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Transcriptional Derepression of the *Saccharomyces cerevisiae* *HSP26* Gene during Heat Shock

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hsp26, the small heat shock protein of *Saccharomyces cerevisiae*, accumulates in response to heat and other types of stress. It also accumulates during the normal course of development, as cells enter stationary phase growth or begin to sporulate (S. Kurtz, J. Rossi, L. Petko, and S. Lindquist, Science 231:1154–1157, 1986). Analysis of deletion and insertion mutations demonstrated that transcriptional control plays a critical role in regulating *HSP26* expression. The *HSP26* promoter was found to be complex and appears to contain repressing elements as well as activating elements. Several upstream deletion mutations resulted in strong constitutive expression of *HSP26*. Furthermore, upstream sequences from the *HSP26* gene repressed the constitutive expression of a heterologous heat shock gene. We propose that basal repression and heat-induced depression of transcription play major roles in regulating the expression of *HSP26*. None of the recombinant constructs that we analyzed separated *cis*-regulatory sequences responsible for heat shock regulation from those responsible for developmental regulation of *HSP26*. Depression of *HSP26* transcription may be the general mechanism of *HSP26* induction in yeast cells. This regulatory scheme is very different from that described for the regulation of most other heat shock genes.

All organisms respond to heat and other physiological stresses by rapidly accumulating the heat shock proteins (hsps). Different organisms employ various combinations of control mechanisms to achieve this response, but in most if not all, transcriptional activation of the heat shock genes is a critical component. The mechanism of transcriptional activation is thought to be universal among eucaryotes (28) and shows a remarkable conservation of *cis*-acting regulatory elements and *trans*-acting regulatory factors.

The *cis*-regulatory component of transcriptional activation is the conserved heat shock element (HSE) (27). HSEs are responsible for the heat shock-inducible transcription of all eucaryotic heat shock genes examined to date (6, 27, 28). These elements are best defined as three or more contiguous repeats of a five-nucleotide element, NGAAN, in alternating orientations (1, 32, 50).

The *trans*-acting component of transcriptional activation is the heat shock transcription factor (HSF). HSF is constitutively present. In *Drosophila* and mammalian cells, it does not bind HSEs or activate transcription under optimal growth conditions (16, 26, 30, 40, 41, 49). With heat shock, its activity is modified. It then binds DNA (as a trimer) and activates transcription (30, 32, 49, 53).

In contrast, the HSF of yeast cells can bind to HSEs constitutively (13, 52) and appears to promote both the basal expression and heat shock-activated expression of the yeast heat shock genes (6, 24, 29, 39). For example, a single HSE from the *SSA1* (*HSP70*) gene, HSE2, is sufficient to provide a heterologous marker gene with high basal expression and heat shock-inducible expression (6, 29). A factor, apparently HStF, from control cell extracts binds to this HSE in vitro (29). For the yeast *HSP82* gene, methylation protection and DNase I footprinting experiments indicate that HSF is constitutively bound to HSE1 and is necessary for constitutive expression (24). Point mutations within HSE1 reduce both HSF binding and constitutive expression of *HSP82*, without affecting the heat-inducible expression. On the basis of such findings, it has been speculated that the yeast HSEs may form functionally distinct subclasses which provide different patterns of basal and inducible expression (6, 24). A dual role for yeast HSF in regulating both basal and heat shock transcription of essential genes (e.g., *SSC1* of the yeast *HSP70* gene family) could explain why HSF is essential in yeast cells (42, 48).

The small hsps present a particularly interesting case in which to examine transcriptional control because they are regulated by both stress and development (21). The small-hsp gene of *S. cerevisiae*, *HSP26*, is expressed during heat shock, during stationary-phase growth, and early after transfer to sporulation medium (20). Our results demonstrate that *HSP26* expression is controlled in large part through the regulation of transcript synthesis. In contrast to other heat shock genes, transcription of *HSP26* appears to be regulated by a mechanism of basal repression during growth at normal temperatures and derepression during heat shock. Nearly all other heat shock genes studied to date are regulated by transcriptional activation. In the large number of deletion mutations analyzed, we were unable to separate the *cis*-regulatory elements responsible for *HSP26* induction following heat shock from those responsible for induction during stationary-phase growth. This result also is unexpected. In the only other well-studied case, that of the *Drosophila* small-hsp genes, *cis*-regulatory sequences involved in stress induction are readily separated from those involved in developmental induction (4, 7, 10, 11, 15, 17, 35).

MATERIALS AND METHODS

Yeast strains and growth conditions. All *Saccharomyces cerevisiae* strains were derived from the nearly isogenic W303-1A and W303-1B haploid strains produced by repeated backcrossing (*ade2-1*, *can1-100*, *his3-11,15*, *leu2-3,12*,

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trp1-1, *ura3-1*; R. Rothstein). The diploid LP112 was derived from mating W303-1A and -1B. The diploid *hsp26* deletion strain (LP150-1) carries an *hsp26*::*HIS3* gene (34) in which the *HIS3* gene replaces the 72-nucleotide *Bgl*II fragment (-129 to -57) upstream of the *HSP26* coding sequence.

Cells were grown at 26°C in either YPDA or YPAc (1% yeast extract, 2% Bacto-Peptone, 10 μg of adenine sulfate per ml, with either 2% dextrose or 2% potassium acetate). Transformants (12) were selected for uracil prototrophy and maintained on minimal SD or SGal medium (0.68% yeast nitrogen base without amino acids, supplemented with 2% adenine sulfate, 2% tryptophan, 3% leucine, 1.2% phthallic acid [pH 5.5], and 2% dextrose or 2% high-purity galactose [containing <0.01% glucose; Sigma]). In minimal media, cultures of strain LP150-1 were supplemented with 2% uracil and cultures of LP112 were supplemented with 2% uracil and 2% histidine. For sporulation, cells were grown to mid-log phase in YPAc and then washed and resuspended in 1% KAc (pH 7.0) (20).

Immunological assay for hsp26. Cultures were grown to mid-log phase (5×10^6 cells per ml) or stationary phase (10^8 cells per ml) in SD. Portions of 1 ml were transferred to 30-ml Corex tubes at 25°C (control) or 39 to 40°C (heat shock) with frequent mixing. Cells were lysed by vigorous agitation with 0.5-mm-diameter glass beads in 0.5 ml of ice-cold absolute ethanol (3) containing 2 mM phenylmethylsulfonyl fluoride, 1 μg of leupeptin per ml, and 1 μg of pepstatin per ml. Proteins were electrophoretically separated on 10% polyacrylamide-sodium dodecyl sulfate (SDS) gels and transferred to nitrocellulose membranes (3). Blots were blocked with 5% powdered milk, reacted with a rabbit anti-yeast hsp26 polyclonal antibody (20, 36), and then reacted with ^{125}I-protein A (ICN; 3). In many different experiments, we have observed a close correspondence between the concentrations of hsp26 message assayed by Northern (RNA) blots and the concentration of hsp26 protein analyzed by Western blots (immunoblots).

β-Galactosidase assays. β-Galactosidase assays (2, 29) were performed on 150-μl portions of a mid-log-phase culture incubated in glass tubes (10 by 75 mm) in a 25 or 39°C water bath for 150 min with frequent mixing. Cells were pelleted and resuspended in 150 μl of buffer Z (2) containing 1 mM phenylmethylsulfonyl fluoride. One drop of 0.1% SDS, 15 μl of chloroform, and 200 μl of 4-mg/ml *o*-nitrophenyl-β-D-galactopyranoside (ONPG) in 0.1 M KH_2PO_4 (pH 7.0) were added (2). Reactions were incubated at 30°C and were terminated by the addition of 500 μl of 1 M Na_2CO_3. Following a brief centrifugation, the A_{420} and A_{556} were measured. β-Galactosidase activities were calculated as previously described (2). Each value reported is the mean of six assays (duplicate assays on each of three independent transformants). For the pβ10 and pβ30 25°C values, the coefficient of variance was ≤0.48. For all other values, the coefficient of variance was between 0.10 and 0.30.

Yeast RNA extraction and analysis. RNAs were extracted from 1-liter cultures (22), denatured (25), and electrophoretically separated on 1.0% agarose gels in phosphate buffer (23). RNAs were transferred to Hybond-N (Amersham) by capillary action according to the manufacturer's recommendations. Membranes were prehybridized in 5× Denhardt solution (23)–4× SSC (0.6 M NaCl and 0.06 M sodium citrate)–100 μg of denatured salmon sperm DNA per ml–0.1% SDS–50% formamide. Hybridization was at 42°C in 1.25× Denhardt solution–4× SSC–100 μg of salmon sperm DNA per ml–0.1% SDS–50% formamide with the nick-translated 1.3-kb *Bgl*II-*Pst*I fragment of the *HSP26* clone, containing the entire *HSP26* open reading frame. Blots were washed for 10 min at 25°C in 2× SSC–0.1% SDS, for 30 min at 65°C in 2× SSC–0.5% SDS with four changes of the wash solution, and finally for 30 min at 25°C in 0.1× SSC–0.1% SDS.

Construction of hsp26 deletion mutations. To generate the pYB set of clones (e.g., pYB10-1 and pYB12-1), a series of 5′ nested deletion mutations of *HSP26* was generated by exonuclease III treatment (8, 45). A *Bam*HI linker was added at each deletion junction, allowing *HSP26* sequences to be transferred to the centromeric vector, YCp50 (14), as *Bam*HI-*Hin*dIII fragments (45).

