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

May M. Luke, Ann Sutton, and Kim T. Arndt
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724-2212

**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.

**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 *sit* through *sit*4 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 *sit*1 alleles are mutations in the gene encoding the largest subunit of RNA polymerase II and the *sit*2 alleles are mutations in the gene encoding the second subunit of RNA polymerase II (Arndt et al., 1989).

The *sit*4 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 GI 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 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, cannot substitute for SIS1.

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 glutamyl-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 PI and ϕλ phase DNA replication is better understood from studies using in vitro replication systems. For in vitro PI ϕλ phase DNA replication, addition of dnaJ and dnaK stimulates DNA replication (Wickner, 1990). The dnaJ protein binds directly to the PI 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 PI replication origin (Wickner et al., 1991). For ϕλ phase DNA replication (Zylicz et al., 1989; Liberek et al., 1990), dimers of αO protein bind to the λ origin of replication and locally unwind the DNA. The dnaB helicase, complexed with αP protein, binds to the λO/ortα complex (at least partially via the λO-αP interaction). However, in this complex, the activity of dnaB is inhibited by αP 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 αP) and for activation of the dnaB helicase. Therefore, the results from the in vitro phase 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 SISI protein to bacterial dnaJ proteins suggests that SISI may perform its cellular functions by a similar mode of action. In this report, we present the isolation and initial characterization of the yeast SISI 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 (Arnold et al., 1989). The carbon source was 2% glucose or 2% galactose except as indicated.

**Isolation of SISI**

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 (SISI 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 (SISI through SIS4) is independently isolated from at least three different fast growing transformants.

**Sequencing**

A 2-kb BstEII/BstEII restriction fragment containing the entire SISI 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 SISI 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 BgII and treating with Klenow enzyme) or at nucleotide +682 (by cutting with KpnI and treating with T4 DNA polymerase) eliminates complementation.

**Genetic Mapping of SISI**

The 2-kb BstEII/BstEII restriction fragment containing the SISI gene was cloned into the yeast URA3 integrating plasmid YIP5. This plasmid was digested with Xhol (cuts at nucleotide +205 of SISI) and transformed into strain W303. Integration was shown to occur at the SISI locus by Southern analysis. Metiotic mapping shows that the distance between SISI::URA3 and pet8 is <1 cM (180 parental diploids, 0 nonparental diploids, and 0 tetraploids in 180 tetrad).

**Preparation of sit2-1**

A 2-kb BstEII/BstEII restriction fragment containing the SISI gene was placed into the XbaI site of pUC118, regenerating the BstEII sites at each end. An oligonucleotide-directed deletion (Kunkel, 1985) removed DNA sequences encoding SISI 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 BgIII site. The resulting plasmid was digested with BstEII and transformed into a W303 diploid, yielding strain CY406. Replacement of the SISI open reading frame with HIS3 at the SISI locus was confirmed by Southern analysis. This null allele of SISI is termed sit2-1. Phenotypes identical to strains containing sit2-2 are obtained with strains containing a smaller deletion of SISI (termed sit2-I). This smaller SISI deletion, which removes SISI amino acids 70-189, was prepared by replacing the sequences between the XbaI and Xhol sites with the HIS3 gene.

**Epitope Tagging of SISI**

To epitope tag the SISI 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 SISI. A duplex oligonucleotide encoding the hemagglutinin epitope was then inserted into these Sipel sites. The sequence of SISI was changed from MVKET...MTSYPYDVPDYASSVKET... to MVKET...MTSYPYDVPDYASSVKET... for the amino-terminal–tagged SISI protein. The sequence of SISI was changed from...DENF(stop) to...DENFSTOP for the carboxyl-terminal–tagged SISI. Both epitope tagged SISI proteins were functional because expression of either one in cells containing a deletion of SISI resulted in a wild-type growth rate.

