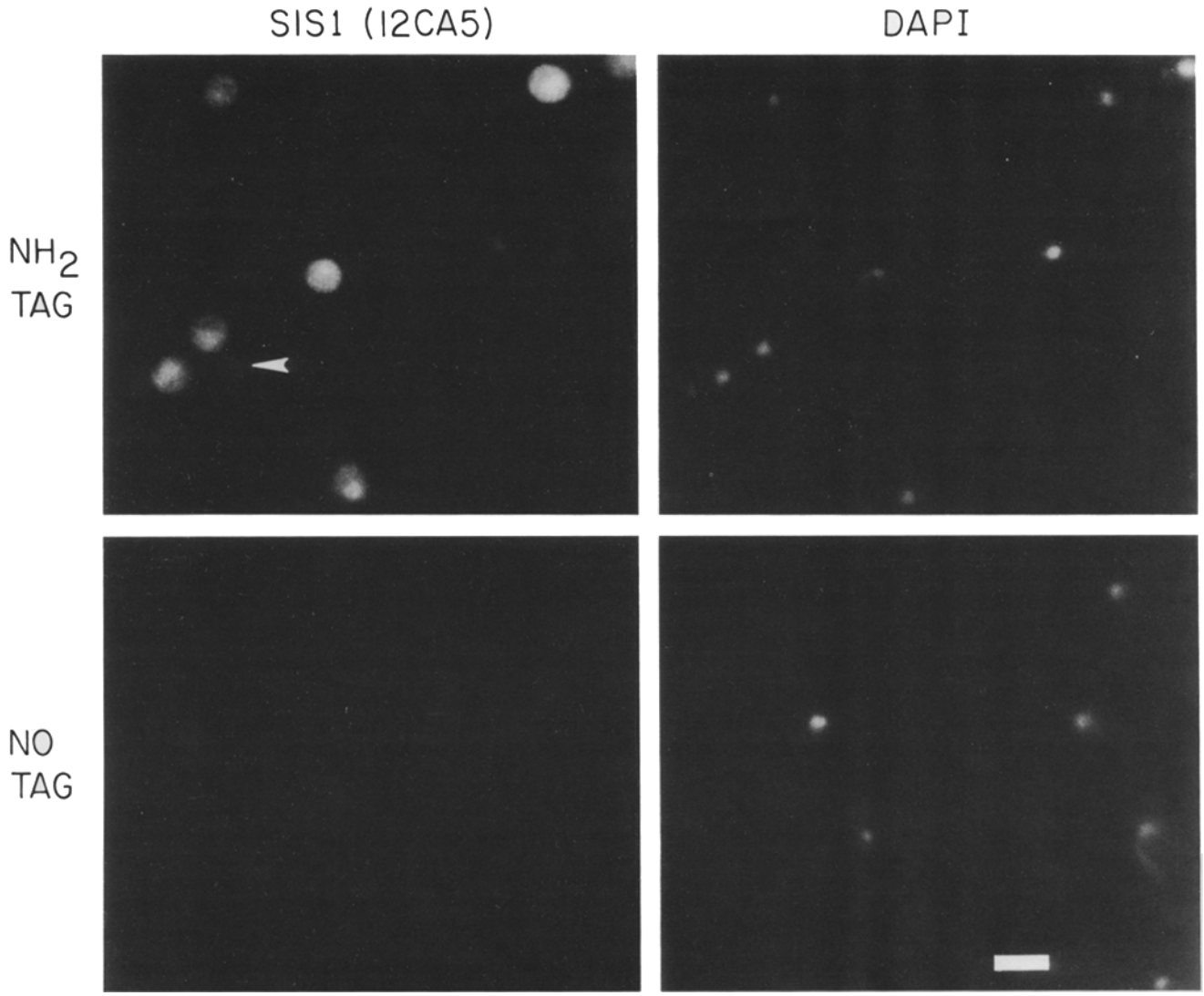

Figure 9. Localization of SIS1 by indirect immunofluorescence. CY455 cells (wild-type SIS1) or CY457 cells (NH_2 epitope-tagged SIS1) were treated with a 1:350 dilution of monoclonal antibody 12CA5 ascites (1.9 mg protein/ml) and then with goat anti-mouse IgG-FITC to visualize the epitope-tagged SIS1 and with DAPI to visualize the nucleus (Materials and Methods). The arrow indicates a budded cell where no staining for SIS1 is apparent in the bud.