The pLFC11 to pLFC21 series of constructs was generated by in vitro site-directed mutagenesis (18, 54). The single-stranded mutagenic template DNA was derived from pYE8, a centromeric clone containing the *hsp26* fragment from pYB10-1 (with an *HSP26* 5′ endpoint at −501) and a 500-bp insert carrying the f1 phage origin insert (51) from pUCf1 (Pharmacia). Synthetic mutagenic oligonucleotides, prepared by Paul Gardner (University of Chicago) by using an Applied Biosystems (Foster City, Calif.) 380B DNA synthesizer, had the following sequences: oligonucleotide 10 (oligo 10), CTTCGTTGTTGATGTTGTCAAAG; oligo 11, GCAGCAGCAACTCCGCGGAATAGTAACCGT; oligo 12, TGTGTACCCGTAAACGTTTACATGAACATC; oligo 13, ATTGTTGGATCGGAATTCCCTCCCCCTTAT; oligo 15, AATATAGGGTGCTCGAGTAATATGCGAGTT; oligo 16, CACTTAGCGTGCTCGAGTGGTATTTCATAA; oligo 17, CAACACAAAATTAAGCTTTTTCACCCTTAT; oligo 19, TCCTAAACATATAAATATTCTACTCTCTTATA; oligo 20, AAATAGGACCTCCATGGTATCCAAAAAAGC; and oligo 21, CACGGATAAAGATATCTAAACAAATTAACA. The mutagenic primers were phosphorylated (2) and annealed to the template DNA (1 μg of single-stranded DNA, 20 pmol of the mutagenic oligonucleotide primer, 2 pmol of the upstream, nonmutagentic primer [54], 20 mM Tris [pH 7.5], 10 mM $MgCl_2$, 50 mM NaCl, 1 mM dithiothreitol [DTT]). Samples were heated to 55°C for 5 min and then maintained at 25°C for 5 min; 10-μl samples of the annealing mixes were then used in 20-μl elongation-ligation reactions by adjusting the reactions to 20 mM Tris (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 4 mM each dATP, dCTP, dGTP, and dTTP, 3 U of T4 DNA ligase, and 2 U of Klenow enzyme. Reaction mixtures were incubated for 6 h at 15° C and then supplemented with 2 mM each deoxynucleoside triphosphate and 2 U of Klenow enzyme. Reactions were continued at 37°C for 90 min. Diluted reaction mixtures were used to transform an *ung*$^+$ strain of *Escherichia coli* (BSJ72). *hsp26* deletion mutations were sequenced and transferred to YCp50 as *Bam*HI-*Hin*dIII fragments. Strain names correspond with names of the mutagenic oligonucleotides described above (e.g., oligo 11 generated the deletion mutation of pLFC11).

To generate pLFC1, the *HSP26* sequence of pVZ26△10-1 (an exonuclease III-generated 5′ deletion with the same *hsp26* sequences as pYB10-1; see Fig. 3A) was digested with *Xba*I. Overhanging ends were filled by treatment with the Klenow enyzme in the presence of all four deoxynucleoside triphosphates and then joined by DNA ligase. The entire *hsp26* sequence was then transferred to YCp50 as a *Bam*HI-*Hin*dIII fragment. To generate pLFC8, the *Bgl*II fragment (−129 to −57) of pVZ26 (45) was removed and the remaining *hsp26* sequences were transferred to YCp50 as a *Bam*HI-*Hin*dIII fragment. To generate pLFC22, the *hsp26* insert from pLFC15 was transferred to the polylinker of pVZ1 (9). This intermediate was cut with *Xba*I and *Xho*I. The ends

were filled and joined as described above. The *Bam*HI-*Hin*dIII fragment from pLFC13 was inserted into the corresponding sites of the pRS312 polylinker (38). To generate pLFC26, the *Xho*I-*Hin*dIII fragment from pLFC16 was inserted into the pRS312 polylinker.

The pβ10 to pβ41 series of constructs was produced by modification of the 2μm-based plasmids pZJHSE2-26 and pZJHSE2-26R, kindly provided by E. A. Craig (29, 39). Sequences from *HSP26* were either added upstream of the HSE from *SSA1* (pβ11, pβ21, and pβ31) or used to replace the HSE (pβ10, pβ20, and pβ30). For pβ10, pβ11, pβ20, and pβ21, the *Sma*I-*Xho*I restriction fragments from pLFC15 and pLFC16 (containing *HSP26* sequences from −501 to −294 and −501 to −262, respectively, with +1 designating the translational start site) were ligated into the corresponding sites of pZJHSE2-26 and pZJHSE2-26R. For pβ30 and pβ31, a synthetic *Xho*I linker was inserted in place of the small *Bgl*II fragment upstream of the *HSP26* coding region in pVZ26. The large *Sma*I-*Xho*I fragment (1,422 nucleotides of *HSP26* sequence) of this intermediate was then transferred to pZJHSE2-26 and pZJHSE2-26R. For pβ40 and pβ41, a small *Nru*I-*Sal*I fragment (321 nucleotides) from the coding region of the tetracycline resistance gene of pBR322 was ligated into pZJHSE2-26 and pZJHSE2-26R.

Primer extension analysis. Synthetic oligonucleotide primers (10 μg) were end labeled in reaction mixtures containing 1 mM [γ-^{32}P]ATP (specific activity, >3,000 Ci/mmol), 50 mM Tris hydrochloride (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, 50 μg of bovine serum albumin, and 20 U of T4 polynucleotide kinase at 37°C for 60 min. Unincorporated ATP was removed with a Sephadex G-50 spin column (Boehringer Mannheim). Primer (200 cpm) was hybridized with 2 to 4 μg of total yeast RNA in 0.5 M NaCl–10 mM Tris hydrochloride (pH 7.5)–2 mM EDTA in 10-μl reaction mixtures, which were heated at 80°C for 4 min and then slowly cooled to room temperature. Reaction mixtures were supplemented to 50 mM Tris hydrochloride (pH 8.3), 6 mM $MgCl_2$, 40 mM KCl, 1 mM DTT, 1 mM each dATP, dCTP, dGTP, and dTTP (Pharmacia ultrapure), and 15 U of RNaseIN (Boehringer Mannheim). Then 200 U of cloned Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) was added, and the volume was brought to 45 μl. Reaction mixtures were incubated at 37°C for 90 to 120 min. Products were precipitated in ethanol, washed, dried, and resuspended in deionized formamide containing 0.03% bromophenol blue and 0.03% xylene cyanol. Samples were heat denatured and separated on 6% Gelmix-6 (Bethesda Research Laboratories) sequencing gels.

RESULTS

***HSP26* transcript analysis.** The first step in elucidating the mechanism of *HSP26* regulation in *S. cerevisiae* was to characterize the transcripts produced from the gene under different conditions. Initially, total cellular RNAs were analyzed by Northern (RNA) blot hybridization (Fig. 1). No *HSP26* hybridization was detectable with RNAs extracted from unstressed, mid-log-phase cells. With cells that had been heat shocked at 39°C for 60 min, a strong band of hybridization was observed with an RNA of approximately 1 kb, the expected size of the *HSP26* message. Intense bands of hybridization were also observed with RNAs isolated from yeast cultures that were transferred to sporulation medium or grown to stationary phase. These RNAs migrated on gels to the same position as did the heat-induced transcript. No hybridization was detected with RNAs extracted from an *hsp26* deletion mutant (LP1501-1), whether or not the cells had been heat shocked. These patterns of *HSP26* transcript accumulation are identical to the patterns of hsp26 protein accumulation previously described. The protein is not detectable during mid-log-phase growth at 25°C but accumulates during heat shock, during stationary-phase growth, and after transfer to sporulation medium (20, 34). Thus, expression of hsp26 appears to be controlled primarily by the regulation of transcript accumulation.

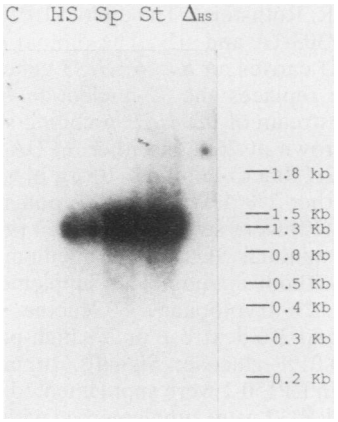


FIG. 1. Northern analysis of heat shock and developmentally induced *HSP26* transcripts. Total RNA was prepared from a diploid wild-type strain (LP112; lanes C, HS, Sp, and St) or an *hsp26* homozygous deletion stain (LP150-1; lane Δ_{HS}). Cells grown in glucose medium at 25°C were harvested in mid-log phase (C), heat stressed for 60 min at 39°C (HS and Δ_{HS}), washed, and transferred to sporulation medium for 6 h (Sp) or grown to stationary phase (St).

Next, we mapped the 5′ ends of the transcripts that accumulated under different induction conditions. Transcripts were analyzed by primer extension with two different and nonoverlapping synthetic oligonucleotide primers. The first primer was 23 nucleotides long and was complementary to nucleotides +30 to +52, relative to the *HSP26* translational start site at +1. The second primer was 28 nucleotides long and was complementary to nucleotides +92 to +119 (45).

The first primer was elongated by reverse transcriptase to 127 nucleotides (Fig. 2, lanes 6 to 8). The second was elongated to 194 nucleotides (lanes 13 to 15). Both results identify a unique 5′ end for *HSP26* transcripts that is 74 nucleotides upstream of the translational start site. Template RNAs from wild-type cells that had been heat shocked, transferred to sporulation medium, or grown to stationary phase directed the synthesis of identical primer extension products.

Two potential problems with primer extension analysis are (i) nonspecific primer hybridization and (ii) premature termination of extension reactions because of secondary structure in the RNA template. To control for the specificity of primer hybridization, extension experiments were performed with no added RNA (Fig. 2, lanes 11 and 18), RNA isolated from heat-shocked cells of the *hsp26* deletion mutant (lanes 9 and 17), or RNA isolated from wild-type cells maintained at 25°C (lanes 5 and 12). No extension products were detected in any of these samples.