**Conditional Expression of SISI**

A BamHI restriction site was created at position -13 of SISI (relative to the A of the SISI ATG) by inserting the self-complementary oligonucleotide 5'-CTAGTATTATTAGTTGGATCCAACTAATAA_TGA-3' (the BamHI site at position-13 of SISI) and replacing these SpeI sites. The sequence of SISI was changed from MVKET... to_MTSYPYDVPDYASSVKET... for the amino-terminal–tagged SISI protein. The sequence of SISI was changed from...DENF(stop) to...DENFSTOP for the carboxyl-terminal–tagged SISI. Both epitope tagged SISI proteins were functional because expression of either one in cells containing a deletion of SISI resulted in a wild-type growth rate.

**Immunoprecipitation of SISI**

Immunoprecipitation of SISI from extracts prepared from 35S-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 SISI, 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 protease 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 protease 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          |
|------------|------------|-----------------------------------------------|-----------------|
| W303       | W          | MATa ade2-1 his3-11,15 leu2-3,112 ura3-1       | R. Rothstein*   |
| CY406      | W          | sis1-2/ SISI W303 diploid                     | This study      |
| CY439      | W          | sis1-2 [SISI on YCp50]                        | This study      |
| CY455      | W          | sis1-2 [SISI on LEU2/cen plasmid]             | This study      |
| CY457      | W          | sis1-2 [NH2-tagged SISI on LEU2/cen plasmid]  | This study      |
| CY461      | W          | sis1-2 [COOH-tagged SISI on LEU2/cen plasmid] | This study      |
| CY661      | W          | sis1-2 [NH2-tagged SISI on YEp24]             | This study      |
| CY719      | W          | sis1-2 [NH2-tagged (2 copies) SISI on LEU2/cen plasmid] | This study |
| CY969      | W          | CY439 [NH2-tagged sis1-79 on LEU2/cen plasmid] | This study      |
| CY701      | W          | CY439 [NH2-tagged sis1-80 on LEU2/cen plasmid] | This study      |
| CY703      | W          | CY439 [NH2-tagged sis1-81 on LEU2/cen plasmid] | This study      |
| CY704      | W          | CY439 [NH2-tagged sis1-82 on LEU2/cen plasmid] | This study      |
| CY705      | W          | CY439 [NH2-tagged sis1-84 on LEU2/cen plasmid] | This study      |
| CY706      | W          | CY439 [NH2-tagged sis1-85 on LEU2/cen plasmid] | This study      |
| CY707      | W          | CY439 [NH2-tagged sis1-86 on LEU2/cen plasmid] | This study      |
| CY708      | W          | CY439 [NH2-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::NH2-tagged sis1-81                | This study      |
| CY889      | W          | sis1-2 LEU2::NH2-tagged sis1-82                | This study      |
| CY891      | W          | sis1-2 LEU2::NH2-tagged sis1-85                | This study      |
| CY893      | W          | sis1-2 LEU2::NH2-tagged sis1-86                | This study      |
| BJ926      |            | MATa trp1- /+ his3-1+ prc1- 126/- pep4-3/-     | E. Jones†       |
|            |            | prbl-1122/- can1-1- gal2-/-                   | (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.
* Columbia University, New York, NY.
† Carnegie Mellon University, Pittsburgh, PA.
‡ University of North Carolina, Chapel Hill, NC.

Preparation of Mutations in SISI

Oligonucleotide directed deletions in the SISI gene that removed 22 amino acid regions of SISI (see Fig. 3) were prepared by the method of Kunkel (1985). Two separate deletion series were prepared: one series used the NH2 epitope tagged SISI and the other series used the COOH epitope tagged SISI as the starting gene. For each sisl mutation, the deletion junction and at least 100 bases on both sides were sequenced to confirm the desired mutation. The results from the NH2 epitope-tagged SISI series and the COOH epitope-tagged SISI series were identical except that strains containing NH2 epitope-tagged SISI-80 were inviable while strains containing COOH epitope-tagged SISI-80 were viable. To demonstrate that the inviability of NH2 epitope-tagged SISI-80 strains was not due to an unidentified mutation outside the region sequenced, a new NH2 epitope-tagged sisl-80 gene was prepared by subcloning a XhoI/Xba I restriction fragment containing the sisl-80 mutation from COOH epitope-tagged sisl-80 into the unmutagenized NH2 epitope-tagged SISI gene. A strain containing the new NH2 epitope-tagged sisl-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 the 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 SISI 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 SISI localization, the cells were blocked with 1% BSA and 2% normal goat serum to reduce nonspecific background staining. Cells were viewed with a Zeiss Axioshot 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 Kalinchich 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 SISI. (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 SISI 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 sisl-2/SISI diploid (CY406) was sporulated and the tetrads dissected. Each tetrad, as shown in a vertical column, contains two large His\(^+\) SISI colonies and two very small colonies each with \(~500\) inviable cells. About half of the sisl-2 cells have a terminal phenotype as a very large sized cells. The growth is shown after 5 d at 30°C. SISI is also essential for viability at lower temperatures because similar results are obtained when the plates are incubated at 23°C.

