

Basal-Level Expression of the Yeast *HSP82* Gene Requires a Heat Shock Regulatory Element

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Previous studies have shown that heat shock factor is constitutively bound to heat shock elements in *Saccharomyces cerevisiae*. We demonstrate that mutation of the heat shock element closest to the TATA box of the yeast *HSP82* promoter abolishes basal-level transcription without markedly affecting inducibility. The mutated heat shock element no longer bound putative heat shock factor, either in vitro or in vivo, but still resided within a nuclease-hypersensitive site in the chromatin. Thus, constitutive binding of heat shock factor to heat shock elements in *S. cerevisiae* appears to functionally direct basal-level transcription.

Transcriptional activation of heat shock genes provides a paradigm for understanding eucaryotic gene regulation (27, 28, 38). Induction is mediated by interaction of heat shock factor (HSF) (37, 53, 57) with heat shock elements (HSEs) (38, 58) within promoters of responsive genes. In yeast cells, HSF constitutively binds to HSEs (20, 36, 45, 47), whereas in multicellular organisms HSF binds to HSEs only after induction (22, 25, 45, 50, 56, 59), although a minor binding activity can be detected in control cells under certain conditions (34). In both systems, HSF is phosphorylated in response to heat shock, but the modification does not influence DNA binding (25, 45, 47). The yeast gene encoding HSF is essential for normal growth (47, 52).

We selected for study the *HSP82* gene of the yeast *Saccharomyces cerevisiae*, because its promoter has the dual function of conferring basal-level transcription and heat shock inducibility (11-13, 33). This gene, formerly termed *HSP90*, has been cloned and sequenced, and its transcription unit and chromatin structure have been delineated (11, 17, 49). It is expressed at a significant basal rate, since the 82-kilodalton protein accounts for about 0.14% of the total protein synthesized in non-heat-shocked cells (33). After heat shock, however, induction primarily due to transcription causes the synthesis rate to increase within 20 min to account for about 4.5% of the total (14, 33). The 273-base-pair (bp) region immediately upstream from the transcription start site is capable of conferring both high basal and heat-shock-inducible expression in a variety of yeast plasmid constructs (12, 13). The encoded protein is highly conserved among the eucaryotes (4, 10, 11, 32), and a homolog has been found in *Escherichia coli* (2), although their functions are unknown (3, 6). The protein has been localized in the cytoplasm of vertebrate cells and has been observed to interact with a variety of other cellular proteins, possibly acting reversibly as an inhibitor (23, 35, 42, 43). Interestingly, in yeasts, *HSP82* is also induced during sporulation (24). Importantly for the studies reported here, mutational analysis in yeast cells is not limited by a primary functional requirement for the *HSP82* gene product, since

this gene is not essential due to the presence of a cognate gene, termed *HSC82*. Deletion of both *HSP82* and *HSC82*, however, is lethal (6).

Like the chromatin structure of other heat shock genes (16), the promoter region of the yeast *HSP82* gene is organized into a constitutive DNase I-hypersensitive site (17, 49). In *Drosophila melanogaster*, it has been shown that these sites contain bound TATA factor (56) and RNA polymerase II molecules (41) even before heat shock induction. We address the importance of the constitutive binding of HSF in yeast cells in determining the 5' hypersensitive site within the *HSP82* gene and how such binding affects gene expression. We mutated the HSE closest to the TATA box within the promoter of the yeast *HSP82* gene and demonstrate that the mutated sequence is incapable of binding putative HSF, either in vitro or in vivo. Surprisingly, while the altered promoter retains substantial inducibility, its basal activity is virtually eliminated, even though the regional chromatin structure is still organized into a constitutive DNase I-hypersensitive site. Thus, HSEs not only mediate heat shock induction in yeast cells but also specify basal-level expression.

