Transcriptional Regulation of the Yeast DnaJ Homologue SIS1*

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The Saccharomyces cerevisiae SIS1 gene encodes an essential heat shock protein with similarity to the Escherichia coli DnaJ protein. In sis1-85 and sis1-86 mutants, the sis1 RNA is induced to high levels at room temperature and without heat shock. The presence of wild type SIS1 in the sis1-85 mutant represses the overexpression of SIS1-85 protein. Furthermore, overexpression of wild type SIS1 reduces the β-galactosidase activity expressed from a SIS1lacZ fusion. These results suggest that SIS1 negatively regulates its own expression. The autoregulation of SIS1 transcription is mediated through a 39-base pair cis-element containing the SIS1 heat shock element plus additional flanking sequences on one side. Although SIS1 transcription is constitutive, it is transiently induced upon heat shock. In addition, SIS1 transcription is regulated by SSA (a class of HSP70 proteins) function. The elevated transcription of SIS1 in ssa1 ssa2 mutants is mediated solely through the SIS1 heat shock element. Therefore, the SIS1 auto-regulatory element is different from the SSA-responsive element, suggesting that the mechanism involved in autoregulation of SIS1 is distinct from regulation of SIS1 by SSA proteins.

All organisms respond to environmental stresses, such as heat shock, by the rapid and transient increase in the rate of synthesis of a group of proteins collectively called the heat shock proteins (Craig et al., 1994; Lindquist and Craig, 1988). The SIS1 gene encodes an essential DnaJ homologue in the yeast Saccharomyces cerevisiae (Luke et al., 1991). The DnaJ protein of Escherichia coli was shown to be a member of the heat shock proteins (Lindquist and Craig, 1988), and 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). The increase in DnaJ expression is rapid and appears to be regulated primarily at the transcriptional level (Georgopoulos et al., 1994). In E. coli, a mutation in the gene encoding any one of three heat shock proteins, DnaJ, Dnak, or GrpE, results in the elevated expression of itself and many other heat shock genes at low temperatures (Straus et al., 1990). This autoregulation is achieved at least partially via a negative feedback mechanism where the synthesis and stability of a heat shock transcriptional activator α2 is regulated (Georgopoulos et al., 1994, Straus et al., 1990).

In eukaryotic cells, the transcription of heat shock genes is normally regulated through a promoter sequence termed the heat shock element (HSE).1 The HSE, which is composed of a 5-bp unit nGAAAn alternating with another 5-bp unit nTCCn (Amin et al., 1988; Xiao and Lis, 1988), has been highly conserved during evolution (Bienz and Pelham, 1987). The heat shock factor binds to the HSE and is required to activate the transcription of certain heat shock genes not only in response to stress but also under conditions of normal growth (Sorger, 1990). In yeast, some heat shock proteins perform essential functions and are constitutively expressed. Other heat shock proteins are expressed, sometimes transiently, only upon a shift to a higher temperature (Craig et al., 1994). Transcriptional regulation of budding yeast HSP70 homologues has been the focus of many studies. SSA1, which encodes one of the SSA class of HSP70 proteins, autoregulates its own transcription via one of its HSEs (Stone and Craig, 1990). In addition, the expression of many heat shock genes, such as SSA3, SSA4, HSC82, and HSP26, is highly induced in ssa1 ssa2 mutants (Boorstein and Craig, 1990b, 1990c; Lindquist and Craig, 1988).

The DnaJ heat shock protein has been conserved in evolution (Caplan et al., 1993). S. cerevisiae is currently known to have eight DnaJ-like proteins: SIS1, YDJ1, SEC63, SCJ1, Zoutin, MDJ1, CAJ1, and XDJ1, all of which contain a region highly similar to the amino-terminal region of bacterial DnaJ (the J-domain) (Luke et al., 1991; Silver and Way, 1993). SEC63 is an integral endoplasmic reticulum membrane protein involved in protein transport across the endoplasmic reticulum (Rothblatt et al., 1989; Sadler et al., 1989). YDJ1 is present in the cytosol and functions to transport polypeptides targeted to both mitochondria and endoplasmic reticulum (Atencio and Yaffe, 1992; Caplan et al., 1992). The SCJ1 gene was identified by its ability, when present on a high copy number plasmid, to cause misorting of a nuclear targeted protein (Blumberg and Silver, 1991). Zoutin was purified from nuclear extracts as a Z-DNA binding protein (Zhang et al., 1992). MDJ1 associates with the inner membrane of mitochondria and is involved in protein refolding and mitochondria biogenesis (Rowley et al., 1994). The functions of CAJ1 and XDJ1 are currently unknown (Mukai et al., 1994; Schwarz et al., 1994). The SIS1 protein is required for the normal initiation of translation and is found associated with ribosomal protein complexes (Zhong and Arndt, 1993).