Western analysis of SISI levels, anti-SISI antiserum (No. 252) directed against the nonconserved central region of SISI was prepared by injecting rabbits with a trpE/SISI fusion protein containing amino acids 74–191 of SISI. At the dilution (1:1000) used for Western analysis of yeast extracts separated on SDS–polyacrylamide gels, this antiserum detected only SISI in the size range of 30–45 kD.

Results

Isolation of SISI

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 ti 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 SISI 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 SISI is shown in Fig. 1 A.

The SISI through SIS4 genes in high copy number are not able to suppress the temperature sensitive phenotype of sit4 strains. In addition, SISI 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 SISI, SIS2, or SIS4 is specific for sit4 strains because none of these genes in high copy number can suppress the growth defect of sitl, sit2, or sit3 strains. In addition, the SIS genes in high copy number do not 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 SISI or SIS2 than with high copy number SIS3 or SIS4 (data not shown). The SIS4 gene (renamed PPH2a) 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 SISI gene.

Genetic Mapping of SISI

The minimal region of the high copy number SISI 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 SISI containing plasmid. This SISI 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 SISI–containing DNA fragment hybridized to chromosome 14, very close to the centromere. The SISI gene maps to a previously unidentified genetic locus, <1 cM from pet8 (see Materials and Methods). One of the original SISI-containing plasmids that contains a 15-kb yeast DNA insert complements the pet8 mutation. However, SISI is not PET8 because the 2.0-kb BstEII/BstEII subclone that contains SISI does not complement pet8.
SISI Encodes a dnaJ Homologue

The DNA sequence of the SISI gene predicts a protein of 352 amino acids with a molecular mass of 37,592 D (Fig. 2). Searches of the databases reveal that SISI is similar to E. coli and M. tuberculosis dnaJ proteins (Fig. 3). SISI is most similar to bacterial dnaJ proteins in the amino-terminal third of the protein (50% identity for SISI and E. coli dnaJ for the first 104 amino acids). Amino acids 75–104 of SISI are very glycine rich, as are both bacterial dnaJ proteins. The carboxyl-terminal third of SISI is also similar to dnaJ proteins. However, the middle third of SISI (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 CxxCxGxG (indicated by arrows in Fig. 3). SISI does not contain these cysteine-rich repeats. In place of two of the cysteine-rich repeats, the SISI protein has a striking glycine/methionine rich sequence; GMGGMPGGMGGMHGGMG-

Figure 2. DNA and predicted protein sequence of SISI. These sequence data are available from EMBL/GenBank/DDBJ under accession number X58460.
GMPGG, which can be viewed either as a (GGMGGMGMP) motif or as containing two direct repeats of the sequences GGMGMPGG (Fig. 3). Within this region of SISI, the six methionines are positioned M M 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 SISI 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-GMP-GMP (Johnson et al., 1989). The function of the carboxyl-terminal regions of these proteins is not known.

SISI 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 SISI or bacterial dnaJ proteins (Rothblatt et al., 1989; Sadler et al., 1989; Fig. 3). The central third region of both YDJ1 and SCJ1 has very little similarity to the corresponding region of YDJ1 or SCJ1. The central third region of both YDJ1 and SCJ1 has very little similarity to the corresponding region of YDJ1 or SCJ1. The central third region of both YDJ1 and SCJ1 has very little similarity to the corresponding region of YDJ1 or SCJ1. The central third region of both YDJ1 and SCJ1 has very little similarity to the corresponding region of YDJ1 or SCJ1.

