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hsp82 Is an Essential Protein That Is Required in Higher Concentrations for Growth of Cells at Higher Temperatures

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hsp82 is one of the most highly conserved and abundantly synthesized heat shock proteins of eucaryotic cells. The yeast *Saccharomyces cerevisiae* contains two closely related genes in the *HSP82* gene family. *HSC82* was expressed constitutively at a very high level and was moderately induced by high temperatures. *HSP82* was expressed constitutively at a much lower level and was more strongly induced by heat. Site-directed disruption mutations were produced in both genes. Cells homozygous for both mutations did not grow at any temperature. Cells carrying other combinations of the *HSP82* and *HSC82* mutations grew well at 25°C, but their ability to grow at higher temperatures varied with gene copy number. Thus, *HSP82* and *HSC82* constitute an essential gene family in yeast cells. Although the two proteins had different patterns of expression, they appeared to have equivalent functions; growth at higher temperatures required higher concentrations of either protein. Biochemical analysis of hsp82 from vertebrate cells suggests that the protein binds to a variety of other cellular proteins, keeping them inactive until they have reached their proper intracellular location or have received the proper activation signal. We speculate that the reason cells require higher concentrations of hsp82 or hsc82 for growth at higher temperatures is to maintain proper levels of complex formation with these other proteins.

When exposed to elevated temperatures or other forms of stress, cells and tissues from a wide variety of organisms synthesize proteins known as the heat shock proteins (HSPs). Coincident with the induction of HSPs, cells and organisms become more tolerant to exposure to extreme temperatures. This observation has led to the general assumption that HSPs provide protection from the toxic effects of heat. The assumption has been reinforced by many lines of evidence. For example, virtually all other stress treatments that induce the HSPs, such as exposure to ethanol, anoxia, and heavy-metal ions, also induce thermotolerance. Moreover, in several cases the kinetics of HSP synthesis and degradation have been shown to parallel the kinetics of thermotolerance induction and decay (reviewed in references 26 and 27).

The results of other experiments, however, conflict with the hypothesis. For example, in some cells, cycloheximide blocks the induction of HSPs without blocking the induction of thermotolerance (25, 48, 49). Genetic investigations have also produced seemingly contradictory results. On the one hand, *Escherichia coli* mutations that block expression of σ^{32} (the transcriptional regulator of the heat shock genes) block both the induction of HSPs and the induction of thermotolerance (20, 32). On the other hand, artificial induction of σ^{32} at normal temperatures leads to induction of the HSPs but does not induce thermotolerance (47).

Mutations in individual HSPs have provided important new insights. Cells of the yeast *Saccharomyces cerevisiae* that carry mutations in the heat-inducible ubiquitin gene are killed more rapidly than wild-type cells by long-term exposure to temperatures just above the maximum growth temperature (19). However, the mutants are killed at the same rate as wild-type cells when exposed to extreme temperatures. Cells carrying mutations in *SSA1* and *SSA2*, two members of the *HSP70* gene family, are temperature sensitive for growth but are less rapidly killed by extreme temperatures than wild-type cells (12). (The constitutive overproduction of other HSPs, including other members of the *HSP70* gene family, in these mutants may explain this result.) Experiments of this type indicate that different proteins may be responsible for different temperature-related phenomena; some proteins may allow cells to grow at the upper end of their normal temperature range, some may help cells survive chronic exposure to supraoptimal temperatures, and some, as yet unidentified, may increase survival after exposure to extreme temperatures.

While attempts to define the roles of the HSPs at high temperature have continued, it has become apparent that many of the proteins, or their close relatives, are synthesized at normal growth temperatures and are induced at specific times in development. Thus, in addition to their putative protective functions during exposure to high temperature and other forms of stress, the proteins may provide essential basal or developmental functions. This has been clearly demonstrated in the case of the hsp70 family of proteins in yeast cells (12, 13). Deletions of certain individual genes and of certain gene combinations in this gene family result in lethal phenotypes.

Here we describe the effects of mutations in the yeast *HSP82* genes. All eucaryotes produce one or more heat-inducible proteins with molecular sizes in the range of 80 to 90 kilodaltons (kDa). Proteins in this family are highly conserved, with those from eucaryotes as distantly related as yeasts, fruit flies, mammals, and trypanosomes sharing at least 50% amino acid sequence identity (3, 17, 18, 30). Moreover, the eucaryotic proteins share approximately 40% amino acid sequence identity with the only known member of this family in *E. coli*, htpG or C62.5 (3). These proteins

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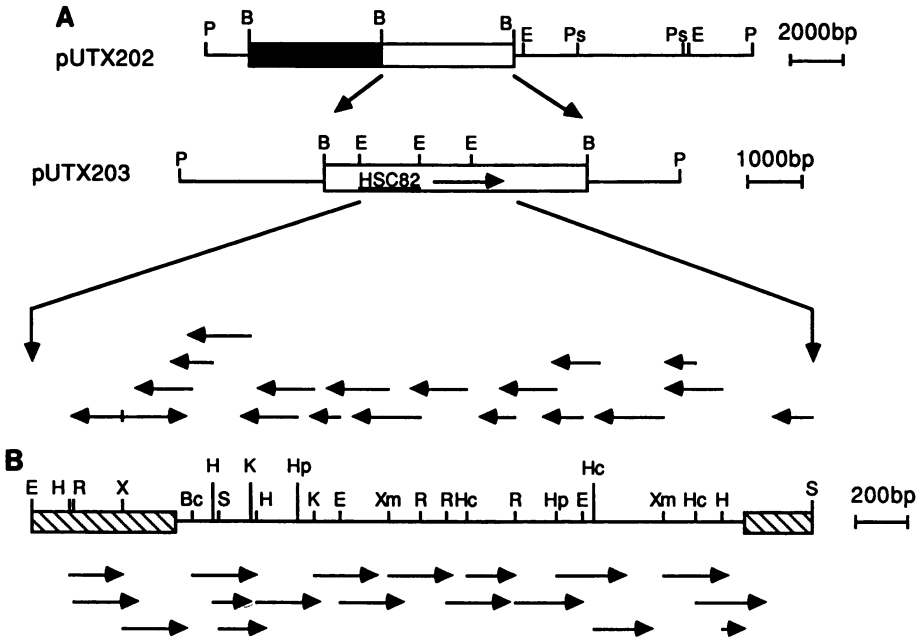

FIG. 1. Subclones and sequencing strategy for the *HSC82* gene from *S. cerevisiae*. (A) Restriction maps of plasmids constructed during subcloning of the *HSC82* gene. Symbols: □, *Bam*HI fragment containing the gene and its flanking regulatory sequences; ■, other flanking yeast sequences; ——, YEp13 (pUTX202)- or pBR322 (pUTX203)-derived sequences. (B) Detailed restriction map of the portion of *HSC82* that was sequenced, 5′ end on the left. Symbols: ——, coding region; ▨, adjoining yeast DNA. Arrows above indicate positions and lengths of sequences determined from the messenger-identical strand; arrows below indicate positions and lengths of sequences determined from the messenger-encoding strand. Abbreviations: B, *Bam*HI; Bc, *Bcl*I; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; Hp, *Hpa*II; K, *Kpn*I; P, *Pvu*II; Ps, *Pst*I; R, *Rsa*I; S, *Sau*3A; X, *Xba*I; Xm, *Xmn*I; bp, base pairs.

have been variously named in the literature, according to their apparent molecular weights on sodium dodecyl sulfate (SDS)-gels. Since nucleic acid sequence analysis of the genes encoding these proteins now reveals that virtually all proteins in the family have predicted molecular sizes of 80 to 84 kDa, we will refer to them here as the hsp82 family. (Two major exceptions exist: the *E. coli* protein has a predicted molecular weight of 62.5 kDa, and vertebrate cells contain additional genes encoding proteins with signal sequences for entry into the endoplasmic reticulum that have predicted molecular weights of 90 to 94 kDa.)

The yeast *S. cerevisiae* produces two proteins in this family with molecular weights of 80,885 (hsc82; this paper) and 81,419 (hsp82; 18). Like the *HSP82* genes of higher eucaryotes, the yeast genes are complexly regulated. They are induced not only in response to high temperatures but also during the approach to stationary phase and during sporulation (24; K. A. Borkovich and S. Lindquist, manuscript in preparation). We have examined the effects of mutations in the yeast *HSC82* and *HSP82* genes on growth at various temperatures, on survival at extreme temperatures, and on sporulation.

MATERIALS AND METHODS

Screening of the genomic library, DNA sequencing, and sequence comparison. The *HSC82* gene was isolated from the Nasmyth-Tatchell YEp13 library (31) by hybridization with the *Hin*dIII-*Eco*RI fragment of the *HSP82* clone pUTX1 as previously described (18) except that the formamide concentration was 30% and the temperature was 42°C. One positive clone, pUTX202, having an insert of approximately 10 kilobases, was chosen for further study (Fig. 1). The *Bam*HI fragment of this clone, containing the *HSC82* gene, was subcloned into pBR322 to give pUTX203 (Fig. 1) and sequenced by the Maxam-Gilbert protocol as previously described (18). Amino acid sequences were aligned by using the program GAP (15), which utilizes the algorithm of Needleman and Wunsch. The percent identity is the number of identical amino acids or bases in the two sequences after alignment divided by the number of units in the first sequence mentioned in the comparison. The GenBank accession number for *HSC82* is M26044.