To control for premature termination, extension reactions were performed with *HSP26* transcripts initiated from the *GAL1* promoter (45). A *GAL1-HSP26* fusion gene was created by joining the *Bgl*II site (at −54) of the *HSP26* gene to the *Bam*HI site of the *GAL1* promoter in plasmid BM150

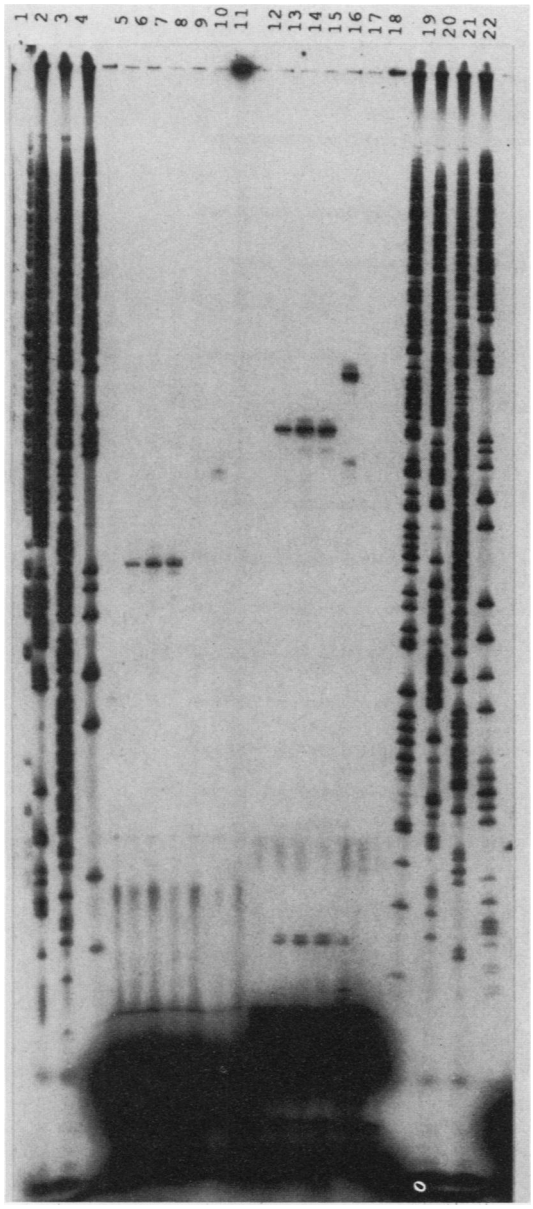


FIG. 2. Mapping the mature 5′ ends of *HSP26* transcripts by primer extension analysis. Total RNA was extracted from a diploid wild-type strain (LP112; lanes 5 to 8 and 12 to 15), a homozygous *hsp26* deletion strain (LP150-1; lanes 9 and 17), or an *hsp26* deletion strain transformed with a centromeric *GAL1-HSP26* chimeric gene (L33; lane 10 and 16). RNAs from wild-type cells were from mid-log-phase cultures maintained in YPDA at 25°C (lanes 5 and 12), heat-shocked at 40°C for 60 min (lanes 6 and 13), transferred to sporulation medium for 6 h (lanes 7 and 14), or grown to stationary phase (lanes 8 and 15). The *hsp26* deletion strain was incubated at 40°C for 60 min (lanes 9 and 17). The *GAL1-HSP26* strain was grown to mid-log phase in SGal minimal medium (lanes 10 and 16). Each RNA sample was analyzed by primer extension, using as primers ^{32}P-end-labeled oligo 10 (lanes 5 to 10) and oligo 24 (lanes 12 to 17). Mock primer extension reactions with no added RNA (lanes 11 and 18) and single-strand sequencing reactions using oligo 10 (lanes 1 to 4) or oligo 24 (lanes 19 to 22) were included as controls.

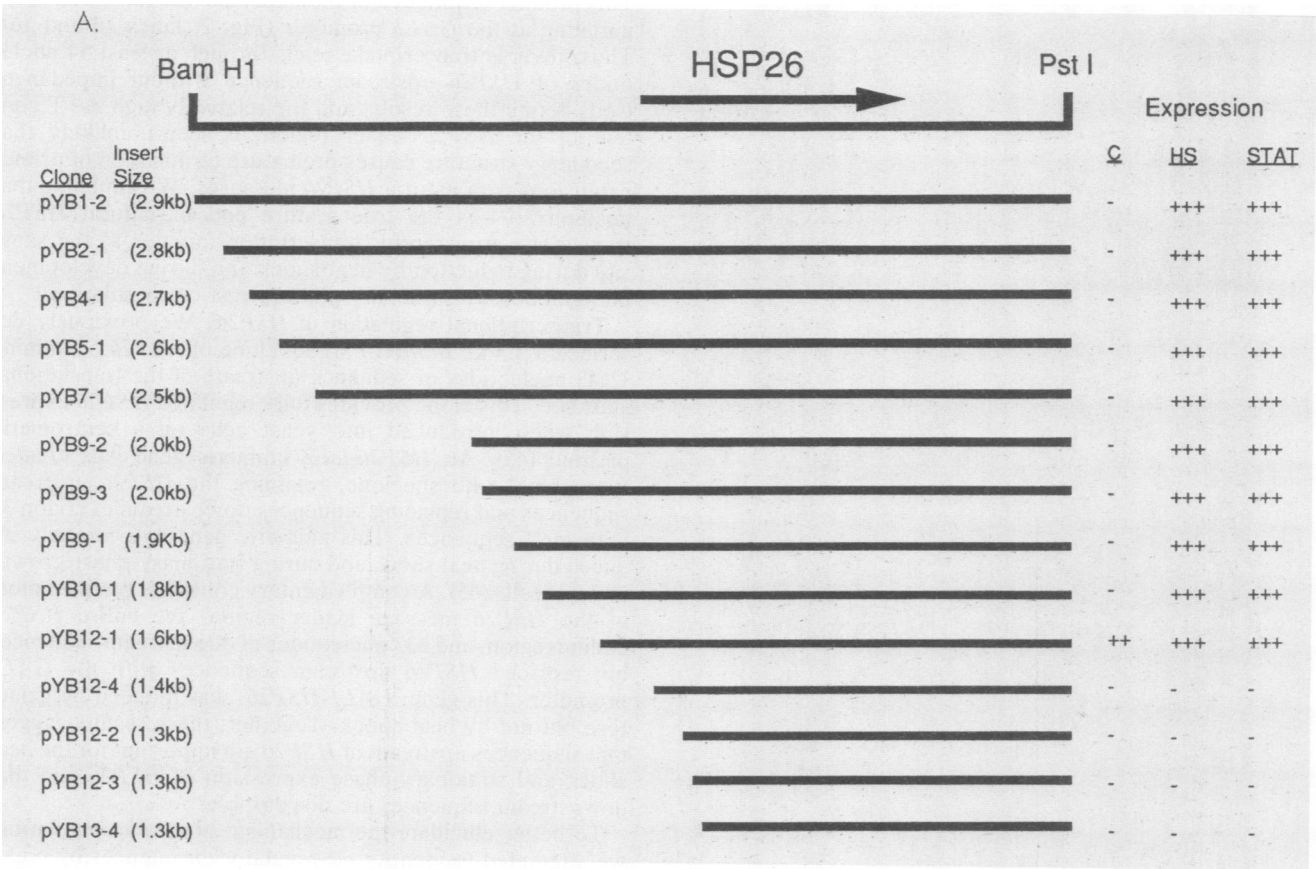
(14). The *hsp26* deletion strain was transformed with this plasmid. RNA isolated from galactose-grown transformants gave a primer extension product 23 nucleotides longer than the extension product from wild-type *HSP26* transcripts. This product terminated at the expected site for a transcript initiating at the *GAL1* promoter (Fig. 2, lanes 10 and 16). Thus, reverse transcriptase reads through at least 54 nucleotides of *HSP26* upstream sequence without impedance. Considering these results and the relatively high A+T content of the *HSP26* leader region, it seems unlikely that secondary structure causes premature termination of primer extensions with natural *HSP26* messages. We conclude that position −74 is the true mature end of natural *HSP26* transcripts. Apparently, transcription initiates at the same site during induction by heat shock and during developmental induction by stationary-phase arrest or sporulation.

Transcriptional regulation of *HSP26*. We previously described a 2.8-kb *Bam*HI-*Pst*I subclone of *HSP26* containing 1,543 nucleotides of sequence upstream of the translational start site. This clone provides fully regulated *HSP26* expression when introduced into yeast cells on a centromeric plasmid (45). An *HSP26-lacZ* chimeric gene was created from this 2.8-kb subclone, retaining the *HSP26* upstream sequences and replacing sequences downstream of codon 36 with *lacZ* sequences. This chimeric gene was strongly induced during heat shock and during stationary-phase growth in yeast cells (45). A complementary construct retained most of the *HSP26* message leader region, the entire *HSP26* coding region, and 633 nucleotides of downstream sequences but replaced *HSP26* upstream sequences with the *GAL1* promoter. This gene, *GAL1-HSP26*, was induced by galactose but not by heat shock. Together, these results suggest that sequences upstream of *HSP26* are important for the heat shock and stationary-phase expression of *HSP26* and that downstream sequences are dispensable.

To better elucidate the mechanism of *HSP26* regulation, we attempted to identify *cis*-regulatory sequences by creating deletion mutations in these upstream sequences. To avoid possible artifacts involved in monitoring chimeric or heterologous protein expression in yeast cells, we chose to monitor hsp26 expression from these mutations directly. This was accomplished by transferring each deletion mutation into the homozygous *hsp26* deletion strain (LP150-1). The validity of this approach depends on two previous observations. First, the strain carrying the *hsp26* deletion mutation (LP150-1) is phenotypically wild type (34). Second, overexpression or underexpression of *HSP26* alters neither the regulation of *HSP26* nor the regulation of any of the other heat shock genes (45). Therefore, the varying levels of hsp26 accumulation observed in these mutant strains should have no phenotypic effect on the cells and no effect on the regulation of *HSP26* or on the regulation of the heat shock response in general.

Transcriptional derepression of *HSP26*. The first mutations that we analyzed were a set of nested deletion mutations, originally created for sequence analysis (45). The deletions were produced by unidirectional nucleolytic digestion from the *Bam*HI site at −1543, the 5′ end of the original 2.8-kb subclone. A *Bam*HI linker was added at each deletion endpoint to allow the transfer of the *HSP26* inserts to a centromeric yeast vector, which should be maintained at approximately one copy per cell. A map of the deletions is shown in Fig. 3A.