MATERIALS AND METHODS

Mutagenesis. Oligonucleotide mutagenesis (60) was performed on a 2.9-kilobase (kb) *EcoRI* fragment of *HSP82* (11) subcloned into M13mp19. The mutated fragment, confirmed by dideoxy sequencing, was inserted into YIp5, cut at a single *BglII* site (at +239), and transformed (19) into haploid yeast strain W303-1B (*MATa leu2-3, 112 his3-11, 15 ura3-1 ade2-1 trp1-1 can1-100*). After selection, cells were plated on 5-fluoroorotic acid to counterselect for elimination of duplicated sequences (5).

Genomic Southern analysis. Cells harboring the mutated sequence were identified by genomic Southern analysis. DNA was isolated as described elsewhere (18). After digestion with *XbaI* or *EcoRI*, 2- μ g samples per lane were separated electrophoretically on a 1% agarose gel in 1 \times TPE buffer (30). DNA was transferred to a Zeta-Probe membrane, and the filter was prehybridized for 2 h at 50°C and then hybridized for 12 h at 50°C as described elsewhere (39).

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with a ^{32}P -end-labeled 35-mer specific for *HSP82* (probe 1 of Fig. 1b). This probe spans -269 to -235 in the upstream region of the *HSP82* gene (11). The filter was washed four times, 20 min each time, at 50°C with $4\times$ SSC (0.6 M NaCl plus 0.06 M trisodium citrate)-20 mM sodium phosphate buffer (pH 7.0)-0.5% sodium dodecyl sulfate. Autoradiograms (Kodak XAR-5) were exposed at -70°C with a Cronex Lightning-Plus intensifying screen (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.).

Northern (RNA) analysis. For assay of basal transcription, samples of wild-type (WT) and mutant cells were withdrawn from cultures growing exponentially at 30°C in YPD medium (1% yeast extract [Difco Laboratories, Detroit, Mich.], 2% peptone [Difco], 2% dextrose) and transferred to sodium azide solution (20 mM final concentration) in Eppendorf tubes on ice. For heat shock induction, the temperature was shifted rapidly from 30 to 39°C by addition of an equal volume of medium prewarmed to 51°C and maintained at 39°C in a shaking water bath (49). Culture samples were withdrawn as described above at various times after induction. RNA was isolated as described elsewhere (24) and assayed colorimetrically by the orcinol procedure. Samples, 5 μg , were treated with 50% formamide, separated on 1.4% agarose gels containing 2.2 M formaldehyde, transferred to a Zeta-Probe membrane, and hybridized (15) with a 100-mer complementary to positions +2186 to +2285 of the 2.3-kb transcript (probe 2 of Fig. 1b). The probe was labeled with [^{32}P]dCTP by primer extension of a complementary 27-mer that abutted the 3' end of the 100-mer. After hybridization for 24 h at 42°C, the filter was washed four times, 20 min each time, at 60°C with $2\times$ SSC-20 mM sodium phosphate buffer (pH 7.0)-0.5% sodium dodecyl sulfate. Blots were exposed for 30 min to 2 weeks to preflashed X-ray films at -70°C (26), and quantitation of hybridization signals was performed by densitometric scanning with a Hoefer densitometer linked to a Bio-Rad integrator (Bio-Rad Laboratories, Richmond, Calif.).