clude the possibility that the apparent size of the p40/SIS1 complex results from its presence in micelles. The very high molecular mass p40/SIS1 complex seems to contain much more p40 than SIS1. Therefore, this complex may contain many p40 molecules for each molecule of SIS1.

Association of SIS1 with p40 Requires Both SIS1 Glycine-rich Regions

To determine the region of SIS1 that is required for association with p40, we prepared eight different *SIS1* mutations (termed *sis1-79* through *sis1-87*), each of which encodes an altered SIS1 protein that lacks a different 22-amino acid region of the protein (see Materials and Methods). The 22-amino acid region deleted in each altered SIS1 protein is shown in Fig. 3. To eliminate the possibility that the 9-amino acid epitope tag could alter the analysis, each of the 8 SIS1 deletions was prepared using the amino-terminal epitope-tagged SIS1 or the carboxyl-terminal epitope-tagged SIS1.

The genes encoding the epitope-tagged SIS1 derivatives on a low copy number *LEU2*/cen plasmid were transformed into strain CY455. CY455 has a deletion of *SIS1* at the chromosomal locus (*sis1-2*) and contains the wild-type *SIS1* gene on a low copy number *URA3*/cen plasmid. Therefore, each of the resulting strains has both an epitope-tagged deletion derivative of SIS1 and the wild-type SIS1 protein without the epitope tag. Immunoprecipitation analysis shows that SIS1-84, SIS1-85, SIS1-86, and SIS1-87 retain their association with p40 (Fig. 8 *A*). In contrast, SIS1-81 (deleted in the region between the glycine-rich region and the glycine/methionine-rich region) and SIS1-82 (deleted for the glycine/methionine-rich region) do not associate with p40. Although the amount of SIS1-80 (deleted for the glycine-rich region) in the immunoprecipitates is reduced (probably due to instability

of the altered SIS1-80 protein), prolonged exposure of the gels show no detectable p40 in these immunoprecipitations. Both SIS1-79 derivatives, which are deleted in the amino-terminal region that is highly conserved between dnaJ proteins and dnaJ homologues, are not detected in the immunoprecipitates and are probably unstable. Therefore, we are not able to determine if this highly conserved amino-terminal region of SIS1 is required for association with p40. Increased exposure of the same gels shows that SIS1-84, SIS1-85, SIS1-86, and SIS1-87 retain their association with p88 while SIS1-81 and SIS1-82 do not associate with p88 (data not shown). We cannot determine if SIS1-79 and SIS1-80 associate with p88 due to their much reduced levels in the immunoprecipitates. It is possible that the regions of SIS1 that are required for its association with p88 are the same regions of SIS1 that are required for its association with p40. The immunoprecipitation analysis also directly shows that SIS1 is multimeric. The mobility of each of the SIS1 deletion derivatives is somewhat variable. In some of the lanes, *SIS1* is present as two separate bands: one band results from the epitope-tagged SIS1 deletion derivative and the other band results from untagged wild-type SIS1 (position indicated by arrow, Fig 8 *A*) that coimmunoprecipitates with the epitope-tagged SIS1. These results show that the regions of SIS1 deleted in the SIS1-84, SIS1-85, and SIS1-86 derivatives are not required for multimerization of SIS1.