Plasmid constructions and yeast transformations. Plasmid YEp13 (7) was cut with *Bgl*II, and the fragment containing the yeast *LEU2* gene was purified. This fragment was inserted at the *Bgl*II site of pUTX17 (*HSP82*) (17), creating plasmid pUTX123. Plasmid pUTX212 was constructed by replacing the *Bcl*I site and the *Eco*RI site site of pUTX203 (*HSC82*) with the 5.1-kilobase *Bam*HI-*Eco*RI fragment of YIp5 (46). Vector pKAT7 was made by digesting pUTX203 to completion with *Kpn*I, treating with T4 DNA polymerase, and religating in the presence of *Bgl*II linkers. Plasmid pKAT12 was constructed by ligating the *Bgl*II *LEU2* fragment from pUTX123 into the *Bgl*II site of pKAT7.

One pair of isogenic **a** and α haploid strains of *S. cerevisiae* were the recipients for all DNA transformations: W3031A (**a** *ade2-1 can1-100 his3-12,16 leu2-3,112 trp1-1 ura3-1*) and W3031B (α *ade2-1 can1-100 his3-12,16 leu2-3,112 trp1-1 ura3-1*). Diploids were created by mating isogenic haploids from independent transformations. Cells were transformed with linear DNA (21, 41) and plated for expression of the appropriate auxotrophic marker as follows: for W303leu, the *Bgl*II *LEU2* gene fragment of pUTX123; for PLD82, the *Xba*I fragment of pUTX123 containing the *LEU2* gene; for strain CLD82, the *Bam*HI fragment of pKAT12

TABLE 1. Yeast strains used

Strain	Relevant genotype	Source
HSC82 HSP82 wild type		
W3031A	*leu2 his3 ura3 HSP82 HSC82 MAT***a**	R. Rothstein
W3031B	*leu2 his3 ura3 HSP82 HSC82 MAT*α	R. Rothstein
LP112	*leu2 his3 ura3 HSP82 HSC82* / *leu2 his3 ura3 HSP82 HSC82*	L. Petko
W303leu	*LEU2 his3 ura3 HSP82 HSC82* / *LEU2 his3 ura3 HSP82 HSC82*	This study
W303ura	*leu2 his3 ura3 HSP82 HSC82 CEN URA3* / *leu2 his3 ura3 HSP82 HSC82 CEN URA3*	This study
hsc82 hsp82 mutant		
CLD82	*leu2 his3 ura3 HSP82 hsc82::LEU2* / *leu2 his3 ura3 HSP82 hsc82::LEU2*	This study
CUD82	*leu2 his3 ura3 HSP82 hsc82::URA3* / *leu2 his3 ura3 HSP82 hsc82::URA3*	This study
PLD82	*leu2 his3 ura3 hsp82::LEU2 HSC82* / *leu2 his3 ura3 hsp82::LEU2 HSC82*	This study
SCC82	*leu2 his3 ura3 hsp82::LEU2 HSC82* / *leu2 his3 ura3 hsp82::LEU2 hsc82::URA3*	This study
SCP82	*leu2 his3 ura3 HSP82 hsc82::URA3* / *leu2 his3 ura3 hsp82::LEU2 hsc82::URA3*	This study
PCD82	*leu2 his3 ura3 HSP82 hsc82::URA3* / *leu2 his3 ura3 hsp82::LEU2 HSC82*	This study

containing the *LEU2* gene; and for strain CUD82, the *Hin*dIII fragment of pUTX212 containing the *URA3* gene. The diploid wild-type strain LP112 was created by mating of W3031A and W3031B (36). Wild-type strain W303ura was constructed by transformation of W3031A and W3031B with the YCp50 vector, followed by mating the resultant haploid transformants. Strain PCD82 was constructed by mating CUD82 and PLD82 haploids. Strain SCC82 was made by transformation of strain PLD82 with the *Hin*dIII fragment of plasmid pUTX212, and strain SCP82 was constructed by transformation of CUD82 with the *Xba*I fragment of plasmid pUTX123.

The five diploid strains examined in greatest detail were W303leu (the wild-type strain transformed with the *LEU2* auxotrophic marker), PLD82 (two wild-type *HSC82* genes and two mutant *hsp82* genes with *LEU* disruptions), CLD82 (two wild-type *HSP82* genes and two mutant *hsc82* genes with *LEU* disruptions), SCP82 (a single copy of the wild-type *HSP82* gene, one mutant *hsp82* gene, and two mutant *hsc82* genes), and SCC82 (a single copy of the wild-type *HSC82* gene, one mutant *hsc82* gene, and two mutant *hsp82* genes). The relevant genotypes of all strains are summarized in Table 1.

Analysis of yeast genomic DNAs. Yeast genomic DNAs were purified from stationary-phase YPDA cultures as previously described (14). DNAs were cut with the indicated restriction enzyme, separated by agarose gel electrophoresis (29), and blotted onto Hybond-N (Amersham Corp.) according to the recommendations of the manufacturer. Prehybridization, hybridization, and washing of blots were done as recommended for this membrane except for the omission of dextran sulfate. A ^{32}P-labeled probe was prepared by nick translation (29) of the *Hin*dIII-*Eco*RI fragment of pUTX1 (*HSP82*), which hybridizes equally well with both *HSP82* and *HSC82* genes (F. W. Farrelly and D. B. Finkelstein, unpublished data).

Heat shock and control conditions and immunological detection of hsp82 and hsc82. Yeast cells were grown in minimal dextrose medium (SD) to mid-log phase (5×10^6 cells per ml). Portions of 1 ml were transferred to glass tubes and incubated at 25°C (control) or 39°C (heat shock) for 1 h. Cells were harvested by centrifugation, and denatured extracts were made by lysis with glass beads in absolute ethanol containing 3 mM phenylmethylsulfonyl fluoride. Total cellular proteins from 10^6 cells were separated by electrophoresis on 7% polyacrylamide-SDS gels and transferred electrophoretically to a nitrocellulose or nylon membrane (36). To increase the resolution between hsp82 and hsc82, electrophoresis was continued until proteins in the range of 80 kDa had run two-thirds of the way down the gel. The blot was preincubated in 5% milk in phosphate-buffered saline, followed by incubation in the same solution containing a 1:500 dilution of a polyclonal antibody specific for hsp82 and hsc82 in yeast cells (Borkovich and Lindquist, in preparation). The sheet was washed in phosphate-buffered saline, and immunoreactive proteins were visualized by using ^{125}I-protein A (ICN Pharmaceuticals Inc.) as previously described (6).

In vivo protein labeling. Strains were cultured in SD medium to a density of 5×10^6 cells per ml at 25°C. Aliquots (1 ml) were incubated at either 25 or 39°C for 30 min, after which 20 μCi of [^{3}H]isoleucine (99 Ci/mmol; Amersham) was added and incubation was continued for 30 min. Cells were pelleted and proteins were extracted as described above. Samples containing 10,000 cpm of base-stable, trichloroacetic acid-precipitable counts were separated by SDS-polyacrylamide gel electrophoresis and prepared for fluorography by incubating the gel in 20% 2,5-diphenyloxazole (PPO) in glacial acetic acid before drying.

Vegetative growth at 25 and 37°C. Strains were grown in liquid YPDA (1% yeast extract, 2% Bacto-Peptone, 2% glucose, 10 μg of adenine sulfate) medium with vigorous aeration at 25°C to mid-log phase and diluted to either 3×10^4 cells per ml (25°C cultures) or 10^4 cells per ml (37°C cultures) in YPDA medium. Samples were taken at various times and counted with a hemacytometer. Genotypes were verified by immunological analysis of proteins extracted from the cells at various times as described above.

Sporulation efficiency and spore viability. Yeast strains were cultured in YPAc (1% yeast extract, 2% Bacto-Peptone, 2% potassium acetate) medium to 3×10^7 to 5×10^7

cells per ml at 25°C. The cultures were centrifuged to pellet the cells, washed once in 1% potassium acetate, and then resuspended to the same cell density in 1% potassium acetate. Equal portions were withdrawn at different times after the transfer to potassium acetate and monitored for the presence of dyads, triads, and tetrads, using a hemacytometer. Percent sporulation was taken as the total number of dyads, triads, and tetrads divided by the total number of cells in the field. To assess spore viability, asci from 4-day sporulation cultures were dissected onto YPDA plates. Plates were incubated at 30°C for 2 to 3 days and then scored for the presence of colonies formed by germinated spores. To recover haploid spores containing no functional copies of *HSP82* or *HSC82*, strain PCD83 was dissected on YPDA plates and incubated at either 30°C for 3 days or 17°C for 3 weeks before scoring. Colonies were patched onto tester plates to check genotypes.

Thermotolerance during vegetative growth at 25°C. (i) YPDA medium. Diploid strains W303leu, PLD82, and CLD82 were inoculated at 2×10^4 cells per ml into YPDA medium, and cultures were grown at 25°C overnight. At various times the next day, equal portions were withdrawn and placed on ice. The samples were sonicated briefly to disaggregate the cells, and 3- to 300-μl portions of each sample were transferred to glass Corning tubes (16 by 80 mm) on ice. Two of the tubes (control and no pretreatment) were placed at 25°C; the third tube was incubated at 37°C (pretreatment) for 30 min. At this time, the control tube was put on ice and the other two were incubated at 50°C for 8 min. After the heating, the last tubes were also placed on ice. Chilled cultures were diluted with ice-cold YPDA medium, and 100-μl portions were spread onto YPDA plates. The plates were incubated at 30°C for 2 days, and the colonies were counted.

(ii) YPAc medium. The protocol described for YPDA-grown cells was used except that the original cell densities varied from 2×10^5 to 1×10^8 cells per ml, the time of incubation at 50°C varied from 3 to 30 min, and cultures were diluted with ice-cold YPAc medium. In some experiments, the cells were plated without dilution.