Gel mobility shift assays. Yeast whole-cell extracts were prepared from the protease-deficient strain BJ926 (*MATa/α trp1/+ his1/+ prc1-126/- pep4-3/- prb1-1122/- can1-11/- gal2/-*) (the gift of E. Jones) before and after heat shock induction of late-log-phase cultures. By using published procedures (47), extracts were prepared by washing cells once in breakage buffer (200 mM Tris hydrochloride, pH 8.0, 10 mM MgCl_2 , 10% glycerol, 1 mM phenylmethylsulfonyl fluoride [PMSF], 10 μg of leupeptin per ml), followed by disrupting cells in the same buffer with a bead beater (Biospec Products), with four 15-s bursts. The homogenate was extracted by addition of ammonium sulfate to a final concentration of 400 mM, followed by agitation for 30 min at 4°C. Cell debris was removed by centrifugation at $10,000\times g$, and the extract was clarified by a high-speed spin at $100,000\times g$ for 1 h at 4°C. Soluble proteins were precipitated by addition of solid ammonium sulfate to a final concentration of 0.35 g/ml, and the pellet was suspended in a minimum volume of dialysis buffer [50 mM $(\text{NH}_4)_2\text{SO}_4$, 1 mM EDTA, 20 mM Tris hydrochloride, pH 8.0, 10% glycerol, 1 mM dithiothreitol, 0.6 mM PMSF, 10 μg of leupeptin per ml] and dialyzed overnight at 4°C. DNA binding assays were performed as described elsewhere (46). Briefly, 0.2 to 0.3 ng of ^{32}P -end-labeled double-stranded probes was incubated with approximately 60 μg of whole-cell extract in the presence of 1 μg of poly(dI-dC) in 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.9)-1 mM MgCl_2 -60 mM KCl-12% glycerol-1 mM dithiothreitol for 20 min at 4°C, yielding a final reaction volume of 20 μl . (Probe 3 of Fig.

1b and those indicated in the Fig. 3 legend were used in these studies. The probe designated DHSE contains the sequence of HSE2 of reference 46.) For competition experiments, unlabeled competitor DNA was added to the binding reaction prior to the addition of the labeled probe. Samples were resolved at 24°C in preelectrophoresed 4% polyacrylamide-bisacrylamide (30:1) gels, using $0.5\times$ TBE buffer-2% glycerol (30). Gels were dried and exposed to X-ray film.

Methylation protection genomic footprinting. Cells cultivated in YPD medium at 30°C to mid-log phase (5×10^7 cells per ml) were heat shocked for 5 or 20 min at 39°C as described above. Induction was terminated by chilling to 0°C and concentrating cells to $10^9/\text{ml}$ in YPD. Dimethyl sulfate was added to 0.5%, and cells were incubated on ice for 7.5 to 22.5 min. Reactions were terminated by 20-fold dilution in 20 mM Tris hydrochloride (pH 8.0)-20 mM sodium azide. Genomic DNA was purified, cut with *Bcl*I, and cleaved with piperidine at methylated guanines (31). Naked DNA was treated with dimethyl sulfate for 0.33 min at 0°C and processed as above. DNA samples (5 to 10 μg per lane) were electrophoresed on 0.35-mm-thick, 8% polyacrylamide-0.27% bisacrylamide sequencing gels and vacuum blotted to GeneScreen, using a novel procedure (15) modified as described in detail elsewhere (D. S. Gross et al., submitted for publication). Briefly, the transfer was effected by using a constant vacuum strength of 300 mtorr for 12 to 16 h. DNA fragments were UV cross-linked to GeneScreen with the Stratolinker model 1800 (Stratagene Cloning Systems, La Jolla, Calif.) set at 300,000 μJ . The covalently linked DNA was then briefly denatured in 0.4 N NaOH, neutralized, and indirectly end labeled with a strand-specific RNA probe spanning +21 to +112 (probe 4 of Fig. 1b) radiolabeled with 0.5 mCi of [α - ^{32}P]UTP (6,000 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.) per ml as precursor (8, 15).