Analysis of Strains Containing Deletion Derivatives of SIS1

We also tested each of the SIS1 deletion derivatives to see if they could substitute for wild-type SIS1. Strains containing only SIS1-79, SIS1-84, or SIS1-87 are not viable; strains containing only SIS1-85 of SIS1-86 grow very slowly and are temperature sensitive; and strains containing only SIS1-81 or SIS1-82 have only a very slight growth defect (Fig. 8 *B*). For SIS1-80, the ability to substitute for SIS1 depends on the position of the epitope tag: a strain containing only NH_2 epitope-tagged SIS1-80 is not viable while a strain containing only COOH epitope–tagged SIS1-80 has only a slight growth defect. Perhaps the 22–amino acid deletion in SIS1-80 is close enough to the amino-terminal end so that, when combined with the insertion of the epitope at the amino-terminal end, it results in a nonfunctional protein.

Some of these SIS1 deletion derivatives cause alterations in plasmid stability. An isogenic set of strains was prepared that has either wild-type *SIS1* or the *SIS1-81*, *SIS1-82*, *SIS1-85*, or *SIS1-86* gene (all NH_2 epitope tagged) integrated at the *LEU2* locus. Each of these strains was transformed with the *URA3*/centromere plasmid YCp50. After growing the strains for 10 generations on medium containing uracil, the percentage of cells containing YCp50 was determined. At least six measurements were performed for each strain. For the strain containing wild-type SIS1, 34% (standard deviation = SD of 7%) of the cells had lost the plasmid. In contrast, the strain containing SIS1-81 had a reproducibly higher rate of plasmid loss: 55% (SD of 11%) of the cells had lost the plasmid. In contrast, the strain containing SIS1-85 had a reproducibly lower rate of plasmid loss: 18% (SD of 6%) of the cells had lost the plasmid. The SIS1-82 strain and the SIS1-86 strain lost the plasmid at a rate similar to the strain containing wild-type SIS1 (31% with SD of 7% and 29% with a SD of 7%, respectively).

Localization of SIS1 by Immunofluorescence

To determine the subcellular localization of SIS1 by indirect immunofluorescence microscopy, we used three isogenic strains: strain CY455 contained only wild-type SIS1, strain CY457 contained only NH_2 epitope–tagged SIS1, and strain CY461 contained only COOH epitope–tagged SIS1. Immunofluorescence microscopy was performed using the monoclonal antibody 12CA5 which is directed against the epitope tag. Therefore, any difference in the staining between these strains will be due only to the epitope-tagged SIS1 proteins. In the absence of the epitope tag, wild-type yeast cells (strain CY455) show only very weak straining (Fig. 9). The staining for strain CY457 shows that the NH_2-tagged SIS1 is localized throughout the cell but is more concentrated in the region of the nucleus, whose position is visualized by DAPI straining (Fig. 9). In almost every case, the staining for SIS1 is very weak or absent in the bud until it gets a nucleus transferred from the mother cell (see *arrow* in Fig. 9). Staining indistinguishable from that shown for NH_2 epitope–tagged SIS1 containing cells is obtained with cells containing COOH epitope–tagged SIS1 protein (data not shown).

Distribution of SIS1 in Subcellular Fractions

Since SIS1 is more concentrated in the region of the nucleus, we determined how SIS1 fractionated with nuclei using the method of Kalinich and Douglas (1989). Yeast spheroplasts containing wild-type SIS1 were lysed in a hypotonic buffer (0.02 M Hepes, pH 7.4, 5 mM $MgCl_2$). The lysate (*Cells* lane in Fig. 10 *A*) was layered onto 20%/30%/40% glycerol step gradients. These gradients separated the lysate into a pellet fraction containing membranes, nuclei, and unlysed cells (*P* in Fig. 10 *A*) and a soluble cytoplasmic fraction (*S* in Fig. 10 *A*). In this procedure, ~90% of the SIS1 fractionates with the pellet. If higher ionic strength buffers are used to lyse the cells, more of the SIS1 fractionates with the supernatant (data not shown). The extracts for the immunoprecipitation analysis shown in Figs. 6–8 were prepared using glass bead lysis and higher ionic strength buffers (0.2 M NaCl, 0.075 M Tris-HCl), which solubilizes >90% of the SIS1.