SISI Is an Essential Gene

A deletion allele of SISI, termed sisl-2, was prepared that replaces DNA sequences encoding amino acids 1–350 of SISI (of 352 total amino acids) with a 1.8-kb DNA fragment that contains the HIS3 gene (see Materials and Methods). When a sisl-2/SISI diploid strain (CY406) is sporulated and the tetrads dissected, each tetrad gives rise to two normal-sized His SISI colonies and two very small colonies, Fig. 1B. 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 sisl-2/SISI diploid containing the wild-type SISI gene on the URA3 centromere plasmid YCp50 is sporulated and the tetrads dissected, haploid sisl-2 (His+) progeny containing the wild-type SISI gene on YCp50 are obtained. When this strain is grown on 5-fluoro-orotic acid medium, which al-
level, at which point the cells are inviable. These second possibility is that the cells cannot divide at all in the absence of the SISI protein and that the cells take nine generations to dilute the SISI protein below some critical level, at which point the cells are inviable. The second possibility is that the cells run out of SISI very rapidly but can divide in the absence of SISI. Here, each division in the absence of sufficient SISI 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 sisl-2 progeny resulting from a sisl-2/SISI diploid shows that not every sisl-2 cell goes through nine generations. Some individual cells are inviable after only three generations from the original sisl-2 spore while other cells (only a few lineages) are viable after 13 generations from the original sisl-2 spore. The second line of evidence comes from the analysis of strain CY719 that conditionally expresses SISI protein. Strain CY719 contains a deletion of SISI at the chromosomal SISI locus and a low copy number URA3/centromere plasmid containing the SISI coding sequences under control of the GAL1 promoter. CY719 is viable when grown on galactose medium (GAL1 promoter induced, SISI expressed) but inviable on glucose medium (GAL1 promoter repressed, SISI not expressed). When CY719 is transferred from galactose to glucose medium, the levels of SISI protein decrease (Fig. 4 A). After 8 h, the levels of SISI 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 SISI 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 SISI 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 SISI are below some critical level but that cell division under these conditions eventually results in inviability.

**Phenotypic Defects Due to Limiting Levels of SISI**

When a sisl-2 strain expressing SISI under control of the GAL1 promoter (CY719) is transferred from galactose to glucose medium, the levels of SISI 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 (pGAL: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 OD600 of 0.1 and 0.75 by dilution). The cells were fixed with formaldehyde and treated with zymolase to remove the cell wall before incubation with the anti-α-tubulin antibody. (B) Nomarski optics and DAPI staining 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.
surface (Fig. 5B) (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. 5B). 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 SISI 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 SISI protein increases about twofold after a heat shock from 23 to 39°C (Fig. 4B). 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 corresponding to a conserved amino-terminal region of yeast HSP70 proteins (data not shown).

The finding that the level of the SISI protein increases upon heat shock is consistent with the presence of the sequence GAACGTTCCAGAAAC_TTCTGGAA (the first G is at -344 relative to the A of the SISI ATG; Fig. 2) in the SISI 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).
**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 aminoterminus 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 35S-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 prepared with RIPA buffer.

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 35S-labeled cells containing NH2-tagged SIS1 (strain CY457) was loaded onto a 1.0 x 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).
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 SISI are present in the immunoprecipitates (only a very small fraction of the SISI protein is associated with p40; see next section). Therefore, while the solubility of SISI is similar in native buffers compared to detergent containing buffers, the p40/SISI complex is much more soluble in buffers containing detergents.