DNase I genomic footprinting. Cultivation and heat shock were performed as described above, and induction was terminated by addition of 20 mM sodium azide (49). Cells were converted to spheroplasts at 30°C, using oxalyticase (Enzogenetics, Corvallis, Ore.), in the presence of 1 mM PMSF. Spheroplasts were lysed by hypotonic shock in spheroplast digestion buffer, consisting of 40 mM HEPES (pH 7.2), 1 mM MgCl_2 , 0.1 mM CaCl_2 , 1 mM PMSF, 2 mM benzamidine, 0.5 μg of chymostatin per ml, 2.0 μg of leupeptin per ml, 2.0 μg of antipain per ml, 1.7 μg of aprotinin per ml, 1.1 of phosphoramidon per ml, 7.2 μg of E-64 per ml, and 2.0 μg of pepstatin per ml. Following a single wash, lysed spheroplasts were suspended in spheroplast digestion buffer to a final concentration of 60 to 300 μg of DNA per ml, equilibrated to 37°C, and digested for 10 min with concentrations of DNase I (DPRF grade; Organon Teknika, Malvern, Pa.) ranging from 3.6×10^{-5} to 52×10^{-5} U/ μg of DNA. Digestions were terminated by addition of EDTA to 10 mM; genomic DNA was isolated and digested with *Bcl*I and *Msp*I as described previously (15). For naked DNA controls, deproteinized DNA was digested with *Bcl*I and *Msp*I, purified by organic extraction, ethanol precipitated, dissolved in 10 mM Tris-1 mM EDTA (pH 8.0), and then extensively dialyzed against 40 mM HEPES (pH 7.5)-1 mM MgCl_2 -0.1 mM CaCl_2 . Digestion was performed at a DNA concentration of 0.3 to 1.0 $\mu\text{g}/\text{ml}$ in the same buffer, using 0.7×10^{-4} to 2.0×10^{-4} U of DNase I per μg of DNA for 10 min at 37°C, and terminated as above. Samples were resolved on sequencing gels, vacuum blotted, and indirectly end labeled with a strand-specific RNA probe spanning -350