The pellet from the glycerol gradient was subjected to two rounds of differential centrifugation in 18% Ficoll to remove unlysed cells and to purify nuclei (Kalinich and Douglas, 1989). The final nuclear pellet fraction contained almost exclusively nuclei (and associated membranes) as determined by DAPI staining. Almost all of the SIS1 in the glycerol gradient pellet is recovered in this nuclear fraction (data not shown). The yeast nuclear fraction was divided into aliquots which were treated separately with DNase plus RNase to digest chromatin and RNA, 1 M NaCl to remove salt soluble components, or 2% Triton X-100 to remove membranes (procedure of Allen and Douglas, 1989). After each treatment, the residual nuclei (P or pellet) were separated from the soluble components (S or supernatant) by centrifugation. Interestingly, when the nuclear fraction was treated with DNase I and RNase A, about one-fourth of the SIS1 was released (Fig. 10 *B*). Separate treatment of the nuclear fraction with RNase A or DNase I shows that treatment with RNase A releases SIS1 while treatment with DNase I does not (Fig. 10 *B*). Subsequent probing of these blots with antiserum against YDJ1 (gift of A. Caplan and M. Douglas) shows that YDJ1 is not released by treatment with either

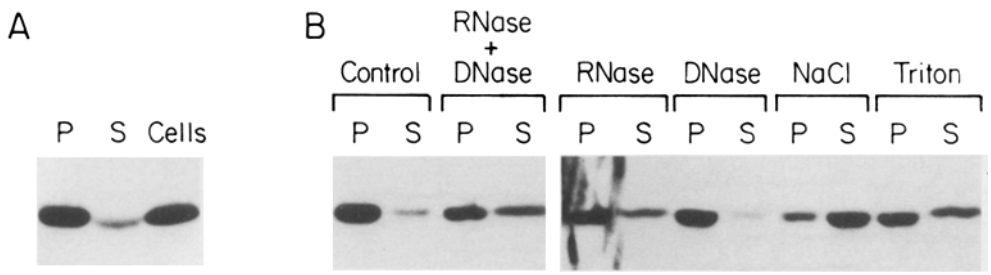

Figure 10. Fractionation of yeast cells and localization of SIS1. (*A*) Cells of strain BJ926 were fractionated as described in Kalinich and Douglas (1989). 20 ml of lysed spheroplasts were layered on a glycerol step gradient and centrifuged. The resulting pellet was resuspended in 20 ml lysis buffer. Then 1.2 ml of the original cell lysate (*cells* lane), 1.2 ml of the gradient top layer (*S* lane), or 1.2 ml of the resuspended pellet were TCA precipitated. (*B*) 0.45-ml aliquots of the nuclear fraction (in digestion buffer) were separately treated in a total volume of 4 ml with buffer (*Control* lanes), RNase + DNase, RNase, DNase, 1 M NaCl, or 2% Triton as described in Allen and Douglas (1989). The samples were centrifuged and the pellets were resuspended in 4 ml of buffer. Then, 1.2 ml of the supernatant fraction (of 4 ml) and 1.2 ml of the pellet fraction (of 4 ml) was TCA precipitated. The TCA precipitates were separated on a SDS/10% polyacrylamide gel, transferred to nitrocellulose membrane, and probed with anti-SIS1 No. 252 antiserum. The sample for the *control* lanes shown in the figure was maintained at 37°C for 25 min as were the RNase + DNase, RNase, and DNase samples. The RNase buffer itself did not release SIS1 into the supernatant because a parallel sample treated with RNase buffer alone gave results identical to the *Control* lanes.

RNase A or DNase I (data not shown; also see Caplan and Douglas, 1991). Treatment of the nuclear fraction with 1M NaCl releases at least two-thirds of the SIS1 protein, confirming the ability of high salt to solubilize SIS1. Treatment of the nuclear fraction with 2% Triton X-100 releases about one-third of the SIS1. When yeast nuclei are treated sequentially with RNase + DNase, NaCl, and then Triton X-100, the residual material is termed the "matrix lamina pore complex." Less than 10% of SIS1 remains associated with this complex (data not shown).