Transformants were analyzed for hsp26 accumulation by reacting electrophoretically separated total cellular protein with an anti-hsp26 antibody (Western immunoblot analysis). For these and all other constructs analyzed, multiple independent transformants were isolated and their proteins were analyzed on multiple blots. The results obtained with each construct were highly reproducible. Moreover, the intensity


A.
Bam H1
HSP26
Pst I
Expression
Clone
Insert Size
C
HS
STAT
pYB1-2 (2.9kb) - +++ +++
pYB2-1 (2.8kb) - +++ +++
pYB4-1 (2.7kb) - +++ +++
pYB5-1 (2.6kb) - +++ +++
pYB7-1 (2.5kb) - +++ +++
pYB9-2 (2.0kb) - +++ +++
pYB9-3 (2.0kb) - +++ +++
pYB9-1 (1.9Kb) - +++ +++
pYB10-1 (1.8kb) - +++ +++
pYB12-1 (1.6kb) ++ +++ +++
pYB12-4 (1.4kb) - - -
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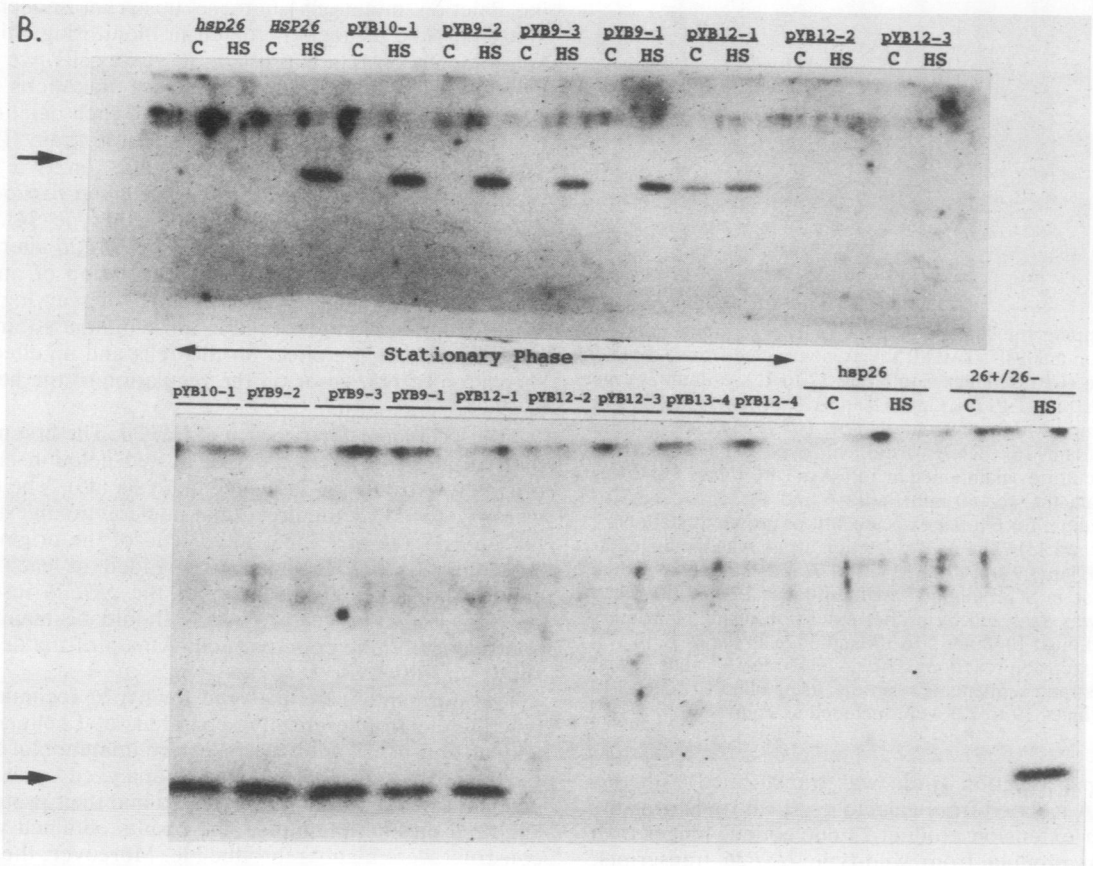

B.
hsp26 HSP26 pYB10-1 pYB9-2 pYB9-3 pYB9-1 pYB12-1 pYB12-2 pYB12-3
C HS C HS C HS C HS C HS C HS C HS C HS C HS
Stationary Phase
pYB10-1 pYB9-2 pYB9-3 pYB9-1 pYB12-1 pYB12-2 pYB12-3 pYB13-4 pYB12-4
hsp26
26+/26-
C HS C HS

FIG. 3. Analysis of *HSP26* nested deletions. (A) Exonuclease III-generated 5′ deletions. The arrow indicates the position and orientation of the *HSP26* open reading frame. Clone designations and relative sizes of the *HSP26* inserts are indicated on the left. Relative levels of hsp26 accumulation observed at 25°C (C), at 39°C (HS), or during stationary-phase growth (STAT) are summarized at the right. (B) Accumulation of hsp26 protein. The 5′ exonucleolytic deletion mutations were introduced into the *hsp26* homozygous deletion (strain LP150-1) as centromeric clones. Accumulation of hsp26 protein was determined by reacting electrophoretically separated proteins transferred to nitrocellulose with an anti-hsp26 antibody (Western blot analysis). Homozygous wild-type diploid (*HSP26*) and heterozygous diploid (26+/26−) strains were included as controls. Cells grown at 25°C were harvested in mid-log phase (C), exposed to 42°C for 60 min (HS), or grown to stationary phase at 25°C.

of the hsp26 reaction with diploid cells homozygous for the *HSP26* gene was reproducibly twice the intensity of the reaction with cells heterozygous for the wild-type and deletion mutations.

All *HSP26* inserts containing 501 nucleotides or more of sequence upstream of the *HSP26* translational start site were fully regulated (Fig. 3B). That is, hsp26 was not detected during log-phase growth at 25°C but accumulated to high levels in mid-log-phase cells heat shocked at 39°C for 60 min and in cells grown to stationary phase at 25°C. The next deletion mutation in this series (YB12-1) contained only 253 nucleotides of upstream sequence. This mutation produced a high level of basal expression. With heat shock, hsp26 expression was two- to threefold further inducible, reaching a level comparable to that observed in heat-shocked wild-type cells. That is, basal expression of hsp26 in log-phase cells carrying YB12-1 was approximately one-third to one-half of the fully heat induced level in wild-type cells. More extensive deletion mutations, containing 125 or fewer nucleotides of upstream sequence (YB12-4, YB12-2, YB12-3, and YB13-4), showed no detectable basal or inducible accumulation of hsp26.

These results demonstrate that 501 nucleotides of upstream sequence is sufficient for fully regulated *HSP26* expression. They also suggest that the 249 nucleotides between −501 and −253 (the YB10-1 and YB12-1 endpoints, respectively; Fig. 4) are involved in reducing the constitutive expression of the *HSP26* gene. An alternative explanation is that the constitutive expression of *HSP26* observed with YB12-1 is due to spurious transcription directed by vector sequences. To distinguish between these two possibilities, we first transferred the *HSP26* insert of YB12-1 from its position in the middle of the tetracycline resistance gene of YCp50 (14) to a new location, within the *lacZ* gene of pRS312 (38), generating RS12 and providing the *hsp26* sequences with a completely different flanking sequence context. Although hsp26 was not produced as abundantly in RS12 as in YB12-1, it showed the same pattern of accumulation. That is, in log-phase RS12 transformants growing at 25°C, *HSP26* was expressed at approximately one-third to one-half the level observed after heat shock (data not shown; R. E. Susek, Ph.D. dissertation, The University of Chicago, Chicago, Ill., 1989).

We also characterized the *HSP26* transcripts produced from YB12-1 by Northern blot hybridization and primer extension analysis. Both the 25 and 39°C transcripts from