Although some of the biological functions of eukaryotic DnaJ-like proteins have been identified, little is known about their transcriptional regulation. Among the promoter regions of these yeast DnaJ-like genes, MDJ1 has several weak matches to the consensus heat shock element (Rowley et al., 1994). YDJ1 and SCJ1 only have one copy of nGAAAnTTTc (Blumberg and Silver, 1991; Caplan and Douglas, 1991). However, SIS1 has a well conserved heat shock element (ATAT-

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1 The abbreviations used are HSE, heat shock element; MOPS, 4-morpholinopropanesulfonic acid; bp, base pair(s); kb, kilobase(s); UAS, upstream activating sequences.
GAACGTTCGAGAACTTCTGAAAAAG) containing 5 total units of nGAn alternating with nTTCn (Luke et al., 1991). In this report, we show that the transcription of SIS1 is transiently increased by heat shock. SIS1 transcription is also induced by a defect in SSA function, and this induction requires only the SIS1 heat shock element. Moreover, SIS1 regulates its own transcription via a specific cis-element that contains the SIS1 heat shock element plus an additional promoter sequence. These findings point to the specificity of the induction of heat shock genes and the multiple mechanisms involved in their transcriptional regulation.

### Table 1

**Yeast strains**

| Strain | Genotype | Source |
|--------|----------|--------|
| AY925  | W303: MATα ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 ssd1-d2 can1-100 | R. Rothstein* |
| CY439  | Y925Δsis1::His3 (SIS1 on YEp50) | Luke et al., 1991 |
| CY57   | Y925Δsis1::His3 (NH$_{2}$-HA-tagged SIS1 on LEU2/CEN plasmid) | Luke et al., 1991 |
| CY706  | CY439 (NH$_{2}$-HA-tagged sis1-85 on LEU2/CEN plasmid) | Luke et al., 1991 |
| CY732  | Y925Δisis1::His3 (NH$_{2}$-HA-tagged sis1-85 on LEU2/CEN plasmid) | Luke et al., 1991 |
| CY733  | Y925Δisis1::His3 (NH$_{2}$-HA-tagged sis1-86 on LEU2/CEN plasmid) | Luke et al., 1991 |
| CY735  | Y925Δisis1::His3 (SIS1 on LEU2/CEN plasmid) | Luke et al., 1991 |
| CY3051 | MATα assb1::LEU2 ura3 leu2 his3 Δtrp1 lys2 | E. Craig* |
| CY3052 | MATα assb1::LEU2 Δssb2::LEU2 ura3 leu2 his3 Δtrp1 lys2 | E. Craig* |
| CY3206 | MATα assb2::LEU2 ura3 leu2 his3 Δtrp1 lys2 | E. Craig* |
| CY3744 | MATα His62::URA3 leu2 his3 ura3 lys2 | S. Lindquist* |
| CY3745 | CY3744 plus hsp82-4 | S. Lindquist* |
| CY3746 | CY3744 plus hsp82-41 | S. Lindquist* |
| CY3747 | CY3744 plus hsp82-33 | S. Lindquist* |
| CY3748 | CY3744 plus hsp82-38 | S. Lindquist* |
| CY3749 | CY3744 plus hsp82-92 | S. Lindquist* |
| CY3766 | MATα URA3/2 his3 Δtrp1 lys2 | E. Craig* |
| CY3767 | MATα Ass1::His3 Ass2::LEU2 ura3 leu2 his3 Δtrp1 lys2 | MW123 of E. Craig* |
| CY3768 | MATα Ass1::His3 Ass2::LEU2 ura3 leu2 his3 Δtrp1 lys2 GAL2 | MW142 of E. Craig* |
| CY3769 | MATα Assa3::TRP1 Ass4::URA3 ura3 leu2 his3 Δtrp1 lys2 | MW42 of E. Craig* |
| CY3770 | MATα Assa3::TRP1 Ass4::URA3 ura3 leu2 his3 Δtrp1 lys2 GAL2 | MW115 of E. Craig* |
| CY3781 | CY372 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY3875 | CY457 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY3998 | CY457 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4003 | CY457 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4232 | CY372 (L39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4288 | CY372 (L39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4241 | CY457 (R39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4235 | CY372 (R39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY3994 | CY457 (31-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY3999 | CY457 (31-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4577 | CY372 (23-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4579 | CY372 (23-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4461 | CY3766 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4464 | CY3767 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4626 | CY3766 (L39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4632 | CY3766 (L39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4633 | CY3767 (L39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4628 | CY3766 (R39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4635 | CY3767 (R39-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4636 | CY3766 (31-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4460 | CY3767 (31-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4367 | CY3766 (23-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4368 | CY3767 (23-ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4369 | CY3767 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4364 | CY3767 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4363 | CY3767 (ΔUAS-HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4557 | CY3767 (ΔUAS-HIS4::lacZ on URA3/2μ plasmid) | This study |
| CY4541 | CY457 (ΔHE-SIS1::lacZ on URA3/2μ plasmid) | This study |
| CY4545 | CY372 (SIS1::lacZ on URA3/2μ plasmid) | This study |
| CY4546 | CY372 (ΔHE-SIS1::lacZ on URA3/2μ plasmid) | This study |
| CY4873 | CY3766 (SIS1::lacZ on URA3/2μ plasmid) | This study |
| CY4874 | CY3766 (ΔHE-SIS1::lacZ on URA3/2μ plasmid) | This study |
| CY4875 | CY3767 (ΔHE-SIS1::lacZ on URA3/2μ plasmid) | This study |
| CY4876 | CY3767 (ΔHE-SIS1::lacZ on URA3/2μ plasmid) | This study |
| CY4900 | CY3766 (HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4902 | CY3767 (HIS4::lacZ on URA3/CEN plasmid) | This study |
| CY4967 | CY4957 (SIS1::lacZ on URA3/2μ plasmid, SIS1 on YEp13 plasmid) | This study |
| CY4968 | AY925 (SIS1::lacZ on URA3/2μ plasmid, sis1-fs on YEp13 plasmid) | This study |
| CY4969 | AY925 (SIS1::lacZ on URA3/2μ plasmid, YEp13 plasmid) | This study |
| CY5020 | CY373 (SIS1::lacZ on URA3/2μ plasmid) | This study |
| CY5026 | CY373 (SIS1::lacZ on URA3/2μ plasmid) | This study |