YDJ1 Can Not Substitute for SIS1

The *SIS1* gene is essential for viability. To determine if overexpression of the yeast dnaJ homologue YDJ1 could substitute for SIS1, a *sis1-2/SIS1* diploid containing the *YDJ1* gene on a high copy number plasmid (pAV5; gift of A. Caplan and M. Douglas) was sporulated and 30 tetrads dissected. All of the *sis1-2* haploid progeny gave colonies of very similar size, each containing ~500 inviable cells (data not shown). We expect ~75% of the *sis1-2* haploid progeny to contain pAV5 because about 75% of the *SIS1* haploid progeny contained pAV5 (are Ura^+). Strains containing pAV5 overexpress YDJ1 about fivefold compared to wild-type strains (Caplan, A., personal communication). Therefore, overexpression of YDJ1 cannot cure the lethality due to deletion of *SIS1*.

We also tested if overexpression of YDJ1 could partially cure the slow growth phenotype of a *sis1-85* strain or a *sis1-86* strain. When a *sis1-85* or *sis1-86* strain was transformed with the high copy number plasmid with no insert, the transformants grew at the same rate as the untransformed strains. In contrast, the *sis1-85* strain or the *sis1-86* strain transformed with the *YDJ1* gene on a high copy number plasmid (pAV5) grew much slower than the untransformed strains (data not shown). An isogenic wild-type *SIS1* strain transformed with pAV5 did not grow slower than when transformed with the control plasmid. Therefore, not only can YDJ1 not substitute for SIS1, but overexpression of YDJ1 interferes with the functions of the SIS1-85 and SIS1-86 proteins. This result contrasts with the suppression of *YDJ1* mutants by high copy number *SIS1* (see Caplan and Douglas, 1991).

Discussion

In addition to SIS1, three proteins with similarity to dnaJ have been identified in the yeast *S. cerevisiae*. The yeast SEC63 protein contains a 70-amino acid region similar to the amino-terminal region of dnaJ and is required for import of proteins into the endoplasmic reticulum (Rothblatt et al., 1989; Sadler et al., 1989). One possible model is that SEC63 functions to unfold proteins for transport across the ER membrane. The yeast YDJ1 and SCJ1 proteins are similar to dnaJ over their entire lengths and, like dnaJ, contain four cysteine rich repeats. Therefore, YDJ1 and SCJ1 may be true dnaJ homologues. The YDJ1 protein was isolated as a component of the matrix lamina pore complex (Caplan and Douglas, 1991). The *SCJ1* gene encodes a protein that is localized in mitochondria and was identified as a high copy number suppressor in a screen to isolate genes whose products alter protein sorting (Blumberg and Silver, 1991). Although it is possible that YDJ1 and SCJ1 function for protein unfolding and/or protein import into various organelles, the functions of YDJ1 and SCJ1 are not known.

The yeast SIS1 protein is similar to bacterial dnaJ proteins in the amino-terminal third and carboxyl-terminal third of the proteins. However, the central third of SIS1 is not similar to any of the other known dnaJ proteins or homologues. This central third of SIS1 contains a striking glycine/methionine-rich sequence that, along with the more amino-terminal glycine-rich region and the sequences in between, is required for association of SIS1 with p40 (and probably p88). Perhaps the corresponding region of bacterial dnaJ proteins and the yeast YDJ1 and SCJ1 proteins, all of which contain the cysteine-rich repeats that are absent in SIS1, mediates the association of these proteins with other specific cellular components.