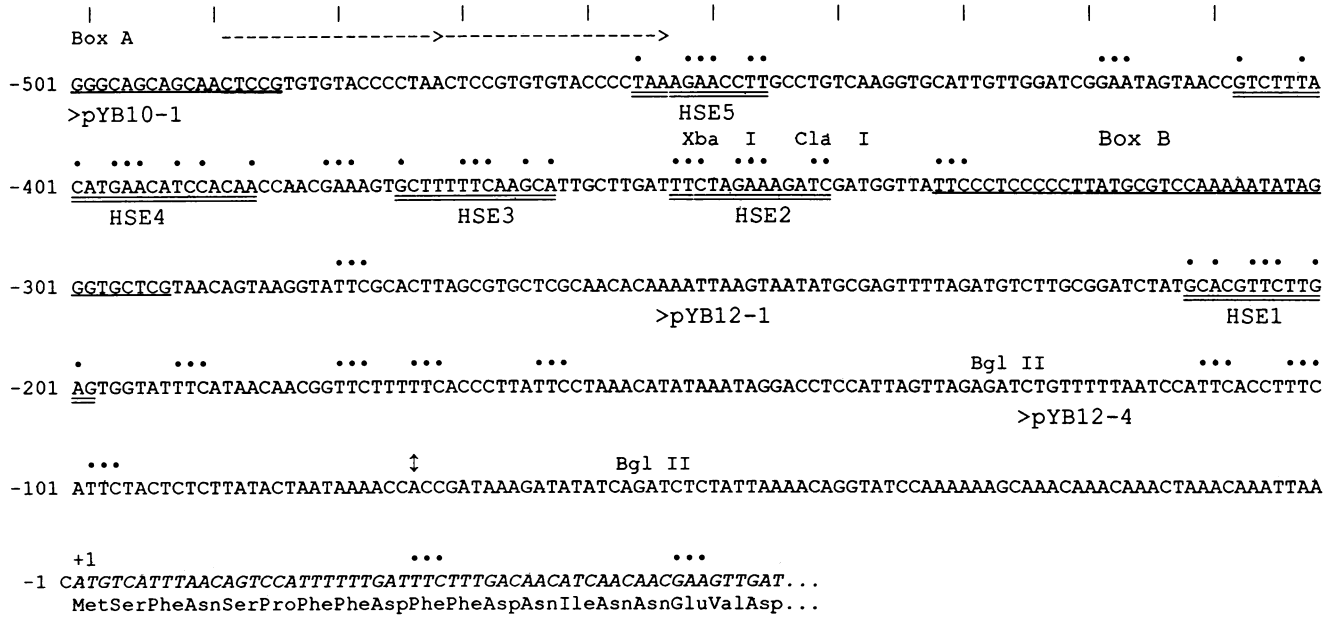
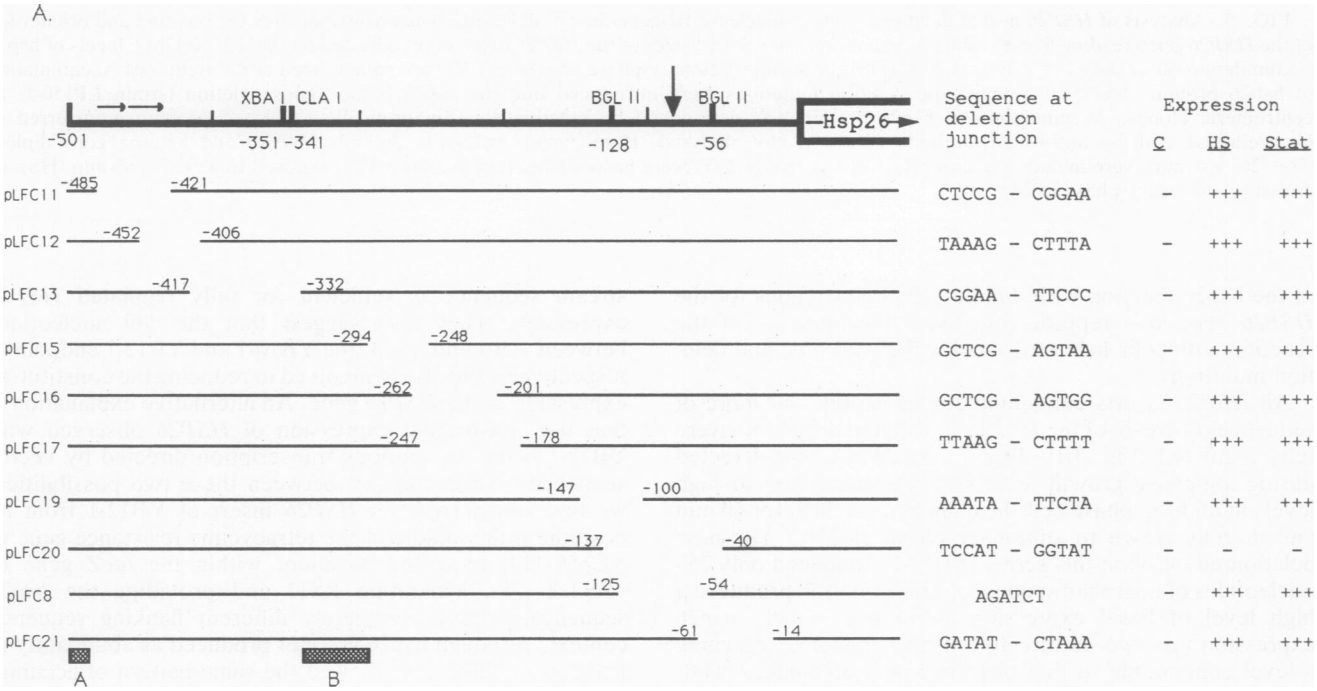


FIG. 4. *HSP26* proximal upstream sequence. Sequence (45) and features of the proximal upstream regulatory sequence are shown. Spacing of 10 nucleotides is indicated at the top (|). Restriction endonuclease recognition sites are indicated. The *HSP26* open reading frame is indicated in italics. The 5′ ends of the *HSP26* transcripts map to −74, relative to the start site of translation. The 5′ boundaries of several exonuclease III-generated deletion mutations are indicated (>; pYB10-1 at −501, pYB12-1 at −253, and pYB12-4 at −125). Matches to the HSE consensus sequence are indicated by double underlines (HSE1 to HSE5). Nucleotides matching the consensus are indicated (●). Nucleotides that are not part of a larger consensus but match an individual HSE subcomponent (GAA or TCC) are also marked. An 18-nucleotide direct repeat (at −489 to −454) is marked by dashed arrows. Single underlines denote sequence boxes A and B described in the legend to Fig. 5.



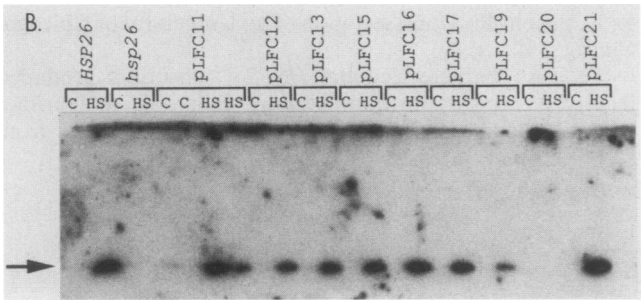


FIG. 5. Analysis of site-directed deletion mutations. (A) Site-directed deletion mutations (pLFC11 to pLFC21). The top line depicts the *HSP26* restriction map. Map positions indicate positions relative to the start of translation at +1. The *HSP26* transcript 5′ end at −74 is marked by a vertical arrow; an 18-nucleotide direct repeat is indicated by adjacent horizontal arrows. Each deletion construct is diagrammed on a separate line, with the deleted sequences denoted by gaps. Exact deletion endpoints are given above the line, and the sequence of the deletion junctions is indicated to the right. All constructs have a common 5′ end at −501, and no new nucleotides have been introduced at the deletion site. Boxes A and B mark upstream sequences that were deleted in pYB12-1 but were not deleted in any of the mutations of this series. The relative levels of hsp26 accumulation shown in panel B are summarized to the right. (B) Accumulation of hsp26 in *HSP26* (LP112) and *hsp26* (LP150-1) diploids and in *hsp26* diploids transformed with centromeric plasmids carrying individual site-directed deletion mutations, analyzed as described for Fig. 4. Cells grown at 25°C were harvested in mid-log phase (C) or exposed to 40°C for 60 min (HS).

YB12-1 migrated to the same position as wild-type heat-induced *HSP26* transcripts during gel electrophoresis (Susek, Ph.D. thesis, 1989). Consistent with the results of protein accumulation, YB12-1 showed a high basal accumulation of *HSP26* transcripts, with a two- to threefold increase in accumulation following heat shock. Primer extension analysis demonstrated that *HSP26* transcripts from the wild-type chromosomal gene and from the YB12-1 mutation had identical mature 5′ ends at −74 (Susek, Ph.D. thesis, 1989). Together, these results indicate that the constitutive expression observed with the YB12-1 construct arises from the deletion or perturbation of sequences regulating the natural *HSP26* promoter rather than from a spurious promoter in the plasmid.

Recombinant *HSP26* genes. These results implicate a 249-nucleotide sequence of *HSP26* (between −501 and −253, relative to the start of translation) in the negative control of *HSP26* transcription. To better elucidate the mechanism(s) of *HSP26* regulation, this putative *cis*-regulatory sequence was the target of additional deletion mutagenesis. Using the technique of oligonucleotide-mediated site-directed mutagenesis (18), we created a series of small overlapping deletion mutations that spanned the region between −501 and −14 (Fig. 5A). Each deletion mutation was marked by a new restriction site generated by joining the deletion endpoints. No heterologous sequences were added.

All but one of the mutations showed a wild-type pattern of expression, with no hsp26 accumulation in log-phase cells at 25°C and strong accumulation after heat shock (Fig. 5B) and during stationary phase (data not shown). The one exception, LFC20, showed no hsp26 protein (Fig. 5B) and no transcript accumulation (data not shown) under any of the conditions tested. This deletion (from −137 to −40) removes the wild-type transcription initiation site at −74 and an extensive part of the leader sequence.

By primer extension analysis, we characterized the *HSP26* transcription initiation sites of several deletion mutations which gave wild-type hsp26 expression patterns. With a single exception, all *hsp26* transcripts had a unique 5′ end at nucleotide position −74, corresponding to that of the wild-type transcript. The exception, LFC8, produced the same total quantity of *hsp26* transcripts (Northern analysis not shown), but the 5′ ends of these transcripts, in roughly equal proportions, mapped to −26, −25, −22, −21, −18, −17, −13, −12, and −8 (Susek, Ph.D. thesis, 1989). The boundaries of the LFC8 deletion (−126 to −54) are nested