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### Materials and Methods

**Yeast Strains and Media**

Yeast strains are listed in Table I. The yeast cells were grown on either YPD medium (1% yeast extract, 2% peptone, 0.2 mliter trytophan, 0.1 mliter adenine, 1 mm KH$_{2}$PO$_{4}$, and 2% glucose) or SC medium (synthetic complete, using 0.2 mliter leucine and 0.1 mliter of all other amino acids, 0.1 mliter uracil, and 0.075 mliter adenine, with 2% glucose) (Rose et al., 1990).

**Plasmid Constructions**

SIS1::lacZ and ΔHE-SIS1::lacZ Translation Fusions—A SpeI restriction site was created just after the SIS1 ATG translation initiation...
.codon (Lueke et al., 1991). A BamHI site was created at the SpeI site by insertion of the oligonucleotide CTAGAGGATCCT (the BamHI site is underlined). For the SIS1:lacZ fusion, the CYC1 promoter region up to the ATG translation start codon in the CYC1::lacZ fusion on the 2 µURA3 vector (Dalan and Treisman, 1992) was replaced in frame by a 1.5-kb BstEII-BamHI fragment containing the entire upstream region of SIS1. For the ΔHSE-SIS1::lacZ fusion, the S1-bp S1::lacZ fusion (sequences 347 to 317 with respect to the A of the S1 ATG translation initiation codon) was deleted by oligonucleotide-directed mutagenesis (Kunkel, 1985).

SIS1 (Promoter Element)-ΔUAS-HIS4::lacZ Fusions—A 53-bp oligonucleotide (TCGACCTTTGGTTTATATGAACTTCTGGAGAACTTCTGGAGAAC- GCT; TCGACATATGAACGTTCCAGAAACTTCTGGAAAAAGC), two 23-bp oligonucleotides (TCGACATTTGGTTTATATGAACTTCTGGAGAAC- GCT; TCGACATATGAACGTTCCAGAAACTTCTGGAAAAAGC), and a 23-bp oligonucleotide (TCGACACATTCTGGAAAAAGC) were cloned into the XhoI site of a ΔUAS-HIS4::lacZ/YCp50 expression plasmid (AB174). The ΔUAS mutation (ΔS2 of Nagawa and Fink (1986)) removes the HIS4 UAS and replaces it with a XhoI site.

sis1::fs Construct—A frame-shift mutation in the SIS1 gene was created by cutting at the XhoI site (at position +200 with respect to the A of the SIS1 ATG initiation codon), filling in the 5' overhangs with Klenow enzyme, and religating the ends.

Heat Shock Treatment and RNA Preparation

Yeast cultures were grown at 24°C to exponential phase. Half of the culture was transferred to a water bath (39°C), while the other half remained at 24°C. 5-ml aliquots of the culture at 24°C and for each time point at 39°C were harvested by centrifugation and washed with ice-cold H2O. Cells were resuspended in 250 µl of 100 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.01 M EDTA, 0.01 M Tris-HCl (pH 7.4), 0.2% SDS and lysed by vortexing for six pulses of 15 s in the presence of 300 µl of LETS-equilibrated phenol and 300 µl of glass beads. After adding an additional 200 µl of LETS buffer, the cells were vortexed again for 10 s and centrifuged at 16,000 × g for 8 min. The supernatant was extracted twice with LETS-equilibrated phenol/chloroform. The RNA was precipitated with 1/10 volume of 5 M LiCl and 2.5 volumes of ethanol at -20°C and resuspended in 50 µl of DEPC-treated H2O.