RESULTS

Characterization of the *HSC82* and *HSP82* genes. Cloning and initial characterization of the yeast *HSP82* gene were described previously (18). Hybridization of this gene to restricted, electrophoretically separated genomic DNAs (Southern blot analysis) revealed a second, closely related gene in the haploid yeast genome. Clones for this related gene were obtained by screening a plasmid library with the *HSP82* gene. Expression of the two genes was analyzed during growth at normal temperatures and during heat shock by hybridizing gene-specific probes to total cellular RNAs (data not shown). RNAs were produced constitutively from both genes at 25°C, with those from the *HSP82*-related gene being much more abundant. The gene was therefore named *HSC82* to denote its relationship to *HSP82* and its higher constitutive level of expression. RNAs from the *HSC82* gene increased modestly in concentration after heat shock, whereas RNAs from the *HSP82* gene increased manyfold. Complementary results were obtained in quantifying expression of the two proteins with a specific antiserum (see Fig. 3C).

The entire coding region of *HSC82* and adjacent sequences sufficient for regulated expression were contained in the 5-kilobase insert of pUTX203 (Fig. 1). The 5′ end of the transcript was mapped at −41 relative to the ATG codon by S1 analysis (data not shown). The largest open reading frame extended from +1 to +2118 and predicted an acidic protein with a molecular weight of 80,885 (Fig. 2). This protein was 97% identical to the yeast hsp82 protein at the amino acid level. There was also a region of 71% base identity in the 5′ upstream regions of the two genes corresponding to positions −233 to −196 for the *HSC82* gene (data not shown). This region had no significant homology to the original purported heat shock consensus sequence CTGGAATNT TCTAGA (34), although the segment from −192 to −179 in the *HSC* gene contained a 9-of-15-nucleotide match. It has been suggested, however, that the heat shock consensus sequence is actually composed of multiple GAA segments arranged in alternating orientations and separated by 2-nucleotide spacers with a 5-base-pair core motif (2). A minimum of three 5-base-pair cores, with one core preceded by two of the nucleotides TA, TC, or GA, is required for optimal induction. An extra core, not containing a GAA or TTC block, may separate two functional cores as long as the proper spacing is maintained. This theory is consistent with results obtained by others (35, 45, 50). Inspection of the yeast *HSC82* and *HSP82* genes revealed that the region of upstream homology (−233 to −196) contained such an element: TTCTA GAANG NNNNN GAANA, with N designating nucleotides that differ between the two genes. Since both *HSP82* and *HSC82* were found to have the same basic element, their different patterns of expression suggest the participation of other elements in their regulation.

The yeast hsp82 and hsc82 proteins shared 62, 62, and 63% amino acid identity with the *Drosophila melanogaster* hsp82 (5), human hsp90 (37), and *Trypanosoma cruzi* hsp90 (17) proteins, respectively. All eucaryotic members of the hsp82 family sequenced to date contain a highly-charged central region of repeated acidic and basic residues. This region is absent in the *E. coli* protein htpG, accounting, in part, for its lower molecular weight (3). For the yeast hsc82 protein, it extends from residues 221 to 259 and contains within it four repeats of the pentapeptide sequence $(\text{acidic amino acid})_3(\text{lysine})_2$, beginning at residues 221, 226, 231, and 246 (Fig. 2). The yeast hsp82 protein shows a similar motif (18), with its higher molecular weight being due to the insertion of an extra EEKKD sequence. The yeast and *E. coli* proteins lack cysteine residues, while the *Drosophila*, *Trypanosoma*, and human proteins contain five, six, and six, respectively, concentrated in the carboxy termini. Finally, the four carboxy-terminal amino acids of the yeast hsc82 and hsp82 proteins are EEVD, found at the carboxy termini of virtually all eucaryotic members of the hsp82 and the apparently unrelated hsp70 protein families. (The major exceptions being those members of the two families which enter the endoplasmic reticulum.)

Construction of disruption mutations in *HSP82* and *HSC82*. Mutations were created in the *HSP82* and *HSC82* genes by inserting restriction fragments containing auxotrophic markers in the protein-coding sequence (Fig. 3A). Linear DNAs containing the disrupted genes were transformed into yeast cells, and gene conversion events replacing the wild-type genes with the mutations were obtained by selecting for expression of the auxotrophic marker (41). Both *LEU2* and *URA3* auxotrophic genes were used to mark disrupted alleles. For purposes of comparison, the original strains were transformed with the same auxotrophic markers. This was essential for correct identification of phenotypes, as the auxotrophic markers influenced certain of the traits we examined (see below). Since transformation itself can be

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-412 AGCTTTTAAC CGTACTAGAT AGTTTATAAC CCATTACGCA TTTGATTATA ATTTGCTTCT TAGGCAAAAT TAATATTTAC GTTCTTTTAT ATTCTTTCTT TTTGT
-307 ATTCATAGAA CAGCAGCCAT TACCAATAGA AAGTTAAAAT AGCCGCCGAT GCATTTTATT ACCCGCCTTT CTGTTTTCTG GGCACTTTTC TTTCTAGAAG GTGAA
-212 AGAACAATTT TTCTCGTTTT CTCGAACTTC CACCAAGCGT TGGGTAATGA GGGAGGAGAT TTGTATAAAA AGAGTGGCAT GTGAACTGCC TACCGTAAGT GACAT
 -97 GAACACATGC ATTATATTTT TTGTGATATA TTCTTTCTCT TGTTTTCTTT TTCTTGAAAC GCTACAGAAC CAATAGAAAA TAGAATCATT CTGAAAT   ATG  GCT
   1                                                                                                              Met  Ala

   7 GGT GAA ACT TTT GAA TTT CAA GCT GAA ATC ACT CAG TTG ATG AGT TTG ATC ATC AAC ACT GTC TAT TCT AAC AAG GAA ATT TTC TTG
   3 Gly Glu Thr Phe Glu Phe Gln Ala Glu Ile Thr Gln Leu Met Ser Leu Ile Ile Asn Thr Val Tyr Ser Asn Lys Glu Ile Phe Leu

  94 AGA GAA CTG ATC TCT AAC GCC TCC GAT GCT TTA GAC AAA ATT AGA TAC CAA GCT TTG TCT GAT CCA AAG CAA TTG GAA ACC GAA CCA
  32 Arg Glu Leu Ile Ser Asn Ala Ser Asp Ala Leu Asp Lys Ile Arg Tyr Gln Ala Leu Ser Asp Pro Lys Gln Leu Glu Thr Glu Pro

 181 GAT TTG TTC ATT AGA ATC ACC CCA AAA CCA GAA GAA AAA GTT TTG GAA ATC AGA GAT TCT GGT ATT GGT ATG ACC AAG GCT GAA TTG
  61 Asp Leu Phe Ile Arg Ile Thr Pro Lys Pro Glu Glu Lys Val Leu Glu Ile Arg Asp Ser Gly Ile Gly Met Thr Lys Ala Glu Leu

 268 ATT AAC AAT TTG GGT ACC ATT GCT AAG TCT GGT ACT AAA GCT TTC ATG GAA GCT CTA TCT GCT GGT GCC GAT GTA TCC ATG ATT GGT
  90 Ile Asn Asn Leu Gly Thr Ile Ala Lys Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Ser Ala Gly Ala Asp Val Ser Met Ile Gly

 355 CAA TTC GGT GTT GGT TTT TAC TCT TTA TTC TTA GTC GCC GAC AGA GTT CAA GTT ATT TCC AAG AAC AAT GAG GAC GAA CAA TAT ATT
 119 Gln Phe Gly Val Gly Phe Tyr Ser Leu Phe Leu Val Ala Asp Arg Val Gln Val Ile Ser Lys Asn Asn Glu Asp Glu Gln Tyr Ile

 442 TGG GAA TCT AAT GCC GGT GGT TCT TTC ACC GTT ACT TTG GAC GAA GTT AAC GAA AGA ATT GGT AGA GGT ACC GTC TTG AGA TTA TTC
 148 Trp Glu Ser Asn Ala Gly Gly Ser Phe Thr Val Thr Leu Asp Glu Val Asn Glu Arg Ile Gly Arg Gly Thr Val Leu Arg Leu Phe

 529 TTG AAA GAT GAC CAA TTG GAG TAC TTG GAA GAA AAG AGA ATT AAA GAA GTC ATC AAG AGA CAT TCT GAA TTC GTT GCT TAC CCT ATC
 177 Leu Lys Asp Asp Gln Leu Glu Tyr Leu Glu Glu Lys Arg Ile Lys Glu Val Ile Lys Arg His Ser Glu Phe Val Ala Tyr Pro Ile

 616 CAA CTT CTA GTC ACC AAG GAA GTC GAA AAG GAA GTT CCA ATT CCA GAA GAA GAA AAG AAA GAC GAG GAA AAG AAG GAT GAA GAT GAC
 206 Gln Leu Leu Val Thr Lys Glu Val Glu Lys Glu Val Pro Ile Pro Glu Glu Glu Lys Lys Asp Glu Glu Lys Lys Asp Glu Asp Asp

 703 AAG AAA CCA AAA TTG GAA GAA GTC GAT GAA GAA GAA GAA GAA AAG AAG CCA AAA ACC AAA AAA GTT AAA GAA GAG GTT CAA GAA TTA
 235 Lys Lys Pro Lys Leu Glu Glu Val Asp Glu Glu Glu Glu Glu Glu Lys Lys Pro Lys Thr Lys Lys Val Lys Glu Glu Val Gln Glu Leu

 790 GAA GAG TTG AAC AAG ACT AAG CCA TTA TGG ACT AGA AAC CCA TCT GAT ATC ACT CAA GAG GAA TAC AAT GCT TTC TAT AAG TCT ATT
 264 Glu Glu Leu Asn Lys Thr Lys Pro Leu Trp Thr Arg Asn Pro Ser Asp Ile Thr Gln Glu Glu Tyr Asn Ala Phe Tyr Lys Ser Ile