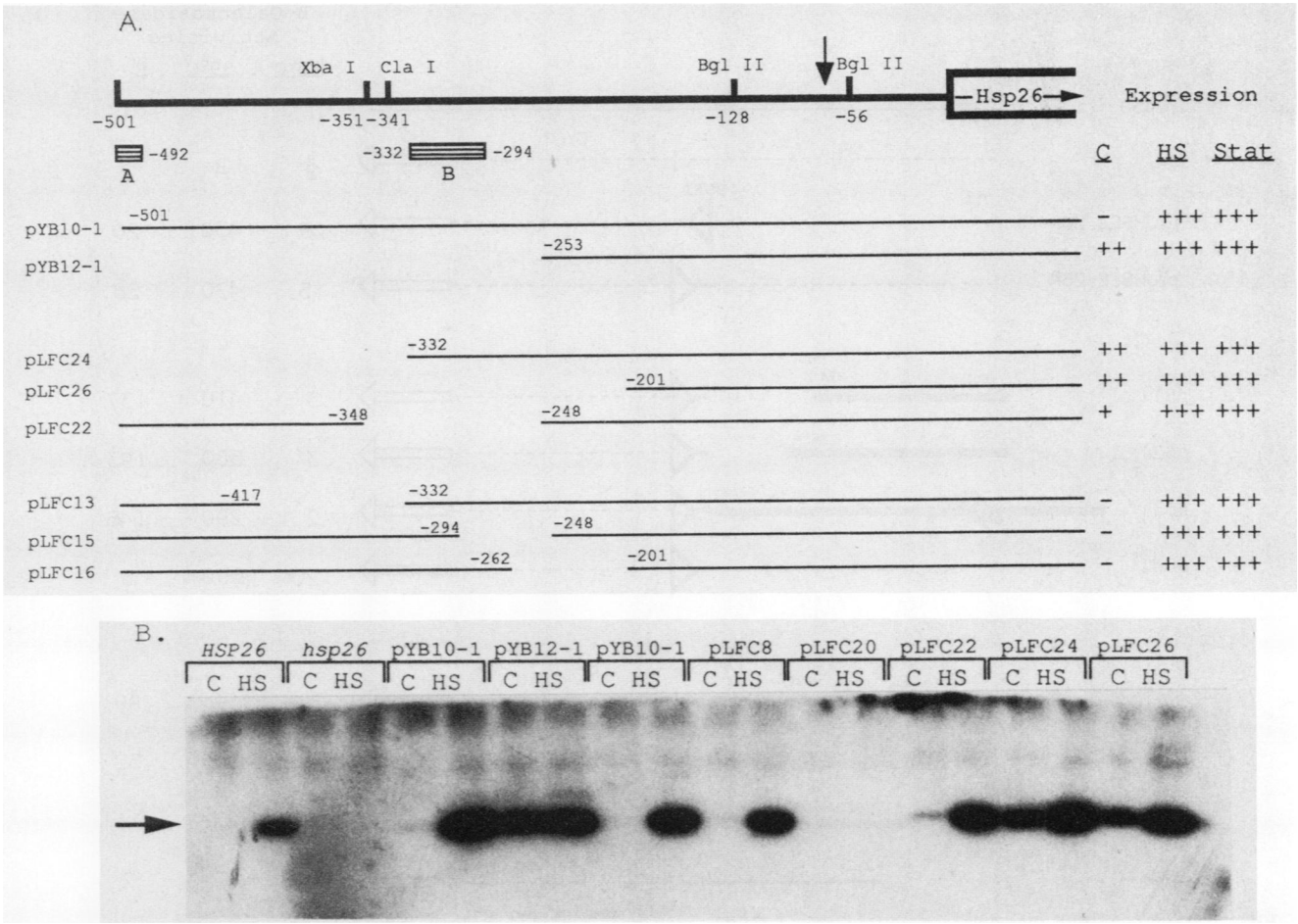


FIG. 6. Constitutive expression of *HSP26*. (A) Structures of constructs. pLFC22, pLFC24, and pLFC26 all show constitutive and heat shock-inducible hsp26 accumulation. The top line diagrams the 501 nucleotides of *HSP26* upstream sequence that are sufficient for a wild-type pattern of regulation. Map positions are relative to the *HSP26* translational start site at +1. The vertical arrow marks the transcript 5′ end at −74. Boxes A and B indicate sequences not deleted in the site-directed deletion series pLFC11 to -21. pLFC13, pLFC15, and pLFC16 are site-directed deletion mutations that were used to create pLFC22, pLFC24, and pLFC26 as described in Materials and Methods. The *HSP26* insert from pYB12-1 was transferred to the centromeric vector pRS312 (38) to generate pRS12. Relative levels of hsp26 accumulation for each mutation are indicated at the right. (B) Accumulation of hsp26 in *HSP26* (LP112), *hsp26* (LP150-1), and *hsp26* cells transformed with centromeric clones containing *hsp26* upstream deletion mutations (pLFC22, pLFC24, and pLFC26), analyzed as described for Fig. 4. Cells grown at 25°C were harvested at mid-log phase (C), heat shocked at 40°C for 60 min (HS), or grown to stationary phase (Stat). Proteins were extracted and analyzed as for Fig. 4. The very faint bands seen in pLFC8 and pYP10-1 were due to loading artifacts and were not observed in other gels. The band pLFC22 was highly reproducible.

inside the boundaries of the LFC20 mutation, and they also remove the wild-type transcription initiation site. Presumably, an initiator element that acts to position transcript start sites (43), but does not affect the efficiency of initiation, was altered or deleted in LFC8. In the more extensive LFC20 deletion (−137 to −40), a critical promoter element was altered, and this prevented expression altogether. The results demonstrate that promoter elements required for transcript initiation and initiation site selection are located between nucleotide positions −137 and −40. These promoter elements are distinct from the sequences involved in basal repression and heat shock inducibility.

None of the small-deletion mutations of this series resulted in strong basal expression, as was observed with YB12-1. One explanation is that the *cis*-regulatory sequences involved in repressing basal expression of hsp26 are functionally redundant. An alternative explanation is provided by the fact that the sequences designated A and B in Fig. 5A were not deleted in this series. These sequences were present in YB10-1, which gave a wild-type pattern of expression, but not in YB12-1, which gave a high level of constitutive expression. If a single sequence element were responsible for the basal repression of *HSP26* transcription, then either box A or box B might contain that element. (Our repeated failure to obtain deletions of these two sequences was presumably due to the instability of the constructs in *E. coli* or to the nature of the oligonucleotides used to generate the mutations.)

Additional recombinant deletion mutations (Fig. 6A) were created to investigate these alternatives. Two of the constructs, LFC24 and LFC26, were created by recombination, using the new restriction sites in LFC13 and LFC16. They contain 332 and 201 nucleotides, respectively, of upstream sequence. Each of these constructs gave a high basal level of hsp26 expression and showed a two- to threefold further increase in expression upon heat shock (Fig. 6B). Their patterns of hsp26 expression were indistinguishable from that of the original constitutively expressed mutation,

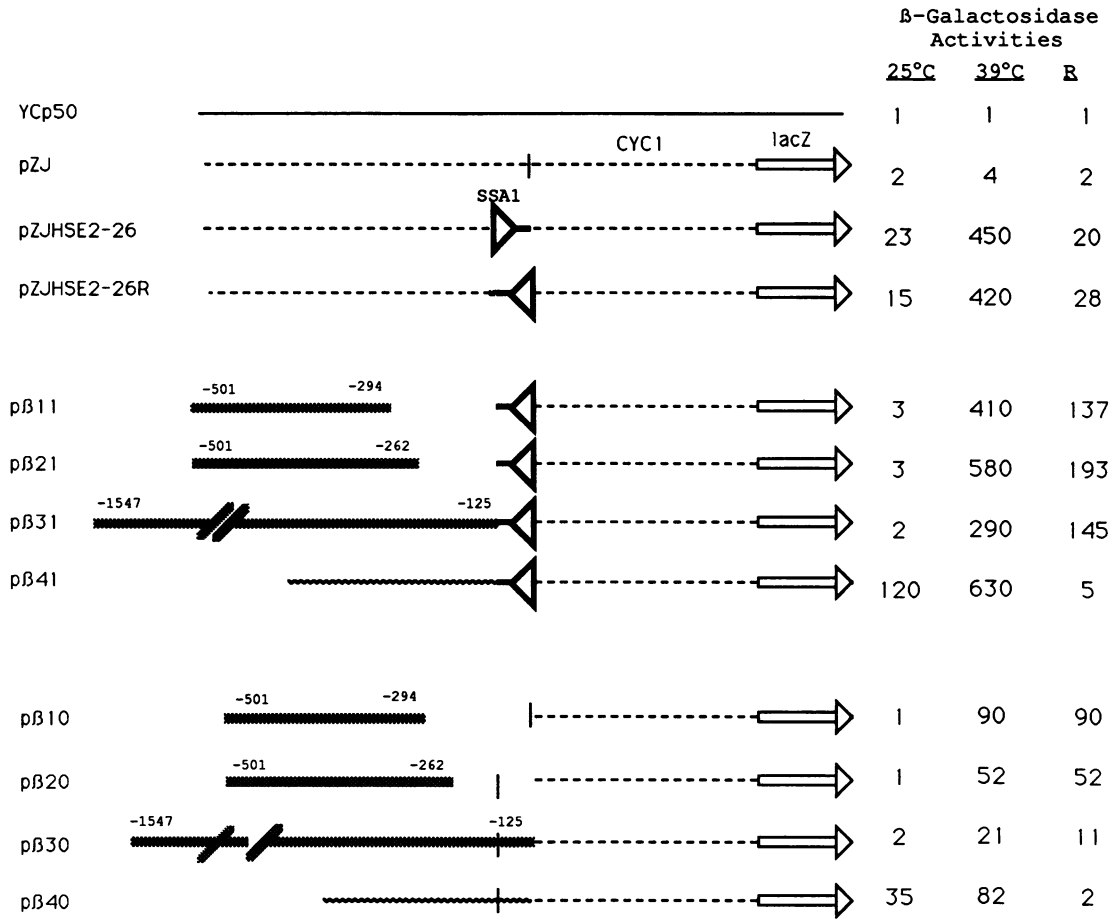


FIG. 7. Demonstration that upstream untranscribed *HSP26* sequences confer heat shock inducibility and constitutive repression on a heterologous gene. *HSP26* upstream sequences were added immediately 5′ of a 26-nucleotide HSE from the yeast *SSA1* gene (HSE2) in plasmid pZJHSE2-26R (pβ11, pβ21, and pβ31). The same *HSP26* inserts were added to pZJ in the absence of the HSE (pβ10, pβ20, and pβ30). pβ40 and pβ41 contain an insert from the coding region of the pBR322 tetracycline resistance gene. YCp50 is a control plasmid that carries the same *URA3* selectable marker but does not contain the *lacZ* gene. β-Galactosidase activity (2) was assayed in mid-log-phase cells maintained at 25°C or in cells incubated at 39°C for 2.5 h. Each value is the mean of six assays (duplicate samples from each of three independent transformants). The ratio of the 39 and 25°C values (R) represents the level of regulated expression. Symbols: ▷, HSE2 from *SSA1*; ▬, *HSP26*; – – –, *CYC1*; ∿∿∿, pBR322.