7.5 µg of RNA was loaded onto each lane of a 1% agarose gel containing 6% formaldehyde, 1 × MOPS (0.02 M morpholinopropane-sulfonic acid, 5 mM sodium acetate, 1 mM EDTA (pH 7.0)). The gels were blotted onto Bio-Trans nylon membranes. The probes used for Northern analyses were the 2.4-kb BstEII fragment of SIS1 from CB547, the 0.9-kb NcoI fragment from SSA1 from CB198, the cloned polymerase chain reaction product encompassing the entire open reading frame of CTT1 from CB2550, the 1.7-kb MluI-Stul fragment of SSA4 from CB1785, the 1.2-kb EcoRI fragment of HSC82 from CB2313, the 0.6-kb AluI fragment of ACT1 from CB882, and the 2.6-kb PvuI fragment of lacZ from AB174. The blots were washed twice (15 min each) at 24°C using 2 × SSC, 0.1% SDS and twice (15 min each) at 65°C using 0.1 × SSC with 0.1% SDS.

Preparation of Cellular Extracts and Immunoblotting

Exponentially growing cells were harvested by centrifugation and washed with ice-cold lysis buffer (100 mM Tris-HCl (pH 7.5), 200 mM NaCl, 1 mM EDTA, 5% glycerol, 0.05% dithiothreitol). Cells were resuspended in 300 µl of lysis buffer (1 mM phenylmethylsulfonyl fluoride; 1 µM pepstatin, 1 mM leupeptin, 1 mM chymostatin, and 1 µg/ml each of leupeptin, antipain, chymostatin, and pepstatin (Sigma)) and lysed by vortexing in the presence of glass beads for four pulses of 15 s. After adding an additional 350 µl of lysis buffer, the cells were vortexed again for 15 s. The liquid was pipetted from the glass beads and centrifuged at 16,000 × g for 8 min to remove cell debris. The protein concentrations of the supernatants were determined using the Bio-Rad protein assay with bovine serum albumin as the standard. The protein extracts were mixed with equal volumes of 2 × Laemmli buffer (Laemmli, 1970), heated for 5 min at 95°C, centrifuged for 3 min at 16,000 × g, and loaded on 10% SDS-polyacrylamide gels. Blots of gels were probed for SIS1-85:HA with ascites containing the monoclonal antibody 12CA5 (Field et al., 1988) using an ECL detection system (Amersham Corp.).

β-Galactosidase Assay

As described previously (Di Como et al., 1995), the cell extracts (0.1 ml of extract for experiments in Fig. 3B; 5 µl of extract for experiments in Figs. 1B, 4, 6B; 10 µl of extract for experiments in Fig. 2B) were mixed with Z buffer and incubated at 28°C. After 5 min, 0.2 ml of o-nitrophenylgalactoside was added. The reactions were stopped by the addition of 0.5 ml of 1 M Na2CO3, and the amounts of product were determined by measuring A420. For each sample, the assay was performed with two separate cultures in at least two independent experiments, and the average (large bar) and the standard deviation (small horizontal line connected to bar with vertical line) are reported.

RESULTS

sis1-85 and sis1-86 Alleles Are Expressed at Very High Levels—At room temperature on YPD plates, sis1-85 and sis1-86 strains form colonies almost as fast as wild type strains but grow very slowly at 37°C (Lueke et al., 1991). In the course of examining the protein levels in sis1-85::HA strains (which have influenza hemagglutinin epitope-tagged SIS1-85 protein), we found that the levels of SIS1-85::HA protein were much higher than those of the SIS1::HA protein in a wild type strain at room temperature (data not shown). To determine if the increased levels of SIS1-85::HA protein were due to the elevated levels of sis1-85 RNA, Northern analysis was performed. We found that the level of sis1-85 or sis1-86 RNA was much higher (about 6-fold) in the sis1-85 or the sis1-86 strain as compared to the SIS1 RNA level in the wild type strain (Fig. 1A).

The increased expression of the sis1-85 and sis1-86 genes might be mediated through the upstream region of SIS1. To test this possibility, we prepared a SIS1::lacZ translation fusion, where the upstream region, including the initiation codon of SIS1, was fused in-frame to the lacZ coding sequence (see "Materials and Methods"). The β-galactosidase activity expressed from the SIS1::lacZ fusion was assayed in isogenic sis1-85, sis1-86, and wild type SIS1 strains. The SIS1::lacZ fusion gave 6-fold higher β-galactosidase levels in the sis1-85 strain and 5.5-fold higher β-galactosidase levels in the sis1-86 strain as compared to the isogenic wild type strain (Fig. 1B). Therefore, the induction of β-galactosidase activity expressed from the SIS1::lacZ fusion in the sis1 mutants was quantitatively similar to the induction of sis1 RNA levels in the sis1 mutants. These results indicate that sequences upstream of the SIS1 initiation codon are responsible for the induction of RNA levels in sis1-85 and sis1-86 strains. Since the sis1-85 and sis1-86 alleles had similar effects on SIS1 expression, subsequent experiments were performed in the sis1-85 strain.