The functions of the SIS1 associated proteins p40 and p88 are not known. The p40/SIS1 complex elutes from a sizing column as a very large complex and may contain many p40 molecules for each SIS1 molecule. Since the p40/SIS1 complex is more soluble in buffers containing detergents, it may be membrane associated. The soluble SIS1 protein present in the cell extracts used for immunoprecipitation analysis

is not detectably associated with yeast HSP70 proteins. Whether or not SIS1 associates with or functions in conjunction with yeast HSP70 proteins in vivo is not known. The *E. coli* dnaJ protein interacts with dnaK, a bacterial homologue of eukaryotic HSP70 proteins (Sell et al., 1990).

We currently do not know the specific function(s) of SIS1. Many of the cells in strains limited for SIS1 appear blocked for nuclear migration and/or become very large in size and contain a large vacuole. However, we can not distinguish if these phenotypic defects result directly or indirectly from limiting SIS1. The very large cells containing a large vacuole could result nonspecifically from cell death. The block in nuclear migration resulting from limiting the SIS1 protein is similar to that seen for *cdc* mutants (such as *cdc2*, *cdc7*, and *cdc9* mutants) that are defective in DNA replication (Pringle and Hartwell, 1981). Specific mutations in *SIS1* cause alterations in plasmid stability, which could result from defects in plasmid replication or segregation. The alterations in plasmid stability resulting from the altered SIS1 proteins could be related to the blocked nuclear migration phenotype in cells limited for SIS1.

The SIS1 protein is localized throughout the cell but is more concentrated in the region of the nucleus. In addition, about a fourth of SIS1 (but none of the YDJ1) is released from a nuclear fraction upon treatment with RNase. This finding may be relevant to the screen used to identify the *SIS1* gene. High copy number *SIS1* dramatically increases the growth rate of strains containing transcriptional suppressor *sit4* mutations. These *sit4* strains have alterations in the lengths and amounts of the mRNA for many diverse genes (Arndt et al., 1989). Therefore, the slow growth phenotype of *sit4* strains may result from the limitation of one or more essential gene products whose transcription is altered due to the *sit4* mutation. Such a limited gene product can not be SIS1 itself since isogenic wild-type and *sit4* strains have very similar amounts of the SIS1 protein (Luke, M.M., A. Sutton, and K. T. Arndt, unpublished data). It is possible that high copy number *SIS1* increases the growth rate of *sit4* strains by increasing the functional amount of a limiting mRNA. That about a fourth of the SIS1 protein is released from a nuclear fraction upon treatment with RNase could be due to the involvement of SIS1 in some aspect of mRNA transcription, processing, or transport. Whether or not SIT4 and SIS1 are both involved in some common step of mRNA transcription remains to be determined. Could SIS1 be a substrate of SIT4? A small minority (<10%) of the SIS1 protein is phosphorylated in vivo such that this form of SIS1 migrates slightly slower than the majority of SIS1 (seen by ^{35}S labeling). Although the overall in vivo phosphorylation state of SIS1 is very similar in wild-type strains as compared to *sit4* strains (Luke, M. M., A. Sutton, and K. T. Arndt, unpublished data), we can not rule out the possibility that SIT4 dephosphorylate only one of many possible phosphorylated residues on the phosphorylated form of SIS1.

SIS1 may have multiple functions required for cellular growth. Some insight into how SIS1 functions may be inferred from studies with *E. coli* dnaJ protein using the in vitro λ phage DNA replication system. In this system, dnaJ seems to be mediating a specific protein/protein dissociation and interacts with the dnaB helicase (Zylicz et al., 1989; Liberek et al., 1990). Perhaps, like dnaJ, the SIS1 protein also mediates specific protein/protein dissociations. Whatever the mechanism of SIS1 action, the function of SIS1 is unique: *SIS1* is an essential gene. Overexpression of the yeast dnaJ homologue YDJ1 (Caplan and Douglas, 1991) cannot substitute for SIS1 and actually inhibits the growth of strains containing *sis1-85* or *sis1-86* mutations. Further genetic and biochemical analysis will be required to determine the precise cellular functions of SIS1.

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References

Allen, J. L., and M. G. Douglas. 1982. Organization of the nuclear pore complex in *Saccharomyces cerevisiae*. *J. Ultrastruct. Mol. Struct. Res.* 102:95–108.