SISI also associates with another protein. Longer exposure of the gel shows that SISI specifically coimmunoprecipitates with a protein of apparent molecular mass of 88 kD (Fig. 6 C). In addition, immunoprecipitation of SISI from a strain containing the amino-terminal epitope–tagged SISI 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 SISI remains to be determined because the same proteins or proteins of the same mobility also precipitate nonspecifically in the absence of SISI (first two lanes of Fig. 6 C). Neither p40, p88, nor the two proteins that increase in amounts in the immunoprecipitates when SISI 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/SISI Complex Has a Very Large Size**

To determine the approximate size of native SISI and the p40/SISI complexes, the cellular extract was fractionated on a Sepharose CL-6B (Pharmacia Inc.) column before immunoprecipitation. The great majority of SISI elutes at ~80 kD (Fig. 7). Therefore, the majority of SISI (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 SISI, the p40/SISI 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 SISI by indirect immunofluorescence. CY455 cells (wild-type SISI) or CY457 cells (NH2 epitope-tagged SISI) 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 SISI and with DAPI to visualize the nucleus (Materials and Methods). The arrow indicates a budded cell where no staining for SISI is apparent in the bud.

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

Association of SISI with p40 Requires Both SISI Glycine-rich Regions
To determine the region of SISI that is required for association with p40, we prepared eight different SISI mutations (termed sisl-79 through sisl-87), each of which encodes an altered SISI 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 SISI 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 SISI deletions was prepared using the amino-terminal epitope-tagged SISI or the carboxyl-terminal epitope-tagged SISI.

The genes encoding the epitope-tagged SISI derivatives on a low copy number LEU2/cen plasmid were transformed into strain CY455. CY455 has a deletion of SISI at the chromosomal locus (sisl-2) and contains the wild-type SISI gene on a low copy number URA3/cen plasmid. Therefore, each of the resulting strains has both an epitope-tagged deletion derivative of SISI and the wild-type SISI protein without the epitope tag. Immunoprecipitation analysis shows that SISI-84, SISI-85, SISI-86, and SISI-87 retain their association with p40 (Fig. 8 A). In contrast, SISI-81 (deleted in the region between the glycine-rich region and the glycine/methionine-rich region) and SISI-82 (deleted for the glycine/methionine-rich region) do not associate with p40. Although the amount of SISI-80 (deleted for the glycine-rich region) in the immunoprecipitates is reduced (probably due to instability
of the altered SISI-80 protein), prolonged exposure of the gels show no detectable p40 in these immunoprecipitations. Both SISI-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 SISI is required for association with p40.

Increased exposure of the same gels shows that SISI-84, SISI-85, SISI-86, and SISI-87 retain their association with p88 while SISI-81 and SISI-82 do not associate with p88 (data not shown). We cannot determine if SISI-79 and SISI-80 associate with p88 due to their much reduced levels in the immunoprecipitates. It is possible that the regions of SISI that are required for its association with p88 are the same regions of SISI that are required for its association with p40. The immunoprecipitation analysis also directly shows that SISI is multimeric. The mobility of each of the SISI deletion derivatives is somewhat variable. In some of the lanes, SISI is present as two separate bands: one band results from the epitope-tagged SISI deletion derivative and the other band results from untagged wild-type SISI (position indicated by arrow, Fig 8A) that coimmunoprecipitates with the epitope-tagged SISI. These results show that the regions of SISI deleted in the SISI-84, SISI-85, and SISI-86 derivatives are not required for multimerization of SISI.

Analysis of Strains Containing Deletion Derivatives of SISI

We also tested each of the SISI deletion derivatives to see if they could substitute for wild-type SISI. Strains containing only SISI-79, SISI-84, or SISI-87 are not viable; strains containing only SISI-85 of SISI-86 grow very slowly and are temperature sensitive; and strains containing only SISI-81 or SISI-82 have only a very slight growth defect (Fig 8B). For SISI-80, the ability to substitute for SISI depends on the position of the epitope tag: a strain containing only N\textsubscript{H\textsubscript{2}} epitope-tagged SISI-80 is not viable while a strain containing only COOH epitope-tagged SISI-80 has only a slight growth defect. Perhaps the 22-amino acid deletion in SISI-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 SISI deletion derivatives cause alterations in plasmid stability. An isogenic set of strains was prepared that has either wild-type SISI or the SISI-81, SISI-82, SISI-85, or SISI-86 gene (all N\textsubscript{H\textsubscript{2}} epitope tagged) integrated at the LEU2 locus. Each of these strains was transformed with the URA3/cenromere 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 SISI, 34% (standard deviation = SD of 7%) of the cells had lost the plasmid. In contrast, the strain containing SISI-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 SISI-85 had a reproducibly lower rate of plasmid loss: 18% (SD of 6%) of the cells had lost the plasmid. The SISI-82 strain and the SISI-86 strain lost the plasmid at a rate similar to the strain containing wild-type SISI (31% with SD of 7% and 29% with a SD of 7%, respectively).