To determine if the expression of other heat shock or stress-induced genes is activated in the sis1-85 mutant, we compared the RNA levels of SSA1 and SSA4 (which encode HSP70 proteins) and CTT1 (a stress-induced gene that encodes catalase T) (Marchler et al., 1993) in the sis1-85 mutant to those in the isogenic wild type strain. At room temperature, SSA1, SSA4, and CTT1 were not highly expressed in the sis1-85 mutant as compared to the wild type strain (Fig. 1C). Upon heat shock (30 min after a shift to 37°C), their RNA levels increased (Fig. 1C). These results suggest that the elevated sis1 expression in the sis1-85 mutant at room temperature is not a general stress or heat shock response. Interestingly, SSA4 RNA was induced by heat shock to a higher level in the sis1-85 mutant than in the isogenic wild type strain (Fig. 1C). Moreover, by 90 min at 37°C, the SSA4 RNA level was maintained at almost the same high level in the sis1-85 mutant, when it was hardly detectable in the wild type SIS1 strain (Fig. 1C). Possibly, the sis1-85 strain could be more sensitive to heat shock.

SIS1 Negatively Regulates Its Own Synthesis—The increased transcription from the SIS1 upstream region in sis1-85 and sis1-86 strains may be due to the absence of wild type SIS1 protein that normally represses its own expression. Since it is not easy to distinguish the sis1-85 RNA from the wild type SIS1 RNA, we compared the level of the HA-tagged
SIS1–85 protein in a sis1–85:HA strain to that in a strain containing both the sis1–85:HA and the wild type SIS1 genes. Because the SIS1–85:HA protein but not the wild type SIS1 protein is tagged with a 9-amino acid HA epitope (Luke et al., 1991), we can uniquely detect the SIS1–85:HA protein using a monoclonal antibody directed against the HA tag. Immunoblot analysis showed that the presence of the wild type SIS1 gene on a centromere plasmid decreased the levels of the SIS1–85:HA protein (Fig. 2A).

If SIS1 protein does negatively regulate its own synthesis, then overexpressing the SIS1 protein might repress the expression of a SIS1::lacZ fusion. β-Galactosidase activity expressed from the SIS1::lacZ fusion was examined in three strains: one

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**Fig. 1. Induction of SIS1 RNA levels.** A. SIS1 RNA is induced to high levels in sis1–85 and sis1–86 mutants. Isogenic strains CY457 (SIS1), CY732 (sis1–85), and CY733 (sis1–86) were grown to exponential phase in YPD medium at 24 °C. 5-ml aliquots of culture for each strain were collected to determine the β-galactosidase activity. Three independent assays were carried out. C, SSA1, SSA4, and CTI1 are not highly expressed in the sis1–85 strain at 24 °C. Isogenic strains CY457 (SIS1) and CY732 (sis1–85) were grown to exponential phase in YPD medium at 24 °C and then shifted to 37 °C. For each strain, 5-ml aliquots of culture were collected at 24 °C and after 30 min and 90 min at 37 °C. Total RNA was prepared. The Northern blot was probed for SSA1, SSA4, CTI1, and ACT1 RNA. The probes used for Northern analysis are as listed under “Materials and Methods.”
due to increased transcriptional activity of the SIS1 promoter.

To further delineate the SIS1 autoregulatory promoter element, smaller regions of the SIS1 promoter element were tested. Surprisingly, the β-galactosidase expressed from the SIS1 heat shock element (31 bp containing 5 units of nGAAAn alternating with nTTCn, plus 3 bp on both sides), which lacks 8 bp from both ends of the 47-bp sequence, was only 2-fold higher in the sis1–85 strain compared to the wild type SIS1 strain (Fig. 3, A and B). Therefore, the 31-bp SIS1 heat shock element does not contain all the sequences necessary for SIS1 regulation.

To determine the SIS1 autoregulatory element sequences present within the 47-bp SIS1 promoter fragment but not contained within the 31-bp SIS1 heat shock element, we constructed L39-ΔUAS-H154lacZ and R39-ΔUAS-H154lacZ reporter plasmids, where the L39-bp and R39-bp digonucleotides contain the SIS1 heat shock element plus eight nucleotides from either the upstream or downstream flanking region of the 31-bp element (Fig. 3A). Similar to the 47-bp element, the R39-bp SIS1 promoter element gave 6-fold more β-galactosidase activity in the sis1–85 strain compared to the isogenic wild type strain (Fig. 3B). However, like the 31-bp SIS1 heat shock element, the L39-bp cis-element gave only 2-fold more β-galactosidase activity in the sis1–85 strain as compared to the isogenic SIS1 strain (Fig. 3B).