Amin, J., J. Ananthan, and R. Voellmy. 1988. Key features of heat shock regulatory elements. *Mol. Cell. Biol.* 8:3761–3769.

Arndt, K. T., C. Styles, and G. R. Fink. 1987. Multiple global regulators control *HIS4* transcription in yeast. *Science (Wash. DC).* 237:874–880.

Arndt, K. T., C. A. Styles, and G. R. Fink. 1989. A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. *Cell.* 56:527–537.

Bardwell, J. C. A., K. Tilly, E. Craig, J. King, M. Zylicz, and C. Georgopoulos. 1986. The nucleotide sequence of the *Escherichia coli* K12 *dnaJ* gene. *J. Biol. Chem.* 261:1782–1785.

Blumberg, H., and P. A. Silver. 1991. A homologue of the bacterial heat-shock gene *DnaJ* that alters protein sorting in yeast. *Nature (Lond.).* 349:627–629.

Boeke, J. D., F. Lacroute, and G. R. Fink. 1984. A positive selection for mutants lacking orotidine-5′ phosphate decarboxylase activity in yeast: 5-fluoroorotic acid resistance. *Mol. Gen. Genet.* 197:345–346.

Caplan, A. J., and M. G. Douglas. 1991. Characterization of *YDJ1*: a yeast homologue of the bacterial dnaJ protein. *J. Cell Biol.* 114:609–621.

Carlson, M., and D. Botstein. 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. *Cell.* 28:145–154.

Field, J., J. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I. A. Wilson, R. A. Lerner, and M. Wigler. 1988. Purification of a *RAS*-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. *Mol. Cell. Biol.* 8:2159–2165.

Gaitanaris, G. A., A. G. Papavassiliou, P. Rubock, S. J. Silverstein, and M. E. Gottesman. 1990. Renaturation of denatured λ repressor requires heat shock proteins. *Cell.* 61:1013–1020.

Georgopoulos, C. P., and I. Herskowitz. 1971. *Escherichia coli* mutants blocked in lambda DNA synthesis. *In* The Bacteriophage Lambda. A. D. Hershey, editor. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 553–564.

Hemmingsen, S. M., C. Woolford, S. M. van der Vies, K. Tilly, D. T. Dennis, C. P. Georgopoulos, R. W. Hendrix, and J. Ellis. 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature (Lond.).* 333:330–334.

Itikawa, H., H. Fujita, and M. Wada. 1986. High temperature induction of a stringent response in the *dnaK*(Ts) and *dnaJ*(Ts) mutants of *Escherichia coli*. *J. Biochem.* 99:1719–1724.

Itikawa, H., M. Wada, K. Sekine, and H. Fujita. 1989. Phosphorylation of glutaminyl-tRNA synthetase and threonyl-tRNA synthetase by the gene products of *dnaK* and *dnaJ* in *Escherichia coli* K-12 cells. *Biochimie.* 71:1079–1087.

Johnson, R. B., K. Fearon, T. Mason, and S. Jindal. 1989. Cloning and characterization of the yeast chaperonin *HSP60* gene. *Gene (Amst.).* 84:295–302.

Kalinich, J. F., and M. G. Douglas. 1989. *In vitro* translocation through the yeast nuclear envelope. *J. Biol. Chem.* 264:17979–17989.

Kilmartin, J. V., and A. E. M. Adams. 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. *J. Cell Biol.* 98:922–933.

Kunkel, T. A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. USA.* 82:488–492.

Lathigra, R. B., D. B. Young, D. Sweetser, and R. A. Young. 1988. A gene from *Mycobacterium tuberculosis* which is homologous to the DnaJ heat shock protein of *E. coli*. *Nucleic Acids Res.* 16:1636.

Liberek, K., J. Osipiuk, M. Zylicz, D. Ang, J. Skorko, and C. Georgopoulos. 1990. Physical interactions between bacteriophage and *Escherichia coli* proteins required for initiation of λ DNA replication. *J. Biol. Chem.* 265:3022–3029.