YB12-1. Since each of these mutations has a different junction with the vector, this result supports our earlier conclusion that it is not the peculiar new juxtaposition of sequences that leads to basal expression of *HSP26* but the deletion of sequences in the *HSP26* upstream region that are normally required for repression. These sequences must lie upstream of nucleotide −332. Therefore, box B cannot be uniquely required for basal repression.

Another mutation, LFC22, was produced by expanding the deletion of LFC15 (−294 to −248) upstream to the *Xba*I restriction site at −351. This mutation showed weak constitutive hsp26 accumulation and was fully inducible with heat shock. Although basal expression was lower than that seen with YB12-1, LFC24, or LFC26, it was clearly and reproducibly higher than that seen with the wild type or with any other construct. Thus, box A cannot be uniquely required for basal repression. Therefore, while sequences between −501 and −332 appear to repress basal expression of *HSP26*, no unique element within that sequence is required.

***HSP26* upstream sequences repress expression of a heterologous gene.** If sequences upstream of *HSP26* repress basal transcription at normal temperatures, then it might be possible to repress the expression of a heterologous gene by introducing *hsp26* sequences upstream of that gene. Obviously, the ideal gene for this experiment should have a high level of basal expression, to provide an assay for repression. However, it should also be expressed at high temperature. This would provide a means of distinguishing regulated repression by *HSP26* upstream sequences from nonspecific inactivation of the heterologous gene.

For this analysis, we chose a *lacZ* fusion gene created by Slater and Craig (39). This gene contains *S. cerevisiae CYC1* upstream sequences and 5′ coding sequences fused to the coding region of the *E. coli lacZ* gene. It also contains a 26-nucleotide fragment from the *S. cerevisiae SSA1* (*HSP70*) gene. This HSE-containing fragment, in either orientation (pZJHSE2-26 and pZJHSE2-26R), enhances basal expression of *lacZ* 10-fold compared with the level observed with the parental construct (pZJ). With heat shock, β-galactosidase activity is further induced 20- to 30-fold (Fig. 7; 29, 39).

The *HSP26* sequences implicated in repression at 25°C were inserted upstream of the HSE in pZJHSE2-26R (Fig. 7; pβ11, pβ21, and pβ31). To control for the effects of unusual sequence juxtapositions or spacing alterations, three dif-

ferent fragments (containing 207, 239, or 1,422 nucleotides of *HSP26* upstream sequences in pβ11, pβ21, and pβ31, respectively) were used. All three fragments have different junctions with the reporter gene, and one has a different junction with the vector sequences. As an additional control, a 321-nucleotide fragment from within the coding region of the pBR322 tetracycline resistance gene was inserted instead of *HSP26* sequences.

Each *HSP26* insert resulted in substantially less basal β-galactosidase activity than in the parental construct (pZJHSE2-26R). With heat shock, however, β-galactosidase activity was as high as it was without the *HSP26* sequences. These results lend support to the hypothesis that *HSP26* upstream sequences function as regulated upstream repressor sequences (URS), repressing expression at normal temperatures.

To examine the effects of *HSP26* upstream sequences in the absence of the HSE from *SSA1*, each *HSP26* insert was used to replace the HSE from pZJHSE2-26. In two of the constructs, basal expression of β-galactosidase was reduced approximately twofold. Since the basal expression of β-galactosidase was already very low in pZJ, the effect was small, and perhaps of doubtful significance, but it was reproducible. More importantly, with each construct (pβ10, pβ20, and pβ30) the expression of β-galactosidase was greatly enhanced after heat shock. Full heat shock induction with *HSP26* sequences alone was significantly less than that observed with the HSE from *SSA1*. However, since the *HSP26* constructs also had substantially lower basal expression, they showed a much greater degree of regulation. The ratio of induced to basal expression was 90 for pβ10, compared with 20 for pZJHSE2-26. Apparently, the sequences located between nucleotides −501 to −294 can repress basal expression of a heterologous gene, but they must also contain elements that can promote transcription during heat shock.

DISCUSSION

hsp26 is the only known member of the small-molecular-weight hsp family in *S. cerevisiae*. The protein is not present in unstressed, vegetative cells but is strongly induced by heat shock, stationary-phase arrest, or nitrogen starvation (sporulation conditions) (19, 20). As reported here, expression of the message is coincident with expression of the protein and is regulated primarily at the level of transcription. Several lines of evidence suggest that the same promoter is employed under all induction conditions. First, the transcripts produced during heat shock, during stationary-phase arrest, and during sporulation all have a unique 5′ end at −74. (Such unique transcription start sites are unusual in *S. cerevisiae*, in which the transcripts of most genes have multiple 5′ ends.) Second, the deletion of 74 nucleotides surrounding the initiation site (the LFC8 mutation) results in the use of many new initiation sites but changes neither the efficiency of transcription nor its pattern of regulation. Third, the deletion of a few additional nucleotides on either side of the LFC8 mutation (the LFC20 mutation) results in the absence of hsp26 expression under all conditions. Finally, none of the deletion mutations in the extensive series analyzed here separated the capacity to produce this protein during heat shock from the capacity to produce the protein during development.

Surprisingly, three different upstream deletion mutations (pYB12-1, pLFC24, and pLFC26) result in very high levels of constitutive hsp26 expression, nearly half the levels achieved with heat shock in wild-type cells. This pattern of constitutive expression is maintained when one of these mutations is transferred to a completely different vector context. Moreover, transcripts produced by these altered genes are initiated at the normal *HSP26* initiation site. When the sequences that are deleted in these mutations are transferred upstream of a heterologous gene containing a heat shock promoter element, they repress its constitutive expression but not its heat shock inducibility. These results implicate the region between nucleotides −501 and −332 (relative to the translational start site) in the basal repression of *HSP26* and indicate that derepression is a key element in its induction by heat shock.

Thus far, it has not been possible to identify any single upstream sequence that is responsible for repressing basal *HSP26* transcription. Instead, the gene appears to contain functionally redundant repression elements. Such a system has not been reported for a heat shock gene before.

Other results reported here indicate that derepression is not the only mechanism employed to regulate *HSP26*. First, the three deletion mutations with high basal expression at 25°C are two- to three-fold further induced at 39°C, to a level comparable to that of the wild-type gene. In the one case tested, YB12-1, transcript concentrations also increase two- to threefold. This might be accomplished either by an increase in transcription or by an increase in the stability of the message. Increased stability of the hsp70 message during heat shock is an important component of its regulation in both *Drosophila* and mammalian cells (26, 33).

The effects of *HSP26* upstream sequences on a heterologous marker gene also suggest that both positive and negative regulatory elements are employed in the regulation of *HSP26*. As discussed above, a 207-nucleotide sequence upstream of *HSP26* (−501 to −294) is sufficient to repress the constitutive expression of a heterologous gene containing a heat shock promoter element. This same sequence also confers strong heat shock inducibility upon a heterologous gene that does not contain a heat shock promoter element. Thus, this sequence is competent for both basal repression and for heat shock activation of transcription.

In the yeast *SSA1*(*HSP70*) and *HSP82* genes, HSEs play a role in both constitutive and heat-inducible expression. It is not clear whether they play a role in the regulation of *HSP26*. The 501-nucleotide sequence that we have studied contains five matches to the HSE consensus. None of these is essential. Any one of them, and several combinations of them, can be deleted without altering *HSP26* expression. This is not uncommon in heat shock promoters; redundant HSEs are the general rule. Most of the HSEs in the *HSP26* upstream sequence are very weak matches to the consensus and would not be expected to be functional on their own. It is possible, however, that they work cooperatively. Since HSF binds yeast HSEs constitutively, they might even account for both basal repression and heat shock activation of *HSP26*. That is, the HSEs of this gene might bind HSF in a nonproductive manner at normal temperatures, thereby repressing basal transcription. With heat shock, a change in the properties of HSF might change their interaction into a productive one. Alternatively, separate, redundant repressor elements might be responsible for basal repression, with the HSEs independently responsible for weak heat shock activation. Sequences retained in the constitutively expressed deletion YB12-1 pose an interesting case. They contain only one rather poor match to the HSE consensus (HSE1; Fig. 4). They do, however, contain an unusual

number of partial elements, TTCs. Are these sequences, through a weak interaction with HSF, able to provide both basal expression and heat shock induction in this mutation? (In the wild type, basal expression would presumably be repressed by the upstream repressor sequences.) Or is transcription of the YB12-1 mutant regulated by other, as yet unidentified transcription elements? Given the redundancy and interdigitation of the elements involved, a much more detailed analysis will be required to unravel the role of HSEs in *HSP26* regulation.

The importance of derepression in the regulation of *HSP26* contrasts with that of most other eucaryotic heat shock genes, which are thought to be controlled primarily by HSF-mediated transcriptional activation. There is, however, evidence that negative regulation may play some role in the transcription of other heat shock genes (29, 46). Specifically, the yeast *SSA1* gene is constitutively expressed at a fairly high level and further induced by heat shock. Numerous promoter modules are dispersed across a large upstream sequence, but the most important elements are the multiple HSEs, which provide both constitutive and heat shock-induced transcription (29, 39). However, one functional HSE overlaps a URS1 element, an upstream repressor sequence with a proposed function in the repression of other yeast promoters (44). (Sequences related to the URS1 consensus, TAGCCGCCG, do not appear in the upstream region of *HSP26*.) Transcription of the *Drosophila HSP70* gene also appears to be negatively regulated, in this case at a step early in transcript elongation (37). Following heat shock, the block in transcription elongation is removed and full-length *HSP70* transcripts are synthesized. In a heterologous system, it has been reported that sequences surrounding the *HSP70* transcriptional start site repress constitutive transcription of the *Drosophila HSP70* gene in mammalian cells (5); however, other analyses suggest that only HSE-mediated activation is important (28, 31).