To determine if the SIS1 autoregulatory element requires the entire SIS1 heat shock element, we prepared the 23-ΔUAS-H154lacZ construct, which contains only 2 basic heat shock units: one each of nGAAAn and nTTCn (a part of the SIS1 heat shock element) (Fig. 3A). The β-galactosidase levels expressed from the 23-bp SIS1 promoter sequence were very low in both the sis1–85 and SIS1 strains, almost the same as those expressed from the original ΔUAS-H154lacZ construct (Fig. 3B). These results suggest that a 23-bp SIS1 promoter sequence containing part of the SIS1 heat shock element plus eight nucleotides on the downstream flanking region is not sufficient for regulation by SIS1.

Since the R39 SIS1 autoregulatory element contains the SIS1 heat shock element, it is possible that the heat shock element is necessary for the induction of SIS1 transcription from the entire SIS1 promoter in the sis1–85 strain. To test this possibility, we fused the upstream region of SIS1, which was deleted for the heat shock element, into the lacZ reporter vector. The induction of β-galactosidase expression from the ΔHSE-SIS1:lacZ fusion in the sis1–85 strain was compared to the isogenic wild type strain. We found that 15 min after heat shock, the level of SIS1 RNA increased by about 4-fold but returned to almost the starting level 90 min after heat shock (Fig. 5). Therefore, transcription of SIS1 is transiently induced by heat shock.
To determine whether the heat shock induction of SIS1 is mediated through the putative SIS1 heat shock element, we determined the effect of heat shock on the lacZ RNA levels expressed from 47-ΔUAS-HIS4: lacZ, L39-ΔUAS-HIS4: lacZ, R39-ΔUAS-HIS4: lacZ, 31-ΔUAS-HIS4: lacZ, and 23-ΔUAS-HIS4: lacZ fusions in a wild type strain. We examined the lacZ RNA levels, and not the β-galactosidase activities, because heat shock only transiently increases SIS1 transcription. Upon heat shock, the 47-, L39-, R39-, and 31-bp SIS1 promoter sequences, which all contained the SIS1 heat shock element, induced lacZ RNA (Fig. 5). By contrast, the lacZ RNA expressed from the 23-bp sequence containing only part of the SIS1 heat shock element (one copy of nGAAnnTTCn) was not activated at all by heat shock (Fig. 5). These observations suggest that induction of SIS1 expression by heat shock requires the SIS1 heat shock element. However, the R39-bp cis-element gave greater SIS1 RNA induction by heat shock than any of the other elements (Fig. 5). The cause of this effect is not known.

The Transcriptional Regulation of SIS1 Expression by HSP70—Because of the coregulation of bacterial DnaJ and DnaK expression, we examined if the level of SIS1 RNA is altered in hsp70 mutants. In S. cerevisiae, there are four genes (SSA1, SSA2, SSA3, and SSA4) in the SSA subfamily of HSP70 genes. The four SSA proteins share 84–99% identity at the amino acid level. SSA1 is most similar to SSA2, while SSA3 is most similar to SSA4. Moreover, SSA1 and SSA2 are expressed constitutively, while SSA3 and SSA4 are expressed transiently upon heat shock (Craig et al., 1994; Lindquist and Craig, 1988). At 24 °C, the levels of SIS1 RNA were induced about 5-fold in ssa1 ssa2 mutants (Fig. 6A), suggesting that SIS1 expression is responsive to defects in SSA activity. The RNA levels of HSC82 (encoding a HSP90 protein) were also increased about 2-fold (Fig. 6A), consistent with previous reports that many heat shock genes (e.g. SSA3, SSA4, and HSC82) have increased RNA levels in ssa1 ssa2 mutants (Boorstein and Craig, 1990b, 1990c; Lindquist and Craig, 1988).

We further examined whether mutations in other HSP70 genes (e.g. SSB1, SSB2, and KAR2) induce the levels of SIS1 RNA. The levels of SIS1 RNA in ssa1 ssa2 mutants were similar to the levels in the respective isogenic wild type strains (Fig. 6C and data not shown). Also, the SIS1 RNA levels were not significantly different in strains containing mutations in HSP90 genes (e.g. HSPB2 and HSC82) (Fig. 6D), suggesting that the induction of SIS1 RNA is somewhat specific to defects in SSA activity.

The increased level of SIS1 RNA in the ssa1 ssa2 mutant is most likely mediated via transcriptional activation of the SIS1 promoter. The levels of β-galactosidase activity expressed from the SIS1:lacZ translation fusion in the ssa1 ssa2 mutant were 5.5-fold higher as compared to the isogenic wild type strain (Fig. 6B). Because there is no heat shock element in the HIS4 promoter, the β-galactosidase activity expressed from the HIS4:lacZ construct, where the upstream region of HIS4 was fused to the lacZ coding region, was not induced in the ssa1 ssa2 mutant (Fig. 6B).