Lindquist, S., and E. A. Craig. 1988. The heat shock proteins. *Annu. Rev. Genet.* 22:631–677.

Ohki, M., H. Uchida, F. Tamura, R. Ohki, and S. Nishimura. 1987. The *Escherichia coli dnaJ* mutation affects biosynthesis of specific proteins, including those of the *lac* operon. *J. Bacteriol.* 169:1917–1922.

Pringle, J. R., and L. H. Hartwell. 1981. The *Saccharomyces cerevisiae* cell cycle. *In* The Molecular Biology of the Yeast Saccharomyces. J. N. Strathern, E. W. Jones, and J. R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 97–142.

Pringle, J. R., R. A. Preston, A. E. M. Adams, T. Stearns, D. G. Drubin, B. K. Haarer, and E. W. Jones. 1989. Fluorescence microscopy methods for yeast. *Methods Cell Biol.* 31:357–435.

Rothblatt, J. A., R. J. Deshaies, S. L. Sanders, G. Daum, and R. Schekman. 1989. Multiple genes are required for proper insertion of secretory proteins into the endoplasmic reticulum in yeast. *J. Cell Biol.* 109:2641–2652.

Sadler, I., A. Chiang, T. Kurihara, J. Rothblatt, J. Way, and P. Silver. 1989. A yeast gene important for protein assembly into the endoplasmic reticulum and the nucleus has homology to *dnaJ*, an *Escherichia coli* heat shock protein. *J. Cell Biol.* 109:2665–2675.

Sell, S. M., C. Eisen, D. Ang, M. Zylicz, and C. Georgopoulos. 1990. Isolation and characterization of *dnaJ* null mutants of *Escherichia coli*. *J. Bacteriol.* 172:4827–4835.

Slater, M. R., and E. A. Craig. 1987. Transcriptional regulation of an hsp70 heat shock gene in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 7:1906–1916.

Straus, D. B., W. A. Walker, and C. A. Gross. 1986. *Escherichia coli* heat shock gene mutants are defective in proteolysis. *Genes & Dev.* 2:1851–1858.

Sutton, A., D. Immanuel, and K. T. Arndt. 1991. The SIT4 protein phosphatase functions in late G_1 for progression into S phase. *Mol. Cell. Biol.* 11:2133–2148.

Tice-Baldwin, K., G. R. Fink, and K. T. Arndt. 1989. BAS1 has a Myb motif and activates *HIS4* transcription only in combination with BAS2. *Science (Wash. DC).* 246:931–935.

Wada, M., Y. Kadokami, and H. Itikawa. 1982. Thermosensitive synthesis of DNA and RNA in *dnaJ* mutants of *Escherichia coli* K-12. *Jpn. J. Genet.* 57:407–413.

Wickner, S. H. 1990. Three *Escherichia coli* heat shock proteins are required for P1 plasmid DNA replication: formation of an active complex between *E. coli* dnaJ protein and the P1 initiator protein. *Proc. Natl. Acad. Sci. USA.* 87:2690–2694.

Wickner, S., J. Hoskins, and K. McKenney. 1991. Function of *DnaJ* and *DnaK* as chaperones in origin-specific DNA binding by RepA. *Nature (Lond.).* 350:165–167.

Xiao, H., and J. T. Lis. 1988. Germline transformation used to define key features of heat-shock response elements. *Science (Wash. DC).* 239:1139–1142.

Zylicz, M., T. Yamamoto, N. McKittrick, S. Sell, and C. Georgopoulos. 1985. Purification and properties of the dnaJ replication protein of *E. coli*. *J. Biol. Chem.* 260:7591–7598.

Zylicz, M., D. Ang, K. Liberek, and C. Georgopoulos. 1989. Initiation of λ DNA replication with purified host- and bacteriophage-encoded proteins: the role of the dnaK, dnaJ, and grpE heat shock proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1601–1608.