 877 TCT AAC GAC TGG GAA GAC CCA TTG TAC GTT AAG CAT TTC TCT GTT GAA GGT CAA TTG GAA TTT AGA GCT ATC TTG TTC ATT CCA AAG
 293 Ser Asn Asp Trp Glu Asp Pro Leu Tyr Val Lys His Phe Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Ile Leu Phe Ile Pro Lys

 964 AGA GCA CCA TTC GAC TTA TTT GAG AGT AAG AAG AAG AAG AAC AAT ATC AAG TTG TAC GTT CGT CGT GTC TTC ATC ACT GAT GAA GCT
 322 Arg Ala Pro Phe Asp Leu Phe Glu Ser Lys Lys Lys Lys Asn Asn Ile Lys Leu Tyr Val Arg Arg Val Phe Ile Thr Asp Glu Ala

1051 GAA GAC TTG ATT CCA GAG TGG TTA TCT TTC GTC AAG GGT GTT GTT GAC TCT GAA GAT TTA CCA TTG AAT TTG TCC AGA GAA ATG TTA
 351 Glu Asp Leu Ile Pro Glu Trp Leu Ser Phe Val Lys Gly Val Val Asp Ser Glu Asp Leu Pro Leu Asn Leu Ser Arg Glu Met Leu

1138 CAA CAA AAT AAG ATT ATG AAG GTT ATT AGA AAG AAT ATT GTC AAG AAA TTG ATT GAA GCC TTC AAC GAA ATC GCT GAA GAC TCC GAG
 380 Gln Gln Asn Lys Ile Met Lys Val Ile Arg Lys Asn Ile Val Lys Lys Leu Ile Glu Ala Phe Asn Glu Ile Ala Glu Asp Ser Glu

1225 CAA TTT GAC AAA TTT TAC TCT GCC TTC GCT AAG AAC ATT AAG CTG GGT GTA CAT GAG GAC ACT CAA AAC AGA GCT GCT TTA GCT AAG
 409 Gln Phe Asp Lys Phe Tyr Ser Ala Phe Ala Lys Asn Ile Lys Leu Gly Val His Glu Asp Thr Gln Asn Arg Ala Ala Leu Ala Lys

1312 TTG CTA CGT TAC AAT TCT ACT AAA TCT GTC GAT GAA TTG ACT TCC TTG ACT GAT TAC GTT ACT AGA ATG CCA GAA CAC CAA AAG AAC
 438 Leu Leu Arg Tyr Asn Ser Thr Lys Ser Val Asp Glu Leu Thr Ser Leu Thr Asp Tyr Val Thr Arg Met Pro Glu His Gln Lys Asn

1399 ATC TAT TAC ATC ACC GGT GAA TCT CTA AAG GCA GTC GAA AAG TCT CCA TTC TTG GAC GCC TTG AAG GCT AAG AAC TTT GAA GTT TTG
 467 Ile Tyr Tyr Ile Thr Gly Glu Ser Leu Lys Ala Val Glu Lys Ser Pro Phe Leu Asp Ala Leu Lys Ala Lys Asn Phe Glu Val Leu

1486 TTC TTG ACC GAC CCA ATT GAT GAA TAC GCT TTC ACT CAA TTG AAG GAA TTC GAG GGT AAA ACT TTG GTT GAC ATT ACT AAA GAT TTC
 496 Phe Leu Thr Asp Pro Ile Asp Glu Tyr Ala Phe Thr Gln Leu Lys Glu Phe Glu Gly Lys Thr Leu Val Asp Ile Thr Lys Asp Phe

1573 GAA TTG GAA GAA ACA GAC GAA GAA AAA GCT GAA AGA GAG AAG GAG ATC AAA GAA TAC GAA CCA TTG ACC AAG GCC TTG AAG GAT ATC
 525 Glu Leu Glu Glu Thr Asp Glu Glu Lys Ala Glu Arg Glu Lys Glu Ile Lys Glu Tyr Glu Pro Leu Thr Lys Ala Leu Lys Asp Ile

1660 TTG GGT GAC CAA GTG GAG AAG GTT GTT GTT TCT TAC AAA TTG CTA GAT GCT CCA GCT GCC ATC AGA ACT GGT CAA TTC GGC TGG TCT
 554 Leu Gly Asp Gln Val Glu Lys Val Val Val Ser Tyr Lys Leu Leu Asp Ala Pro Ala Ala Ile Arg Thr Gly Gln Phe Gly Trp Ser

1747 GCT AAC ATG GAA AGA ATC ATG AAG GCT CAA GCC TTG AGA GAC TCT TCC ATG TCC TCC TAC ATG TCT TCC AAG AAG ACT TTC GAA ATT
 583 Ala Asn Met Glu Arg Ile Met Lys Ala Gln Ala Leu Arg Asp Ser Ser Met Ser Ser Tyr Met Ser Ser Lys Lys Thr Phe Glu Ile

1834 TCT CCA AAA TCT CCA ATT ATT ATT GAA ACG AAA AAG AGA GTT GAT GAG GGT GGT GCA CAA GAT AAG ACC GTC AAA GAT TTG ACT AAC
 612 Ser Pro Lys Ser Pro Ile Ile Ile Glu Thr Lys Lys Arg Val Asp Glu Gly Gly Ala Gln Asp Lys Thr Val Lys Asp Leu Thr Asn

1921 TTA TTA TTC GAG ACC GCT TTG TTG ACT TCT GGT TTC AGT TTG GAA GAA CCA ACT TCT TTT GCA TCA AGA ATA AAT AGA TTG ATT TCT
 641 Leu Leu Phe Glu Thr Ala Leu Leu Thr Ser Gly Phe Ser Leu Glu Glu Pro Thr Ser Phe Ala Ser Arg Ile Asn Arg Leu Ile Ser

2008 TTA GGT TTG AAC ATT GAT GAG GAT GAA GAA ACA GAA ACC GCT CCA GAA GCT TCT ACC GAA GCT CCA GTT GAA GAG GTT CCA GCT GAC
 670 Leu Gly Leu Asn Ile Asp Glu Asp Glu Glu Thr Glu Thr Ala Pro Glu Ala Ser Thr Glu Ala Pro Val Glu Glu Val Pro Ala Asp

2095 ACC GAG ATG GAA GAA GTT GAT TGA  TCTCTT  TTTTCGCCTT CATGTTTTAT ATATTATATA AATTTGTTTA CTTATTTTTA CTATTTGTAA TAATGATTCC
 699 Thr Glu Met Glu Glu Val Asp End

2195 TGCTTTACGC GCCTTTAAAAAG
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FIG. 2. Nucleotide and deduced amino acid sequences of *HSC82* from *S. cerevisiae*. The sequence presented is the messenger-identical or plus strand. The nucleotide position (+1) corresponds to the translation origin of *HSC82*. Numbering of both nucleotides and amino acids is shown on the left.

mutagenic, in each case several independent haploid transformants were selected and compared with respect to growth at 25 and 37°C. In all but one case, all haploids from a given transformation reaction displayed the same characteristics. The exceptional strain was discarded. Finally, to minimize the chance that extraneous mutations might influence our results, **a** and α haploid transformants were mated to produce diploids. Any random recessive mutations in one haploid would be expected to be covered by a wild-type allele in the other. The use of diploid cells also allowed us to construct strains with a broader variation in *HSP82* and *HSC82* gene copy number.