To define the SSA-responsive SIS1 promoter element, we examined the β-galactosidase activities expressed from the 47-, L39-, R39-, and 31-bp SIS1 promoter sequences in the UAS-HIS4:lacZ reporter plasmid in the ssa1 ssa2 mutant versus the isogenic wild type strain. The 47-, L39-, R39-, and 31-bp SIS1 promoter cis-element all gave more than 100-fold induction in the ssa1 ssa2 mutant as compared to the wild type strain (Table II). However, in either the ssa1 ssa2 mutant or the wild type strain, the β-galactosidase activity expressed from the 23-bp SIS1 promoter element was almost as low as from the original ΔUAS-HIS4:lacZ vector (Table II). Further-
more, deletion of the SIS1 heat shock element abolished the induction of the SIS1:αZ fusion in the ssa1 ssa2 mutant (Table II). These results suggest that the 31-bp SIS1 heat shock element is both necessary and sufficient to induce SIS1 RNA in the ssa1 ssa2 mutant. Therefore, the SSA-responsive SIS1 promoter element is different from the SIS1 autoregulatory element, which requires the SIS1 heat shock element plus additional adjacent sequences.

**DISCUSSION**

Mutations in SIS1 result in increased levels of ssi1 RNA. This finding led us to investigate if SIS1 can regulate its own transcription. Since the sis1–85 and sis1–86 mutations were made by deleting non-overlapping 66-bp regions of SIS1 (which deletes 22 amino acid regions in the carboxyl terminus of SIS1) (Luke et al., 1991), the high levels of sis1–85 and sis1–86 RNAs could be due to three possibilities. One possibility might be that the 66-bp sequences, which were deleted in sis1–85 or sis1–86, act as a cis-element to control expression or stability of the SIS1 RNA. A second possibility was that SIS1–85 and SIS1–86 proteins were gain-of-function proteins that induced the expression of SIS1. The third possibility was that the wild type SIS1 protein may act in trans to negatively regulate its own synthesis, and SIS1–85 and SIS1–86 proteins lose the function for self-regulation. Our results showed that the last possibility is correct. The increased level of SIS1–85 protein can be repressed to a normal level by the wild type SIS1 protein. Also, overexpression of wild type SIS1 protein (SIS1 gene on a 2μ plasmid) decreases the β–galactosidase levels expressed from a SIS1:αZ translational fusion. Furthermore, a specific SIS1 promoter element confers SIS1 regulation to a heterologous promoter.

In the sis1–85 mutant at 24 °C, the levels of SIS1 RNA are highly induced, but the levels of other heat shock genes, including SSA1, SSA4, and HSC82,2 are not highly induced. In contrast, in the ssa1 ssa2 mutant, the levels of SIS1 RNA as well as the RNA of many other heat shock genes (e.g. SSA3, SSA4, and HSC82) are highly induced (Boorstein and Craig, 1990b, 1990c; Lindquist and Craig, 1988). These observations led us to investigate the specificity of the cis-elements in the SIS1 promoter that respond to different conditions (e.g. SIS1 function.

To delineate the SIS1 promoter elements, experiments were performed to show that the SIS1 heat shock element is necessary, but is not sufficient, for SIS1 autoregulation. Downstream adjacent sequences from the SIS1 heat shock element (within AATGGGAT) are also required for the induction of SIS1 by the sis1 mutations. Although AATGGGAT has similarity to the SSA3 post-diauxic shift element (TTAGGGAT) (Boorstein and Craig, 1990a), the SIS1 RNA was not induced after the post-diauxic shift.2 These results suggest that the AATGGGAT sequence in the SIS1 promoter is not functionally equivalent to the SSA3 post-diauxic shift element. Moreover, the post-diauxic shift elements in the CTT1 promoter are not highly induced in a sis1–85 mutant at 24 °C (Fig. 1C). Therefore, we propose that the SIS1 heat shock element plus downstream adjacent sequences within AATGGGAT define a specific SIS1 autoregulatory element.