Finally, our results suggest that the regulation of *HSP26* transcription differs from that of other small heat shock genes in yet another way. *HSP26* of *S. cerevisiae* and the small-hsp genes of other organisms share the property of being expressed not only during heat shock but also at specific times during the normal course of development. For example, yeast hsp26 is induced under conditions of nutrient deprivation that lead to stationary-phase arrest and sporulation in diploids. This physiological response is distinct from a heat shock induction in several ways: the full set of heat shock genes is not induced, a large number of other genes are induced, and the trigger is not the same (19, 20, 47). (The trigger for the heat shock response is believed to be protein denaturation, aggregation, and misassembly.) Certain of the *Drosophila* small-hsp genes are induced during oogenesis, and members of the small-hsp gene family in lilies are induced during meiosis. For the *Drosophila* small-hsp genes, elements regulating heat shock induction are widely dispersed and readily separated from those regulating developmental expression (4, 7, 10, 11, 15, 17, 35). In contrast, none of our many *HSP26* deletion mutations separated heat shock regulatory elements from developmental regulatory elements. It may be that these elements are indeed separate in *S. cerevisiae* but too closely interdigitated to be dissected in this manner. It is also possible, if not probable, that a common regulatory mechanism is employed for *HSP26* transcription in response to heat shock, nitrogen starvation, and stationary-phase arrest.

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LITERATURE CITED

1. **Amin, J., J. Ananthan, and R. Voellmy.** 1988. Key features of heat shock regulatory elements. Mol. Cell. Biol. **8**:3761–3769.
2. **Ausubel, F. M., R. Brent, R. L. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1988. Current protocols in molecular biology Green Publishers Associates and Wiley-Interscience, New York.
3. **Borkovich, K. A., F. W. Farrelly, D. B. Finkelstein, J. Taulien, and S. Lindquist.** 1989. hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Mol. Cell. Biol. **9**:3919–3930.
4. **Cohen, R. S., and M. Meselson.** 1985. Separate regulatory elements for the heat-inducible and ovarian expression of the *Drosophila hsp26* gene. Cell **43**:737–746.
5. **Corces, V., and A. Pellicer.** 1984. Identification of sequences involved in the transcriptional control of a Drosophila heat-shock gene. J. Biol. Chem. **259**:14812–14817.
6. **Craig, E. A., W. Boorstein, H.-O. Park, D. Stone, and C. Nicolet.** 1989. Complex regulation of three heat inducible *Hsp70* related genes in *Saccharomyces cerevisiae*. UCLA Symp. Mol. Cell. Biol. **96**:51–62.
7. **Glaser, R. L., and J. T. Lis.** 1990. Multiple, compensatory regulatory elements specify spermatocyte-specific expression of the *Drosophila melanogaster hsp26* gene. Mol. Cell. Biol. **10**:131–137.
8. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
9. **Henikoff, S., and M. K. Eghtedarzadeh.** 1987. Conserved arrangement of nested genes at the *Drosophila Gart* locus. Genetics **117**:711–725.
10. **Hoffman, E. P., S. L. Gerring, and V. G. Corces.** 1987. The ovarian, ecdysterone, and heat-shock-responsive promoters of the *Drosophila melanogaster hsp27* gene react very differently to pertubations of DNA sequence. Mol. Cell. Biol. **7**:973–981.
11. **Hultmark, D., R. Klemenz, and W. J. Gehring.** 1986. Translational control elements in the untranslated leader of the heat shock gene hsp22. Cell **44**:429–438.
12. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact cells treated with alkali cations. J. Bacteriol. **153**:163–168.
13. **Jakobsen, B. K., and H. R. B. Pelham.** 1988. Constitutive binding of yeast heat shock factor to DNA in vivo. Mol. Cell. Biol. **8**:5040–5042.
14. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1440–1448.
15. **Kelley, S. E., and I. L. Cartwright.** 1989. Perturbation of chromatin architecture on ecdysterone induction of *Drosophila melanogaster* small heat shock protein genes. Mol. Cell. Biol. **9**:332–335.
16. **Kingston, R. E., T. J. Scheutz, and Z. Larin.** 1987. Heat-inducible human factor that binds to a human *hsp70* promoter. Mol. Cell. Biol. **7**:1530–1534.
17. **Klemenz, R., and W. Gehring.** 1986. Sequence requirement for expression of the *Drosophila melanogaster* heat shock protein *hsp22* gene during heat shock and normal development. Mol. Cell. Biol. **6**:2011–2019.
18. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**: 488–492.
19. **Kurtz, S., and S. L. Lindquist.** 1984. The changing pattern of gene expression in sporulating yeast. Proc. Natl. Acad. Sci.

USA **81**:7323–7327.
20. **Kurtz, S., J. Rossi, L. Petko, and S. Lindquist.** 1986. An ancient development induction: heat-shock proteins induced in sporulation and oogenesis. Science **231**:1154–1157.
21. **Lindquist, S., and E. A. Craig.** 1988. The heat-shock proteins. Annu. Rev. Genet. **22**:631–677.
22. **Lindquist, S.** 1981. Regulation of protein synthesis during heat shock. Nature (London) **293**:311–314.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **McDaniel, D., A. J. Caplan, M.-S. Lee, C. C. Adams, B. R. Fishel, D. S. Gross, and W. T. Garrard.** 1989. Basal-level expression of the yeast *HSP82* gene requires a heat shock regulatory element. Mol. Cell. Biol. **9**:4789–4798.
25. **McMaster, G. K., and G. G. Carmichael.** 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. USA **74**:4835–4838.
26. **Morimoto, R. I., D. Mosser, T. K. McClanahan, N. G. Therodorakis, and G. Williams.** 1989. Transcriptional regulation of the human Hsp70 gene. UCLA Symp. Mol. Cell. Biol. **96**:83–94.
27. **Munro, S., and H. Pelham.** 1985. What turns on heat shock genes? Nature (London) **317**:476–478.
28. **Nover, L.** 1987. Expression of heat shock genes in homologous and heterologous systems. Enzyme Microb. Technol. **9**:130–144.
29. **Park, H.-O., and E. A. Craig.** 1989. Positive and negative regulation of basal expression of a yeast *hsp70* gene. Mol. Cell. Biol. **9**:2025–2033.
30. **Parker, C. S., and J. Topol.** 1984. A Drosophila RNA polymerase II transcription factor binds to the regulatory site of an *hsp70* gene. Cell **37**:273–283.
31. **Pelham, H. R. B.** 1982. A regulating upstream promoter element in the Drosophila HSP70 heat-shock gene. Cell **30**:517–528.
32. **Perisic, O., H. Xiao, and J. T. Lis.** 1989. Stable binding of Drosophila heat shock factor to heat-to-head and tail-to-tail repeats of a conserved 5bp recognition unit. Cell **59**:797–806.
33. **Petersen, R., and S. Lindquist.** 1988. The *Drosophila* hsp70 message is rapidly degraded at normal temperatures and stabilized by heat shock. Gene **72**:161–168.
34. **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.
35. **Riddihough, G., and H. R. B. Pelham.** 1986. Activation of the Drosophila *hsp27* promoter by heat shock and by ecdysone involves independent and remote regulatory sequences. EMBO J. **5**:1653–1658.
36. **Rossi, J., and S. Lindquist.** 1989. Intercellular location of hsp26 in yeast cells varies with metabolism. J. Cell Biol. **108**:425–439.
37. **Rougvie, A. E., and J. T. Lis.** 1988. The RNA polymerase II molecule at the 5′ end of the uninduced hsp70 gene of *D. melanogaster* is transcriptionally engaged. Cell **54**:795–804.
38. **Sikorsky, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122**:19–27.
39. **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.
40. **Sorger, P. K., M. J. Lewis, and H. R. B. Pelham.** 1987. Heat shock factor is regulated differently in yeast and HeLa cells. Nature (London) **329**:81–84.
41. **Sorger, P. K., and H. R. B. Pelham.** 1987. Purification and characterization of a heat-shock element binding protein from yeast. EMBO J. **6**:3035–3041.
42. **Sorger, P. K., and H. R. B. Pelham.** 1988. Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. Cell **54**:855–864.
43. **Struhl, K.** 1987. Promoter activator proteins and the mechanism of transcriptional initiation in yeast. Cell **49**:295–297.
44. **Sumadra, R. A., and T. G. Cooper.** 1987. Ubiquitous upstream repression sequences control activation of the inducible arginase gene in yeast. Proc. Natl. Acad. Sci. USA **84**:3997–4001.
45. **Susek, R. E., and S. L. Lindquist.** 1989. hsp26 of *Saccharomyces cerevisiae* is related to the superfamily of small hsps but is without a demonstrable function. Mol. Cell. Biol. **9**:5265–5271.
46. **Tanaka, K., T. Yatomi, K. Matsumoto, and A. Toh-e.** 1989. Transcription of the heat shock genes by cyclic-AMP and heat shock in yeast. UCLA Symp. Mol. Cell. Biol. **96**:63–72.
47. **Werner-Washburne, M., J. Becker, J. Kosic-Smithers, and E. A. Craig.** 1989. Yeast Hsp70 RNA levels vary in response to the physiological status of the cell. J. Bacteriol. **171**:2680–2688.
48. **Wiederrecht, G., D. Seto, and C. S. Parker.** 1988. Isolation of the gene encoding the *S. cerevisiae* heat shock transcription factor. Cell **54**:841–853.
49. **Wu, C.** 1984. Activating protein factor binds *in vitro* to upstream control sequences in the heat shock gene chromatin. Nature (London) **311**:81–84.
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. **Zagursky, R. J., and M. L. Berman.** 1984. Cloning vectors that yield high levels of single-stranded DNA for rapid DNA sequencing. Gene **27**:183–191.
52. **Zimarino, V., C. Tsai, and C. Wu.** 1990. Complex modes of heat shock factor activation. Mol. Cell. Biol. **10**:752–759.
53. **Zimarino, V., and C. Wu.** 1987. Induction of sequence-specific binding of *Drosophila* heat shock activator protein without protein synthesis. Nature (London) **327**:727–728.
54. **Zoller, M. J., and M. Smith.** 1985. Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template. Methods Enzymol. **154**:329–350.