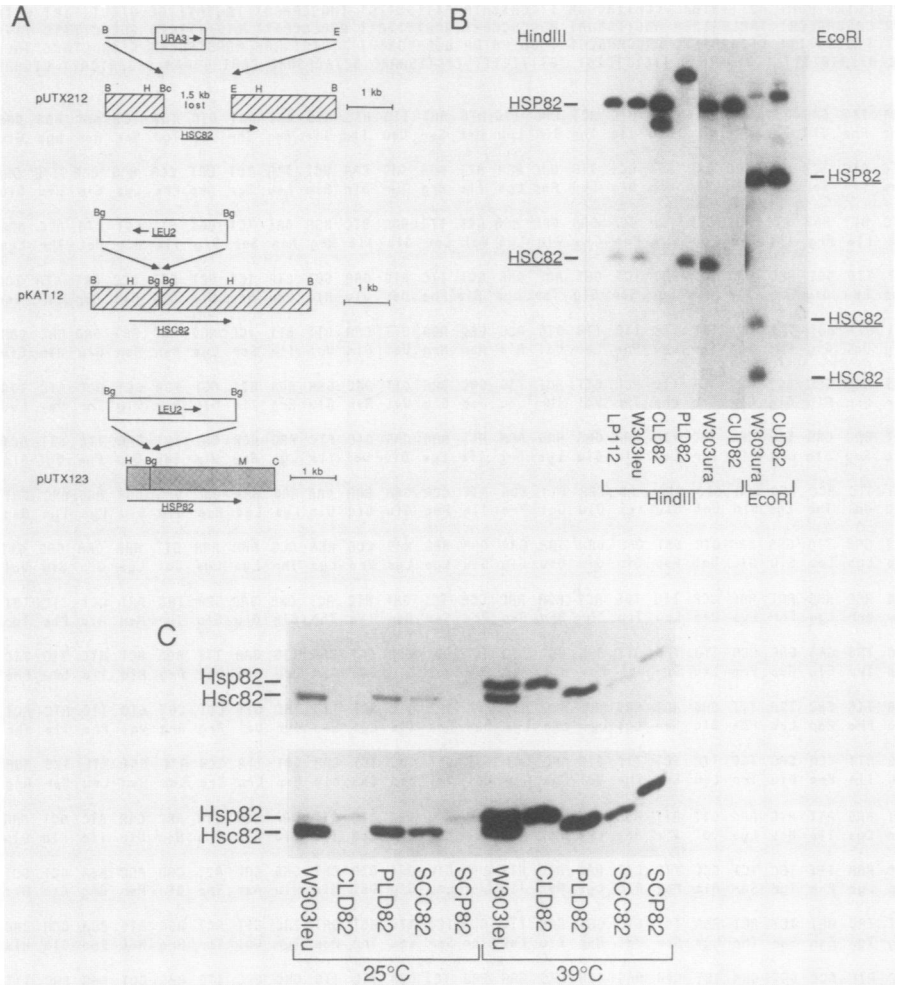

FIG. 3. Construction and characterization of mutations in *HSC82* and *HSP82*. (A) Plasmid constructions. A *URA3* or *LEU2* marker was inserted into *HSC82* or *HSP82* as shown. (Adjoining pBR322 sequences are not shown.) (B) Southern blot hybridization of a portion of *HSP82* to yeast genomic DNA from wild-type or mutant cells. The *HSP82* fragment was labeled by nick translation and hybridized to electrophoretically separated yeast DNAs cleaved with the indicated enzyme. Positions of fragments containing the wild-type *HSC82* and *HSP82* genes are marked. (C) Expression of hsc82 or hsp82 in mutant and wild-type cells after incubation at 25 or 39°C. Total cellular proteins from the indicated strains were electrophoretically separated, transferred to a nylon membrane, and reacted with a polyclonal antiserum specific for hsp82 and hsc82, followed by ^{125}I-protein A. Two different autoradiographic exposures of the same blot are shown.

That the transformations had resulted in disruption of the targeted genes and not their relatives was confirmed by Southern blot analysis of genomic DNAs (Fig. 3B). To confirm that expression of the appropriate protein was eliminated by the mutations and to examine the effects of the mutations on expression of the related gene, total cellular proteins from various diploid strains were electrophoretically separated, transferred to a nylon membrane, and reacted with an antiserum specific for the hsp82 and hsc82 proteins. Since wide variations in expression were observed in these experiments, two different exposures of the 80-kDa region of the blot are displayed in Fig. 3C. Wild-type strains produced both proteins at 25°C, with hsc82 being produced at a much higher level than hsp82 (approximately 10 times greater by densitometry; data not shown). Cells homozygous for the *hsc82* mutations (strain CLD82) produced no hsc82 protein and showed a very slight induction of hsp82 relative to wild-type cells. Cells homozygous for the *hsp82* mutations (strain PLD82) produced no hsp82 and showed no change in the level of hsc82 relative to wild-type cells. Finally, cells carrying a single wild-type *HSP82* or *HSC82* gene (SCP82 and SCC82) produced roughly half the protein of cells carrying two wild-type alleles of the same genes. Therefore, the genes have a very limited capacity for dosage compensation.

To examine the inducibility of the proteins at high temperatures, matched samples of cells were shifted from 25 to 39°C, the temperature that gives maximal induction of hsp82, for 90 min, and total cellular proteins were prepared and processed as described above. Wild-type cells showed a strong induction of hsp82 and a moderate induction of hsc82 (Fig. 3C). (The induction of hsc82 is more readily apparent on the shorter exposure.) In mutant strains, each gene appeared to be induced independently. That is, cells carrying a single copy of the *HSP82* gene produced about half as much hsp82 protein as cells carrying two copies of the gene. Moreover, they produced the same quantities of hsp82 regardless of whether they also produced hsc82. Thus, as was the case at 25°C, the genes have little, if any, capacity for dosage compensation at high temperatures.

Comparison of total cellular proteins from these experiments on stained gels demonstrated that hsc82 was one of

the most abundant proteins in the cell at all temperatures, accounting for one of the most intense Coomassie-stainable bands (data not shown). hsp82 produced a very lightly stained band at 25°C but, consistent with the results of Western blot (immunoblot) analysis, accumulated to approximately the same level as hsc82 after 90 min at 39°C. In separate experiments, hsp82 accumulated to approximately the same level as hsc82 after 4 h at 37°C.

Expression of one member of the *HSP82* gene family is essential for growth. Wild-type haploids were readily transformed with constructs designed to produce disruption mutations in either the *HSP82* or the *HSC82* gene. However, haploids that already carried one of these disruption mutations could not be transformed with constructs designed to disrupt the other gene despite repeated attempts. If these single-disruption haploids were first transformed with an extrachromosomal plasmid containing an *HSP82* or *HSC82* gene, they could then be readily transformed with constructs designed to disrupt the other chromosomal gene.

These results suggested that the proteins produced by the two genes serve identical or nearly identical functions and that these functions are essential. The results of several other experiments confirmed that this is the case. First, diploid cells heterozygous for mutations in the *HSP82* and *HSC82* genes were sporulated at 30°C and dissected. Figure 4A shows the results obtained with strain PCD82, carrying a *URA3*-marked mutation in the *HSC82* gene and a *LEU2*-marked mutation in the *HSP82* gene. No colonies were obtained from Leu^+ Ura^+ spores, whereas other spores produced colonies at the expected frequencies. Microscopic examination of the spores that did not form colonies revealed that they either did not germinate or germinated and divided only two or three times. To determine whether spores lacking the *HSP82* and the *HSC82* genes might grow and germinate at lower temperatures, the experiment was repeated at 17°C. The same results were obtained as at 30°C (Fig. 4B). Additionally, dissection of 58 tetrads produced no evidence for linkage of *HSP82* and *HSC82* (data not shown).

These analyses demonstrate that cells must produce either the hsp82 or hsc82 protein for spore outgrowth. To confirm that the proteins are required for vegetative growth, heterozygous diploids were constructed in which mutations in both the *HSP82* and *HSC82* genes were marked with *LEU2*. The strain was then transformed with a 2μm plasmid containing the wild-type *HSC82* gene and the *URA3* gene, sporulated, and dissected. In tetrads showing 2:2 segregation of the *LEU2* gene, Leu^+ cells were chosen for further analysis. These cells carried mutations in both the chromosomal *HSP82* and the chromosomal *HSC82* genes and carried a wild-type *HSC82* gene on the 2μm plasmid (confirmed on Southern blots). They grew at normal rates at 25°C in YPDA medium. To select for loss of the *URA*-containing plasmid, the cells were plated onto medium containing 5-fluoro-orotate. No colonies were recovered from 10^4 cells plated. Many colonies were obtained on 5-fluoro-orotate if cells carrying the *URA HSC82* plasmid also carried a chromosomal *HSC82* gene, a chromosomal *HSP82* gene, or an extrachromosomal *HSC82* gene on a plasmid marked with *HIS*.

Individual mutations in *HSP82* or *HSC82* affect growth at high temperatures. The effects of the *hsp82* and *hsc82* mutations on vegetative growth were examined in several different media at different temperatures. The five strains shown in Fig. 3C were examined in greatest detail. All grew at the same rate at 25°C in rich dextrose medium, with a doubling time of 1.8 h, and all achieved the same final cell

A

TETRAD	SPORE	YPDA	SD-LEUCINE	SD-URACIL	COMMENTS
1	1	+	−	+	
	2	−	(+)	(+)	D
	3	+	−	−	
	4	+	+	−	
2	1	−	(+)	(+)	D
	2	−	(+)	(+)	D
	3	+	−	−	
	4	+	−	−	
3	1	+	−	+	
	2	+	+	−	
	3	−	(+)	(+)	D
	4	+	−	−	
4	1	−	(+)	(+)	D
	2	+	−	−	
	3	+	−	−	
	4	−	(+)	(+)	D
5	1	+	−	−	
	2	−	(+)	(+)	D
	3	+	−	+	
	4	+	+	−	
6	1	−	(+)	(+)	D
	2	+	−	−	
	3	+	−	−	
	4	−	(+)	(+)	D
7	1	+	+	−	
	2	+	−	+	
	3	+	−	+	
	4	+	+	−	
8	1	+	−	−	
	2	+	−	−	
	3	−	(+)	(+)	D
	4	−	(+)	(+)	D
9	1	−	(+)	(+)	D
	2	+	+	−	
	3	+	−	−	
	4	+	−	+	
10	1	−	(+)	(+)	D
	2	+	+	−	
	3	+	−	+	
	4	+	−	−	
11	1	+	−	+	
	2	+	+	−	
	3	+	−	−	
	4	−	(+)	(+)	D
12	1	+	−	+	
	2	−	(+)	(+)	D
	3	+	+	−	
	4	+	−	−	

B

TETRAD	SPORE	YPDA	SD-LEUCINE	SD-URACIL	COMMENTS
1	1	−	(+)	(+)	D
	2	−	(+)	(+)	D
	3	+	−	−	
	4	+	−	−	
2	1	+	+	−	
	2	+	−	+	
	3	+	+	−	
	4	+	−	+	
3	1	+	−	+	
	2	+	−	−	
	3	+	+	−	
	4	−	(+)	(+)	D
4	1	+	−	−	
	2	+	−	+	
	3	−	(+)	(+)	D
	4	+	+	−	
5	1	+	−	+	
	2	+	+	−	
	3	+	−	+	
	4	+	+	−	
6	1	+	−	−	
	2	−	(+)	(+)	D
	3	−	(+)	(+)	D
	4	+	−	−	

FIG. 4. Analysis of the viability of Hsc^- Hsp^- spores. SD-uracil and SD-leucine indicate synthetic dextrose medium minus uracil and leucine, respectively. Growth scores: +, growth; −, no growth. Scores in parentheses are those expected for dead spores based on the scores of other spores in the tetrad. A D comment marks double-disruption (or *hsc82 hsp82*) mutant spores.

density (Fig. 5A). When the cells were diluted and transferred in the log phase of growth from 25 to 37.5°C, cells homozygous for the *hsp82* or *hsc82* mutation grew more slowly than wild-type cells (Fig. 5B). The slow-growth phenotype was proportional to gene dosage. Cells carrying only a single copy of the *HSP82* or *HSC82* genes virtually stopped growing. When the mutants were transformed with an extrachromosomal centromeric plasmid carrying the *HSC82* gene, growth rates were restored to the expected values (data not shown). Thus, the defect in growth at high temperatures in these mutants was due to reduced synthesis of the hsc82 and hsp82 proteins.