The SIS1 heat shock element-containing DNA fragments that were cloned into the HIS4 promoter in place of the normal HIS4 upstream activating sequences were at least 100-fold more active in the ssa1 ssa2 mutant as compared to the wild type strain. This amount of induction by a defect in SSA activity is similar to that reported when the SSA3 and SSA4 heat shock elements were used as cis-elements (Boorstein and Craig, 1990b, 1990c). That SIS1 is transcriptionally activated in the ssa1 ssa2 mutant via the SIS1 heat shock element supports the hypothesis that SSA1 and SSA2 are negative regulators of the heat shock response. In the ssa1 ssa2 mutant, transcription from the intact SIS1 promoter was induced only about 5-fold (as measured by β–galactosidase activity from a SIS1:αZ fusion and by RNA levels from the SIS1 gene), while the SIS1 heat shock elements that were cloned into the HIS4 promoter were induced more than 100-fold. These findings suggest that either the promoter context determines the level of induction of the SIS1 heat shock element in ssa1 ssa2 mutants or the intact SIS1 promoter has a specific element that prevents the very high induction of the heat shock element in ssa1 ssa2 mutants. If such a putative element exists in the SIS1 promoter, it would have to lie at least partially outside the 47-bp sequence because in the 47-bp sequence (in place of the HIS4 5′ end) was induced more than 100-fold in the ssa1 ssa2 mutant. Since the SSA3 autoregulatory element requires the SIS1 heat shock element plus additional downstream adjacent sequences, the promoters of both SSA1 and SSA4 contain the conserved heat shock element similar to the SIS1 heat shock element (31 bp) that gave a 2-fold induction of β–galactosidase activity in the sis1–85 mutant (see Fig. 3B). The RNA levels of SSA1 or SSA4 increased slightly in the sis1–85 mutant at 24 °C (see Fig. 1C). The lack of high expression of SSA1 and SSA4 in the sis1–85 mutant may be explained by the absence of specific SIS1 responsive element in their promoters. By contrast, the increased expression of many heat shock genes, including SIS1 and SSA4, in the ssa1 ssa2 mutant could be solely due to the presence of the conserved heat shock element in their promoters. Therefore, unlike bacterial DnaJ and Dnak, which are involved in the autoregulation of the heat shock response by the same mechanism (Georgopoulos et al., 1994), SIS1 and SSA appear to be differently involved in regulating the expression of certain heat shock genes.

Among known genes encoding DnaJ-like proteins in S. cerevisiae, MDJ1 has several weak matches to the nGAAnTTChn sequence (Rowley et al., 1994). YDJ1 and SCJ1 only have a copy of nGAAnTTChn (Blumberg and Silver, 1991; Caplan and Douglas, 1991). However, one copy of nGAAnTTChn is not sufficient to respond to heat shock (Xiao and Lis, 1988). Consistent with this finding, we found that the 31ΔUAS-HIS4:

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2 T. Zhong, M. M. Luke, and K. T. Arndt, unpublished data.
lacZ construct containing the SIS1 heat shock element was transcriptionally activated upon heat shock, but the 23-ΔUAS-H1S4lacZ construct containing only one copy of nGAAAnnTTCn did not respond to heat shock at all.

We also examined SIS1 RNA levels in mutants that are defective in certain cellular processes, including nuclear transport, protein synthesis, and protein degradation. Interestingly, the only other condition where we found increased SIS1 RNA levels (about 2-fold) was in cim3, cim5, and cim3 cim5 mutants.2 Cim3 and Cim5 possibly encode components of a 26 S protease complex (Ghislain et al., 1993). These results raise the possibility that, like bacterial DnaJ, the SIS1 protein may be involved in the process of protein degradation. How this would relate to the role of SIS1 for the initiation of translation (Zhong and Arndt, 1993) is not known.

The induction of SIS1 RNA in the s1-85 mutant is mediated through the R39-bp SIS1 promoter sequence (SIS1 autoregulatory element), whereas the induction of SIS1 RNA in the ssa1 ssa2 mutant is via the 31-bp SIS1 heat shock element (SSA-responsive element). The different cis-element requirements for induction of SIS1 transcription in s1 mutants versus ssa1 ssa2 mutants most likely reflect the different molecular mechanisms for transcriptional control. In the autoregulation of the heat shock response in E. coli, DnaJ/DnaK/GrpE specifically interact with σ32 to control its function (possibly regulating interaction of σ32 with the holoenzyme RNA polymerase core) and synthesis (regulating translation and proteolysis) (Georgopoulos et al., 1994). In budding yeast, SSA protein, possibly in conjunction with SIS1, may be involved in regulating heat shock factor for activation from heat shock elements. For SIS1 autoregulation, SIS1 might regulate either heat shock factor or a SIS1 specific trans-acting factor. How could SIS1 regulate such a transcription factor? We have previously shown that SIS1 is required for translation initiation (or for a very early heat shock elongation step) (Zhong and Arndt, 1993). Perhaps SIS1 regulates the translation of the transcription factor. Alternatively, that SIS1 RNA levels are induced in cim3, cim5, and cim3 cim5 mutants raises the possibility that SIS1 might stabilize the transcription factor. In addition, similar to how E. coli DnaJ functions to convert repA dimers into repA monomers for binding to the P1 phage origin of replication (Wickner et al., 1991), perhaps SIS1 regulates (via a chaperon-like function) the binding of the transcription factor to DNA. Further delineation of these models will require the identification of the transcription factor that mediates the autoregulation of SIS1.

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