Localization of SISI by Immunofluorescence

To determine the subcellular localization of SISI by indirect immunofluorescence microscopy, we used three isogenic strains: strain CY455 contained only wild-type SISI, strain CY457 contained only N\textsubscript{H\textsubscript{2}} epitope-tagged SISI, and strain CY461 contained only COOH epitope-tagged SISI. 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 SISI proteins. In the absence of the epitope tag, wild-type yeast cells (strain CY455) show only very weak staining (Fig 9). The staining for strain CY457 shows that the N\textsubscript{H\textsubscript{2}}-tagged SISI is localized throughout the cell but is more concentrated in the region of the nucleus, whose position is visualized by DAPI staining (Fig 9). In almost every case, the staining for SISI 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 N\textsubscript{H\textsubscript{2}} epitope-tagged SISI containing cells is obtained with cells containing COOH epitope-tagged SISI protein (data not shown).

Distribution of SISI in Subcellular Fractions

Since SISI is more concentrated in the region of the nucleus, we determined how SISI fractionated with nuclei using the method of Kalinchich and Douglas (1989). Yeast spheroplasts containing wild-type SISI were lysed in a hypotonic buffer (0.02 M Hepes, pH 7.4, 5 mM MgCl\textsubscript{2}). The lysate (Cells lane in Fig 10A) 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 10A). In this procedure, ~90% of the SISI fractionates with the pellet. If higher ionic strength buffers are used to lyse the cells, more of the SISI 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 SISI.

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 SISI 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 SISI was released (Fig 10B). Separate treatment of the nuclear fraction with RNase A or DNase I shows that treatment with RNase A releases SISI while treatment with DNase I does not (Fig 10B). Subsequent probing of these blots with antisera against YDJ1 (gift of A. Caplan and M. Douglas) shows that YDJ1 is not released by treatment with either
RNase A or DNase I (data not shown; also see Caplan and Douglas, 1991). Treatment of the nuclear fraction with 1 M NaCl releases at least two-thirds of the SISI protein, confirming the ability of high salt to solubilize SISI. Treatment of the nuclear fraction with 2% Triton X-100 releases about one-third of the SISI. 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 SISI remains associated with this complex (data not shown).

**YDJ1 Can Not Substitute for SISI**

The **SISI** gene is essential for viability. To determine if overexpression of the yeast **dnaJ** homologue **YDJ1** could substitute for SISI, a **sisl-2/SISI** 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 **sisl-2** haploid progeny gave colonies of very similar size, each containing \sim 500 inviable cells (data not shown). We expect \sim 75% of the **sisl-2** haploid progeny to contain pAV5 because about 75% of the SISI 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 **SISI**.

We also tested if overexpression of YDJ1 could partially cure the slow growth phenotype of a **sisl-85** strain or a **sisl-86** strain. When a **sisl-85** or **sisl-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 **sisl-85** strain or the **sisl-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 SISI strain transformed with pAV5 did not grow slower than when transformed with the control plasmid. Therefore, not only can YDJ1 not substitute for SISI, but overexpression of YDJ1 interferes with the functions of the SISI-85 and SISI-86 proteins. This result contrasts with the suppression of **YDJ1** mutants by high copy number SISI (see Caplan and Douglas, 1991).

**Discussion**

In addition to SISI, 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 SISI protein is similar to bacterial **dnaJ** proteins in the amino-terminal third and carboxyl-terminal third of the proteins. However, the central third of SISI is not similar to any of the other known **dnaJ** proteins or homologues. This central third of SISI 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 SISI 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 SISI, mediates the association of these proteins with other specific cellular components.