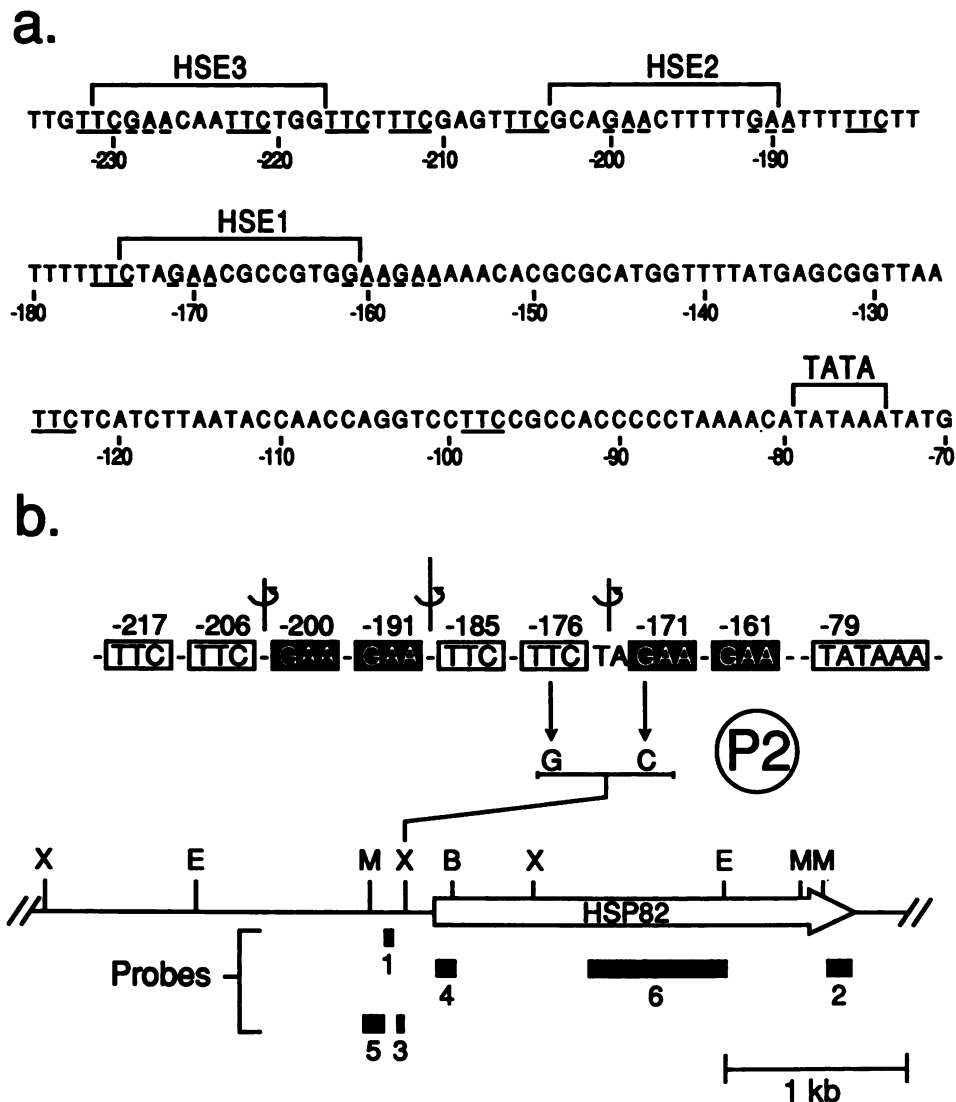


FIG. 1. DNA sequence of the *HSP82* promoter region (adapted from reference 11) (a) and schematic organization of the promoter of the *HSP82* gene (b). (a) Numbering is relative to the 5' end of the transcript as identified by S1 nuclease mapping (11). The HSEs and TATA box indicated by brackets bear similarities to the consensus sequences of Pelham (38) and Chen and Struhl (7), respectively. Also shown as either solid or dashed underlines depending on orientation are HSE half-sites (TTC) as defined by Xiao and Lis (58) and Amin et al (1). (b) Positions of HSE half-sites, dyad axes, and the TATA box are indicated. The bases at -171 and -175 were changed, generating the P2 mutation that eliminates an *Xba*I (X) site. Other restriction sites shown are *Eco*RI (E), *Msp*I (M), and *Bcl*II (B), relative to the *HSP82* gene transcription unit depicted by the arrow. Shown below are probes used in various analyses: 1, a 35-mer spanning -269 to -235 used in Southern analysis (Fig. 2b); 2, a 100-mer spanning +2186 to +2285 used in Northern analysis (Fig. 3); 3, a 27-mer spanning -188 to -162 used in gel mobility shift assays (Fig. 4); 4, a riboprobe spanning +21 to +112 used in methylation protection experiments (Fig. 5); 5, a riboprobe spanning -350 to -273 used in DNase I genomic footprinting (Fig. 6); 6, a 755-bp *Xmn*I fragment spanning +843 to +1598 used in DNase I-hypersensitive site mapping (Fig. 7).

to -273 (probe 5 of Fig. 1b) as reported elsewhere (15), with the modifications described above.

Nuclease-hypersensitive site mapping. Cells were subjected to heat shock for 10 min (WT) or 15 min (P2) and treated with 20 mM sodium azide as described above. Nuclei were isolated as reported elsewhere (49) except for the following modifications. Cells were incubated with oxalyticase (800 U/ml) in spheroplast buffer (1.4 M sorbitol, 40 mM HEPES [pH 7.5], 0.5 mM MgCl₂, 1 mM PMSF, 20 mM sodium azide, 0.5% mercaptoethanol) for 60 to 90 min at 30°C. Cell lysates were transferred onto glycerol step gradients—5 ml of 40%, 10 ml of 30%, and 5 ml of 20% glycerol in 8% Ficoll, 20 mM PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)] (pH