In rich dextrose medium (YPDA), a difference in growth rates was not observed between mutant and wild-type cells

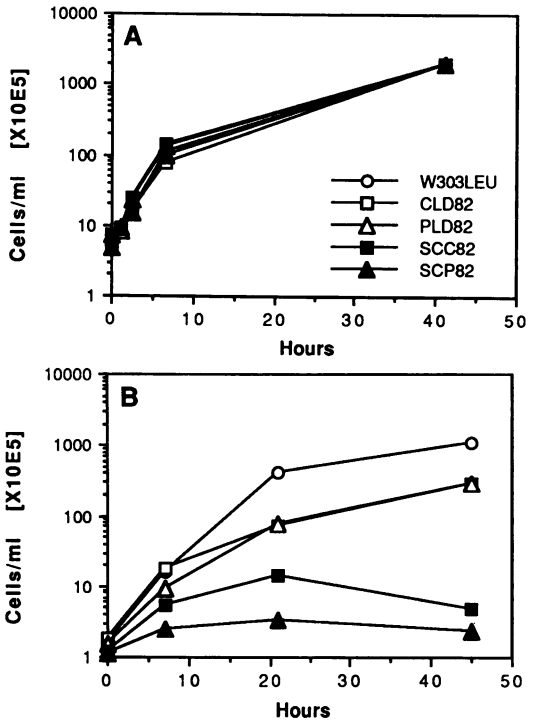

FIG. 5. Demonstration that mutations in *HSC82* or *HSP82* affect growth at 37.5°C but not 25°C. (A) Growth at 25°C. Cells were inoculated in YPDA at 25°C and counted at the indicated times with a hemacytometer. (B) Growth at 37.5°C. Cells were inoculated into YPDA at 37.5°C and counted as for panel A. Key to symbols is the same as in panel A.

at temperatures below 36°C. Between 36 and 38°C, differences between mutant and wild-type cells were more pronounced the higher the temperature. (As is typical of *S. cerevisiae*, the wild-type strain does not grow above 39°C.) Curiously, the negative effects of the mutations on growth at high temperatures were somewhat dependent on cell densities. That is, the effect was more pronounced when log-phase cells were diluted to 10^4 or 10^5/ml rather than 10^6/ml. Cultures of mutant cells also grew more slowly at high temperatures when stationary-phase cells were used for inoculation (data not shown).

Microscopic analysis of the strains examined in Fig. 3 and 5 after 13 h at high temperature revealed a large number of double cells and cell clusters. The cells were much bigger than wild-type cells, and their associations were not disrupted by sonication. Occasionally, the large cells were observed to lyse, leaving behind ghosted cell walls. Surprisingly, a substantial number of these cells retained their colony-forming capacity; after 25 h at 37.5°C, 10% of the cells of strain SCC82 and 1% of the cells of strain SCP82 produced colonies when plated at 25°C on YPDA medium (data not shown).

Sporulation efficiencies and spore viability. As discussed above, hsp82 protein is induced both during stationary phase and during sporulation (Borkovich and Lindquist, in preparation). Sporulation efficiencies were therefore compared in various strains at 18, 25, 30, and 34°C in liquid medium and on plates. (As is commonly observed in yeast strains, sporulation efficiencies were strongly and adversely affected in the parental stain by temperatures above 35°C; sporulation was therefore not examined in the mutants above 34°C.)

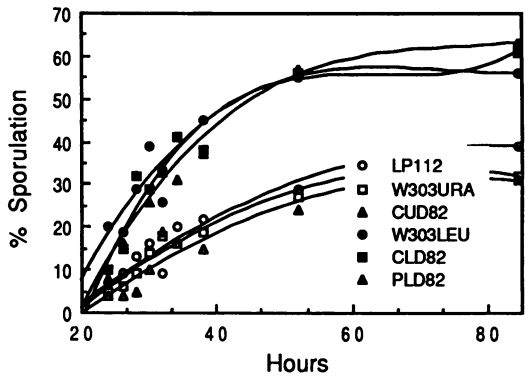

FIG. 6. Demonstration that mutations in *HSC82* or *HSP82* do not influence sporulation efficiency. Cells from the indicated strains were inoculated into sporulation medium at 30°C. Percent asci in the culture (calculated as the sum of two-, three-, and four-spored asci) was determined by microscopic inspection of the cultures at the indicated times.

Figure 6 displays the time course of sporulation for strains W303leu, PLD82, CLD82, LP112, W303ura, and CUD82 at 25°C in liquid medium. The only significant difference in sporulation efficiency among these strains at this temperature was due to a difference in the nutritional markers; *LEU2 ura3* strains sporulated faster and to a higher final efficiency than *leu2 URA3* strains. In separate experiments comparing the five strains depicted in Fig. 3 and 5 (W303leu, CLD82, PLD82, SCC82, and SCP82, all marked with *LEU2*) at 18, 25, 30, and 34°C in liquid medium and on plates, no consistent, substantial differences in sporulation efficiencies were observed (data not shown). Finally, spore viability was examined in dissected asci from cells sporulated at 25°C (Table 2). For the six strains examined, spore viabilities were approximately the same, with *LEU2* spores having a slight advantage over *leu2* spores.

Thermotolerance. The effects of mutations in the *HSP82* and *HSC82* genes on thermotolerance (that is, on the ability of cells to survive brief exposures to extreme temperatures) were examined in dextrose medium, which supports growth by fermentation, and in acetate medium, which supports growth by respiration. The results of a typical thermotolerance assay, obtained by exposing cells growing in rich dextrose medium at 25 to 50°C for 10 min, are presented in Fig. 7A. Survival rates for all strains increased as cells went from the early log phase to the stationary phase of growth (Fig. 7B). Survival rates increased by more than 2 orders of

TABLE 2. Spore viability

Strain	Relevant genotype	% Spore viability
W303leu	*LEU2 ura3 HSP90 HSC82* *LEU2 ura3 HSP90 HSC82*	100
CLD82	*leu2 ura3 HSP82 hsc82::LEU2* *leu2 ura3 HSP82 hsc82::LEU2*	95
PLD82	*leu2 ura3 hsp82::LEU2 HSC82* *leu2 ura3 hsp82::LEU2 HSC82*	100
W303ura	*leu2 ura3 HSP82 HSC82 CEN URA3* *leu2 ura3 HSP82 HSC82 CEN URA3*	83
CUD82	*leu2 ura3 HSP82 hsc82::URA3* *leu2 ura3 HSP82 hsc82::URA3*	80
LP112	*leu2 ura3 HSP82 HSC82* *leu2 ura3 HSP82 HSC82*	90

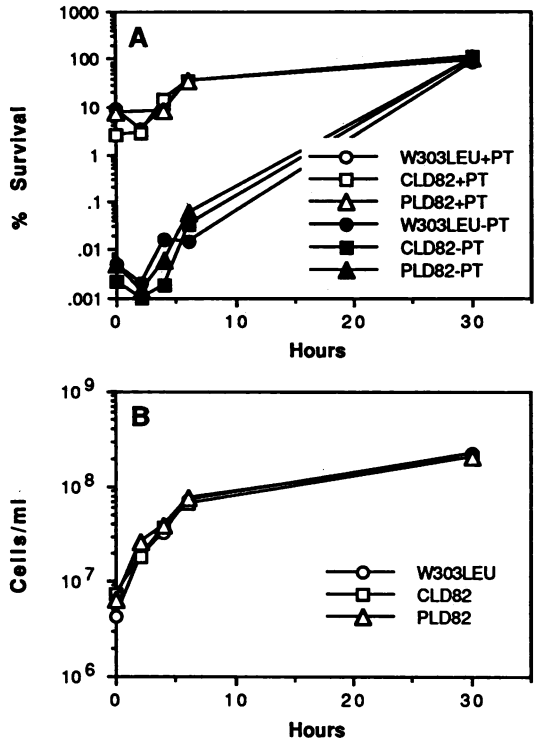

FIG. 7. Demonstration that *hsp82* and *hsc82* mutants are as thermotolerant as wild-type cells in YPDA. (A) Thermotolerance in YPDA. Cells of the designated strain were inoculated in YPDA at low density and cultured at 25°C. Equal portions were withdrawn at various times, incubated at 50°C for 8 min, and plated onto YPDA medium to determine the fraction of surviving cells as described in Materials and Methods. +PT, Cells were pretreated at 37°C for 30 min before the 50°C incubation; −PT, cells did not receive a pretreatment. (B) Growth curve in YPDA. Cell densities of the cultures analyzed in panel A were determined at the indicated times by counting with a hemacytometer.