The functions of the SISI associated proteins p40 and p88 are not known. The p40/SISI complex elutes from a sizing column as a very large complex and may contain many p40 molecules for each SISI molecule. Since the p40/SISI complex is more soluble in buffers containing detergents, it may be membrane associated. The soluble SISI protein present in the cell extracts used for immunoprecipitation analysis...
is not detectably associated with yeast HSP70 proteins. Whether or not SISI 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 SISI. Many of the cells in strains limited for SISI appear blocked for nuclear migration and/or become very large in size and contain a large vacuole. However, we cannot distinguish if these phenotypic defects result directly or indirectly from limiting SISI. The very large cells containing a large vacuole could result nonspecifically from cell death. The block in nuclear migration resulting from limiting the SISI protein is similar to that seen for cdc mutants (such as cdc2, cdc7, and cdc9 mutants) that are defective in DNA replication (Fringe and Hartwell, 1981). Specific mutations in SISI cause alterations in plasmid stability, which could result from defects in plasmid replication or segregation. The alterations in plasmid stability resulting from the altered SISI proteins could be related to the blocked nuclear migration phenotype in cells limited for SISI.

The SISI protein is localized throughout the cell but is more concentrated in the region of the nucleus. In addition, about a fourth of SISI (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 SISI gene. High copy number SISI 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 SISI itself since isogenic wild-type and sit4 strains have very similar amounts of the SISI protein (Luke, M.M., A. Sutton, and K. T. Arndt, unpublished data). It is possible that high copy number SISI increases the growth rate of sit4 strains by increasing the functional amount of a limiting mRNA. That about a fourth of the SISI protein is released from a nuclear fraction upon treatment with RNase could be due to the involvement of SISI in some aspect of mRNA transcription, processing, or transport. Whether or not SIT4 and SISI are both involved in some common step of mRNA transcription remains to be determined. Could SISI be a substrate of SIT4? A small minority (<10%) of the SISI protein is phosphorylated in vivo such that this form of SISI migrates slightly slower than the majority of SISI (seen by 15S labeling). Although the overall in vivo phosphorylation state of SISI 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 dephosphorylates only one of many possible phosphorylated residues on the phosphorylated form of SISI.

SISI may have multiple functions required for cellular growth. Some insight into how SISI 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 (Zylorzic et al., 1989; Liberek et al., 1990). Perhaps, like dnaJ, the SISI protein also mediates specific protein/protein dissociations. Whatever the mechanism of SISI action, the function of SISI is unique: SISI is an essential gene. Overexpression of the yeast dnaJ homologue YDJ1 (Caplan and Douglas, 1991) cannot substitute for SISI and actually inhibits the growth of strains containing sit1-s5 or sit1-s6 mutations. Further genetic and biochemical analysis will be required to determine the precise cellular functions of SISI.

We thank A. Caplan and M. Douglas for YDJ1 antisera, the YDJ1 gene on a high copy number vector, sharing data before publication, and continued collaboration. We thank E. Craig for antisera to the conserved peptide of yeast HSP70 proteins, C. Georgopoulos for an introduction to dnaJ proteins, and L. Riles, and M. Olsen for mapping SISI to their yeast physical map. We also thank C. Devlin and M. Tyers for comments on the manuscript.

This research was supported by National Institutes of Health grant GM39892 to K. T. Arndt.

Received for publication 2 April 1991 and in revised form 10 May 1991.

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. Zylorzic, 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 K. H. Herkowitz. 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 dnaT(Ts) and dnaD(Ts) mutants of Escherichia coli. J. Bacteriol. 99:1719-1724.

Itikawa, H., M. Wada, K. Sekine, and H. Fujita. 1989. Phosphorylation of glutamyl-tRNA synthetase and threonyl-tRNA synthetase by the gene products of dnaK and dnaD in Escherichia coli K-12 cells. Biochim. Biophys. Acta. 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.

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

Kilmarin, J. V., and A. E. M. Adams. 1984. Structural rearrangements of yeast 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.

Lamberta, R. B., D. B. Young, D. Sweitzer, 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. Tanura, 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, L., A. Chiang, T. Kurthara, 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, 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. Iikawa. 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 PI plasmid DNA replication: formation of an active complex between E. coli dnaJ protein and the PI 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.