6.5), 1 mM MgCl₂, 0.5 mM PMSF, and nuclei were pelleted for 40 min at 25,000 × *g* in a JA20 rotor (Beckman Instruments, Inc., Fullerton, Calif.). Nuclei were digested with DNase I, and DNA was purified as described elsewhere (49). For naked DNA controls, genomic DNA was purified from WT and P2 nuclei isolated from heat-shocked cells. DNA was digested with *Eco*RI and separated on a 1.5% agarose gel in 1× TPE buffer (30). *Eco*RI plus *Cla*I- or *Bcl*II-restricted genomic DNA and *Hind*III-digested lambda DNA were used for molecular size markers. DNA was transferred to Zeta-Probe membranes, and filters were prehybridized for 2 h at 65°C and hybridized with a radiolabeled 755-bp *Xmn*I fragment (probe 6 of Fig. 1b) for 24 h at 65°C as described

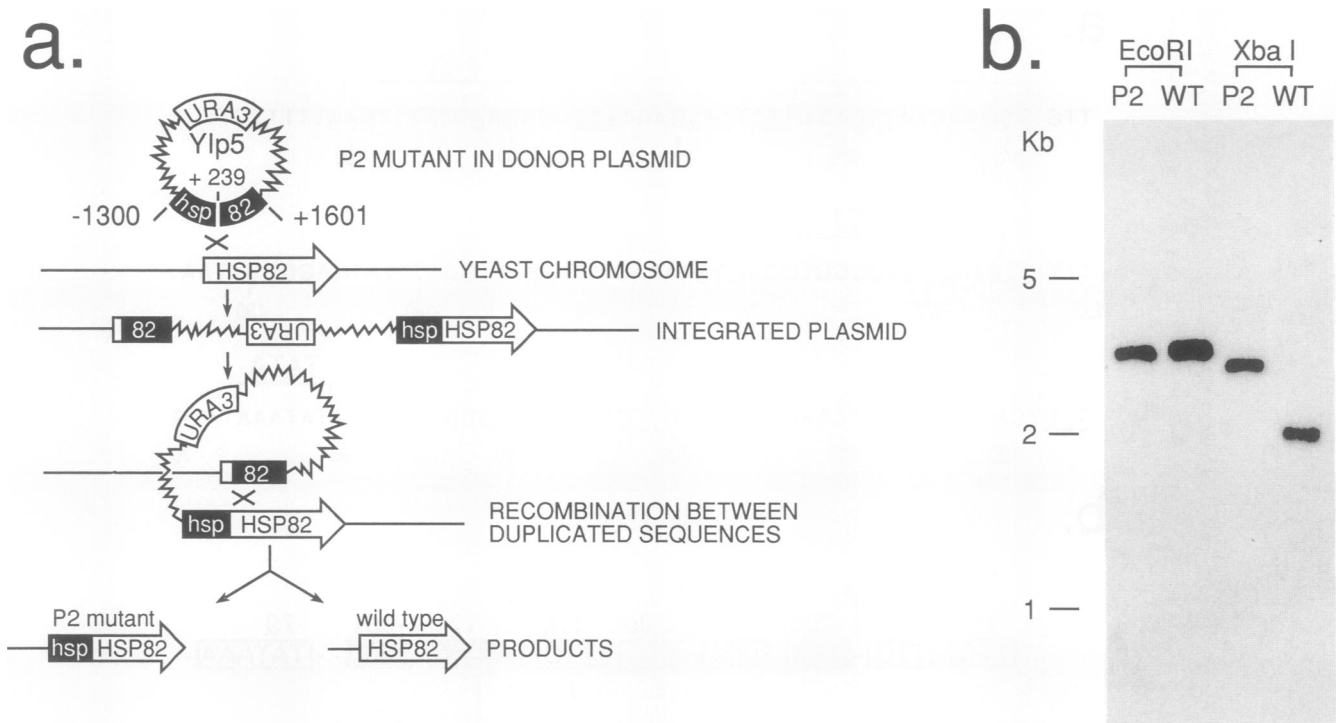


FIG. 2. Strategy for introducing the P2 mutation into the *HSP82* locus (adapted from reference 5) (a) and demonstration of replacement of the wild-type (WT) *HSP82* gene with the P2 mutant (b). (a) The mutated *Eco*RI fragment of the *HSP82* locus spanning -1300 to +1601 from M13mp19 was inserted into *Ylp5*, and the donor plasmid was cleaved at the single *Bgl*II site (position +239 relative to the *HSP82* transcription start site). Haploid yeast cells were transformed with the linearized donor plasmid and plated on medium selecting for *URA3* expression. Genomic Southern analyses were performed to verify site-specific integration and the absence of multiple tandem copies (data not shown). Recombinants between adjacent duplicated sequences were then selected by plating on medium containing 5-fluoroorotic acid, leading to those retaining the mutation (*hsp82*) or those retaining the wild-type sequence (*HSP82*). (b) DNA from WT and P2 cells was isolated, digested with either *Eco*RI or *Xba*I, separated on a 1% agarose gel, and transferred for hybridization with a 35-mer 32 P-end-labeled probe spanning -269 to -235 of the *HSP82* upstream region (probe 1 of Fig. 1b).

elsewhere (39). The probe was labeled with [α^{32} P]dCTP by random primer synthesis, using an oligo-labeling kit (Pharmacia, Inc., Piscataway, N.J.). The filter was washed four times, 20 min each time, at 65°C with 0.1× SSC–20 mM sodium phosphate (pH 7.0)–1% sodium dodecyl sulfate. Autoradiograms (Kodak XAR-5) were exposed with an intensifying screen (Dupont Cronex) at -70°C for 12 to 36 h.

RESULTS