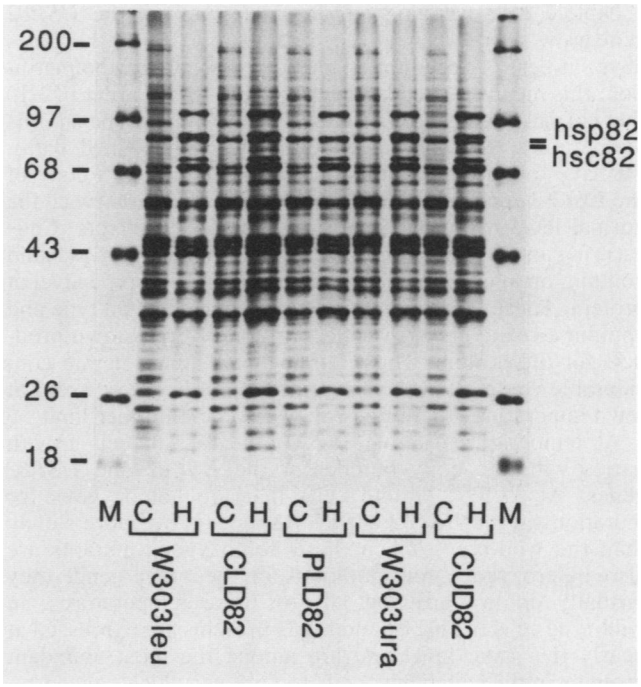

FIG. 8. Demonstration that mutations in *HSC82* or *HSP82* do not affect the expression of other cellular proteins at 25 or 39°C. Cells grown at 25°C were incubated for 30 min at 25 or 39°C. [^{3}H]isoleucine was added, and incubation was continued for 30 min more. Total cellular proteins were separated on an SDS-polyacrylamide gel and visualized by fluorography.

magnitude when cells were pretreated at 37°C for 30 min before the 10-min treatment at 50°C. As observed previously, pretreatments had lesser effects on thermotolerance as cells entered stationary phase, with late-stationary-phase cells showing no effect at all. At equivalent points in the growth curve, mutant and wild-type strains showed similar rates of survival without a pretreatment and similar increases in survival with a pretreatment.

Thermotolerance was also examined in cells grown in acetate, which forces them into respiratory metabolism. As was the case with growth in glucose, there was an increase in thermotolerance in all strains as cells transited from the early log to the stationary phase of growth. In initial experiments, mutant and wild-type cells showed no differences in thermotolerance at any point in the growth curve when shifted directly from 25 to 50°C. However, when the cells were given a conditioning treatment at 37°C before the shift to 50°C, there was a marked difference in the temperature sensitivity of the strains. Mutants were more readily killed than wild-type cells at virtually every point in the growth curve, with the greatest differences between strains (on the order of 10-fold) observed in early-log-phase and late-stationary-phase cells. To investigate this difference in thermotolerance in more detail, a variety of modifications were made to the basic protocol. In initial experiments, cells were diluted into ice-cold medium before plating. When the cells were plated directly, without dilution, differences between the strains disappeared. Apparently, the mutations make the cells more sensitive to manipulation after heat treatment. Depending on the protocol used, this may or may not manifest itself as a difference in thermotolerance.

Finally, we examined the patterns of protein synthesis in wild-type and mutant cells at normal temperatures and during heat shock to determine whether reduced synthesis of hsp82 or hsc82 in the mutant cells had any effect on the expression of other proteins (Fig. 8). No differences could be found in control or heat-shocked cells from the wild type or the mutants after visual inspection of the fluorograph from a one-dimensional gel.

DISCUSSION

We have shown that the haploid yeast genome contains two closely related genes in the *HSP82* gene family, *HSC82* and *HSP82*, with an overall amino acid identity of 97%. The product of the *HSC82* gene, hsc82, is expressed at a very high level at 25°C and is one of the most abundant proteins in the cell. It is induced 1.5- to 2-fold at 39°C. The product of the *HSP82* gene, hsp82, is expressed at a much lower level at 25°C. It exhibits an approximately 20-fold increase in expression at 39°C and accumulates to nearly the same level as the hsc82 protein. Haploid spores that do not contain a functional *HSC82* or *HSP82* gene do not germinate or germinate and divide only a few times. Such cells can be rescued by an extrachromosomal plasmid carrying a wild-type *HSC82* gene. Vegetatively growing cells that lose their plasmid die but can themselves be rescued by transformation with an *HSP82* gene. Thus, although their patterns of expression vary, the two proteins serve identical or nearly identical functions, with expression of one or the other being essential for growth at normal temperatures.

Diploid cells carrying only a single copy of the *HSP82* gene grow as well as wild-type cells at 25°C. Since the genes have, at best, a very limited capacity for dosage compensation, this means that cells which express on the order of 1/10 the normal level of protein exhibit no phenotype at this temperature. In separate experiments (unpublished data), we have found that haploid cells carrying an extra copy of the *HSC82* gene on a *CEN* plasmid produce nearly twice the normal level of protein, with no apparent ill effects. Cells carrying an extra copy of the *HSC82* gene on a 2μm plasmid contain, on average, roughly five times the wild-type level of protein. These cells do not grow as well as the wild type and contain a relatively high concentration of breakdown products for the protein. Thus, although cells can tolerate considerable variation in the concentration of hsp82 or hsc82 at low temperature, there does appear to be an upper limit.

At temperatures above 36.5°C, the rate of cell growth varies with the copy number of the *HSC82* and *HSP82* genes. At 37.5°C, diploid cells that are homozygous for mutations in the *HSP82* or *HSC82* genes grow more slowly than the wild-type. When these homozygous mutants are also heterozygous for mutations in the other gene, they virtually do not grow at all. At these temperatures, in wild-type cells the hsp82 and hsc82 proteins are expressed at nearly the same level and are among the most abundant proteins in the cell. Thus, cells require very high concentrations of these proteins for growth at high temperatures, on the order of 10 to 20 times as much protein as they require for growth at 25°C. In light of these results, the different patterns of expression observed for these two functionally equivalent proteins make a great deal of sense. One gene is expressed constitutively at a very high level (probably at close to the maximal level for any yeast gene). This provides enough protein to act as a buffer against a broad range of temperature fluctuations. Toward the upper extreme of the natural growth range, however, this gene cannot supply a sufficient quantity of protein by itself and another, heat-inducible gene is employed.

Although little is known about the biochemical associations of hsp82 in yeast cells, the proteins of avian and mammalian cells, usually referred to as hsp90, have been reported to associate with several different proteins, with only a small percentage of the total protein pool found in complex with any one protein. For example, hsp90, together with another cellular phosphoprotein of 50 kDa (pp50), has been shown to associate with several newly synthesized transforming kinases in virus-infected cells. These kinases have little activity while in this cytosolic complex. When the complex reaches the plasma membrane, hsp90 and pp50 dissociate, and the kinase becomes phosphorylated on tyrosines and acquires full activity (1, 8, 9, 11, 28, 51). hsp90 also binds to untransformed steroid hormone receptors. Transformation of the receptor to the DNA-binding state, upon the addition of hormone, coincides with dissociation of hsp90 from the receptor (16, 22, 38, 39, 42, 43). hsp90 also shows an affinity for tubulin (44), F-actin and calmodulin (23, 33), protein kinase C (F. O. Fields and J. Thorner, personal communication), and eIF2α kinase (40), although the specific nature of these associations is less clear.

On the surface, these associations would appear to be very different in nature, but there does seem to be an underlying theme. The general function of hsp82 may be to bind to a wide variety of proteins, keeping them quiescent, until they have arrived at their proper intracellular location or have received the proper signal for activation. One might expect that such a protein would be essential for growth at any temperature, and this is what we find. Our results also indicate that cells need higher concentrations of the hsp82 or hsc82 protein for growth at higher temperatures. This result is also in keeping with the model. Protein-protein interactions are profoundly affected by increases in temperature, since the association process involves large changes in the entropy of the proteins and the solvent, water (summarized in reference 10). We suggest that the stability of interactions between hsp82 or hsc82 and other proteins decreases as the temperature increases. Thus, higher protein concentrations would be required to maintain proper levels of complex formation. In this case, it would be most efficient and economical to induce only hsp82 or hsc82, since the protein is shared by a variety of complexes and since cells are able to tolerate a considerable excess of the protein without ill effect.

Comparison of our results with the *HSP82* gene family in *S. cerevisiae* with the results of similar experiments in *E. coli* reveals important similarities and differences. Deletion mutants in the *htpG* gene (which encodes the homologous C62.5 protein in *E. coli*) display only an extremely subtle disadvantage for growth at normal temperatures, but this disadvantage becomes more pronounced at extreme temperatures (4). This phenotype is similar to the phenotypes of individual mutations in the yeast *HSP82* and *HSC82* genes and suggests a similarity in function. However, in *E. coli* there appears to be only one gene in this family, and the deletion is viable. It is doubtful that a closely related gene covers the essential function of *htpG*, since no cross-reacting material was detected with polyclonal antibodies and no cross-hybridizing material was detected with low-stringency Southern blot analysis. It appears, then, that this protein is essential in yeast cells but serves only an auxiliary, growth-enhancing role in *E. coli*. Given the extraordinary degree of conservation in this protein (40% amino acid identity between the yeast and *E. coli* proteins [3]), it may be that the function of the protein is the same in the two organisms but that this function is simply not essential in *E. coli*. If, for example, the function is to keep particular target proteins inactive during intracellular transport, the smaller size of bacterial cells and the lack of internal membranes may make this a valuable but nonessential function. Alternatively, the protein may have acquired novel functions in eucaryotic evolution (perhaps related to the appearance of the highly charged domain that is only found in the eucaryotic proteins), and it may be these novel functions that are essential. Extending the biochemical methods of analysis used with this protein family in vertebrate cells to organisms as amenable to genetic analysis as *E. coli* and *S. cerevisiae* should provide an answer to these questions.

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LITERATURE CITED

1. **Adkins, B., T. Hunter, and B. M. Sefton.** 1982. The transforming proteins of PRCII virus and Rous sarcoma virus form a complex with the same two cellular phosphoproteins. J. Virol. **43:** 448–455.
2. **Amin, J., J. Ananthan, and R. Voellmy.** 1988. Key features of heat shock regulatory elements. Mol. Cell. Biol. **8:**3761–3769.
3. **Bardwell, J. C. A., and E. A. Craig.** 1987. Eukaryotic M_r 83.000

heat shock protein has a homologue in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:5177–5181.
4. **Bardwell, J. C. A., and E. A. Craig.** 1988. Ancient heat shock gene is dispensible. J. Bacteriol. **7**:2977–2983.
5. **Blackman, R. K., and M. Meselson.** 1986. Interspecific nucleotide sequence comparisons used to identify regulatory and structural features of the *Drosophila HSP82* gene. J. Mol. Biol. **188**:499–515.
6. **Borkovich, K. A., and R. L. Weiss.** 1987. Purification and characterization of arginase from *Neurospora crassa*. J. Biol. Chem. **262**:7081–7086.
7. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **8**:121–133.
8. **Brugge, J. S., E. Erikson, and R. L. Erikson.** 1981. The specific interaction of the Rous sarcoma virus transforming protein, $pp60^{src}$, with two cellular proteins. Cell **25**:363–372.
9. **Brugge, J. S., W. Yuonemoto, and D. Darrow.** 1983. Interaction between the Rous sarcoma virus transforming protein and two cellular phosphoproteins: analysis of the turnover and distribution of this complex. Mol. Cell. Biol. **4**:2697–2704.
10. **Clothia, C., and J. Janin.** 1975. Principles of protein-protein recognition. Nature (London) **256**:705–708.
11. **Courtneidge, S. A., and J. M. Bishop.** 1982. Transit of $pp60^{v\text{-}src}$ to the plasma membrane. Proc. Natl. Acad. Sci. USA **79**: 7117–7121.
12. **Craig, E. A., and K. Jacobsen.** 1984. Mutations of the heat-inducible 70 kilodalton genes of yeast confer temperature sensitive growth. Cell **38**:841–849.
13. **Craig, E. A., J. Kramer, and J. Kosic-Smithers.** 1987. *SSC1*, a member of the 70-kDa heat shock protein multigene family of *Saccharomyces cerevisiae*, is essential for growth. Proc. Natl. Acad. Sci. USA **84**:4156–4160.
14. **Davis, R. W., M. Thomas, J. Cameron, T. P. St. John, S. Scherer, and R. A. Padgett.** 1980. Rapid DNA isolations for enzymatic and hybridization analysis. Methods Enzymol. **65**: 404–411.
15. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
16. **Dougherty, J. J., R. K. Puri, and D. O. Toft.** 1984. Polypeptide components of two 8 S forms of chicken oviduct progesterone receptor. J. Biol. Chem. **259**:8004–8009.
17. **Dragon, E. A., S. R. Sias, E. A. Kato, and J. D. Gabe.** 1987. The genome of *Trypanosoma cruzi* contains a constitutively expressed, tandemly arranged multicopy gene homologous to a major heat shock protein. Mol. Cell. Biol. **7**:1271–1275.
18. **Farrelly, F. W., and D. B. Finkelstein.** 1984. Complete sequence of the heat shock-inducible *HSP90* gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **259**:5745–5751.
19. **Finley, D., E. Ozkaynak, and A. Varshavsky.** 1987. The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation and other stresses. Cell **48**:1035–1046.
20. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The htpR gene of *Escherichia coli* is a sigma factor for heat-shock promoters. Cell **28**:282–290.
21. **Ito, H., Y. Fukada, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
22. **Joab, I., C. Radanyi, M. Renoir, T. Buchou, M.-G. Catelli, N. Binart, J. Mester, and E.-E. Baulieu.** 1984. Common non-hormone binding component in non-transformed chick oviduct receptors of four steroid hormones. Nature (London) **308**: 850–853.
23. **Koyasu, S., E. Nishida, T. Kadowaki, F. Matsuzaki, K. Iida, F. Harada, M. Kasuga, H. Sakai, and I. Yahara.** 1986. Two mammalian heat shock proteins, HSP90 and HSP100 are actin-binding proteins. Proc. Natl. Acad. Sci. USA **83**:8054–8058.
24. **Kurtz, S., and S. L. Lindquist.** 1984. The changing pattern of gene expression in sporulating yeast. Proc. Natl. Acad. Sci. USA **81**:7323–7327.
25. **Lee, Y. J., and W. C. Dewey.** 1988. Thermotolerance induced by heat, sodium arsenite or puromycin: its inhibition and differences between 43°C and 45°C. J. Cell. Physiol. **136**:991.
26. **Li, G. C., and A. Laszlo.** 1985. Thermotolerance in mammalian cells: a possible role for the heat shock proteins, p. 349–371. *In* B. G. Atkinson and S. B. Walden (ed.), Changes in gene expression in response to environmental stress. Academic Press, Inc. (London), Ltd., London.
27. **Lindquist, S., and E. A. Craig.** 1988. The heat shock proteins. Annu. Rev. Genet. **22**:631–677.
28. **Lipsich, L. A., J. R. Cutt, and J. S. Brugge.** 1982. Association of the transforming proteins of Rous, Fujinami, and Y73 avian sarcoma viruses with the same two cellular proteins. Mol. Cell. Biol. **2**:875–880.
29. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Mazzarella, R. A., and M. Green.** 1987. ERp99, an abundant, conserved glycoprotein of the endoplasmic reticulum, is homologous to the 90-kDa heat shock protein (hsp90) and the 94-kDa glucose regulated protein (GRP94). J. Biol. Chem. **262**:8875–8883.
31. **Nasmyth, K. A., and K. Tatchell.** 1980. The structure of transposable yeast mating type loci. Cell **19**:753–764.
32. **Neidhardt, F., R. VanBogelen, and E. Lau.** 1983. Molecular cloning and expression of a gene that controls the high temperature regulon in *Escherichia coli*. J. Bacteriol. **153**:597–603.
33. **Nishida, E., S. Koyasu, H. Sakai, and I. Yahara.** 1986. Calmodulin-regulated binding of the 90-kDa heat shock protein to actin filaments. J. Biol. Chem. **261**:16033–16036.
34. **Pelham, H. R. B.** 1982. A regulatory upstream promoter element in the *Drosophila* Hsp70 heat-shock gene. Cell **30**:517–528.
35. **Pelham, H. R. B.** 1985. Activation of heat-shock genes in eukaryotes. Trends Genet. **1**:31–35.
36. **Petko, L., and S. Lindquist.** 1986. Hsp26 is not required for growth at high temperatures, nor for thermotolerance, spore development, or germination. Cell **45**:885–894.
37. **Rebbe, N. F., J. Ware, R. M. Bertina, P. Modrich, and D. W. Stafford.** 1987. Nucleotide sequence of a cDNA for a member of the human 90-kDa heat-shock protein family. Gene **53**:235–245.
38. **Redeuilh, G., B. Moncharmont, C. Secco, and E.-E. Baulieu.** 1987. Subunit composition of the molybdate-stabilized "8-9 S" nontransformed estradiol receptor purified from calf uterus. J. Biol. Chem. **262**:6969–6975.
39. **Renoir, J.-M., T. Buchou, and E.-E. Baulieu.** 1986. Involvement of a non-hormone-binding 90-kilodalton protein in the nontransformed 8S form of the rabbit uterus progesterone receptor. Biochemistry **25**:6405–6413.
40. **Rose, D. W., R. E. H. Wettenhall, W. Kudlicki, G. Kramer, and B. Hardesty.** 1987. The 90-kilodalton peptide of the heme-regulated eIF-2a kinase has sequence similarity with the 90-kilodalton heat shock protein. Biochemistry **26**:6583–6587.
41. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
42. **Sanchez, E. R., P. R. Housley, and W. B. Pratt.** 1986. The molybdate-stabilized glucocorticoid binding complex of L-cells contains a 98-100 Kdalton steroid binding phosphoprotein and a 90 Kdalton nonsteroid-binding phosphoprotein that is part of the murine heat-shock complex. J. Steroid Biochem. **24**:9–18.
43. **Sanchez, E. R., S. Meshinchi, W. Tienrungroj, M. J. Schlesinger, D. O. Toft, and W. B. Pratt.** 1987. Relationship of the 90-kDa murine heat shock protein to the untransformed and transformed states of the L cell glucocorticoid receptor. J. Biol. Chem. **262**:6986–6991.
44. **Sanchez, E. R., T. Redmond, L. C. Scherrer, E. H. Bresnik, M. J. Welsh, and W. B. Pratt.** 1988. Evidence that the 90-kilodalton heat shock protein is associated with tubulin-containing complexes in L cell cytosol and in intact PtK cells. Mol. Endocrinol. **2**:756–760.
45. **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.
46. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High frequency transformation in yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:

1035–1039.
47. **VanBogelen, R. A., M. A. Acton, and F. C. Neidhardt.** 1987. Induction of the heat shock regulon does not produce thermotolerance in *Escherichia coli*. Genes Dev. **1**:525–531.
48. **Watson, K., G. Dunlop, and R. Cavicchioli.** 1984. Mitochondrial and cytoplasmic protein synthesis not required for heat shock acquisition of ethanol- and thermotolerance in yeast. FEBS Lett. **169**:267–273.
49. **Wiedlitz, R. B., B. E. Magun, and E. W. Gerner.** 1986. Effects of cycloheximide on thermotolerance expression, heat shock protein synthesis, and heat shock protein mRNA accumulation in rat fibroblasts. Mol. Cell. Biol. **6**:1088–1094.
50. **Xiao, H., and J. T. Lis.** 1988. Germline transformation used to define key features of heat-shock response elements. Science **239**:1139–1142.
51. **Ziemiecki, A.** 1986. Characterization of the monomeric and complex-associated forms of the *gag-onc* fusion proteins of three isolates of feline sarcoma virus: phosphorylation, kinase activity, acylation, and kinetics of complex formation. Virology **151**:265–273.