Mutagenesis of the TATA-proximal HSE within the *HSP82* gene promoter. We wished to investigate the role that HSEs play in transcriptional regulation and 5' hypersensitive site formation within the yeast *HSP82* gene. Analysis of the region upstream of the single TATA box at -79 revealed three HSEs exhibiting a 11-of-14 match to the minimal consensus sequence of Pelham (38), CNNGAANNTT CNNG (at -174, -203, and -231) (e.g. 5 of 8 matches to the 8-bp core sequence while maintaining correct nucleotide spacing) (Fig. 1a). However, a particularly striking DNA sequence organization was uncovered by comparing the same region with the revised *Drosophila* HSE consensus sequence of Xiao and Lis (58), NTTCNNGAAN, in which the 10-bp HSE itself exhibits 5-bp dyad symmetry relative to the half-site TTC. Of the 15 half-sites found upstream of the TATA box, 8 are spaced at either 10-nucleotide + 1-bp or 5-nucleotide \pm 1-bp intervals depending on orientation, symmetrically disposed about a major dyad axis at -187, with minor dyads at -173 and -202 (Fig. 1b). This perio-

dicity leads to an array of potential HSF binding sites that likely reside on the same side of the helix, spanning at least five helical turns, and suggests that multiple functional contacts between HSF molecules may require the proper alignment of HSEs, as proposed previously (9). Furthermore, a yeast *HSP70* gene, which also exhibits both basal and induced expression (36, 44), has a very similar organization of such half-sites (data not shown), and the parent 10-bp consensus sequence appears to be common among the promoter elements of other yeast heat shock genes (51). We therefore decided to mutate the first complete pair of these half-sites located upstream and closest to the TATA box. We created the mutant termed P2 by introducing single transversion mutations into nucleotides at positions -175 and -171 (Fig. 1b). These base substitutions eliminate the *Xba*I site residing at -174.

Replacement of the wild-type *HSP82* gene by site-directed integration. To investigate the effects of the mutated HSE on gene expression and chromatin structure within its native chromosomal environment, the mutated *HSP82* gene was targeted into the *HSP82* locus of haploid yeast cells. This approach overcomes potential artifacts caused by chromosomal position effects, flanking foreign DNA sequences, altered gene copy number, and timing of replication during S phase. Haploid yeast cells were transformed with the mutated *Eco*RI fragment, which had been inserted into *Ylp5*, and linearized at the single *Bgl*II site (position +239) (Fig. 2a). After initial selection for *URA3* expression, those cells

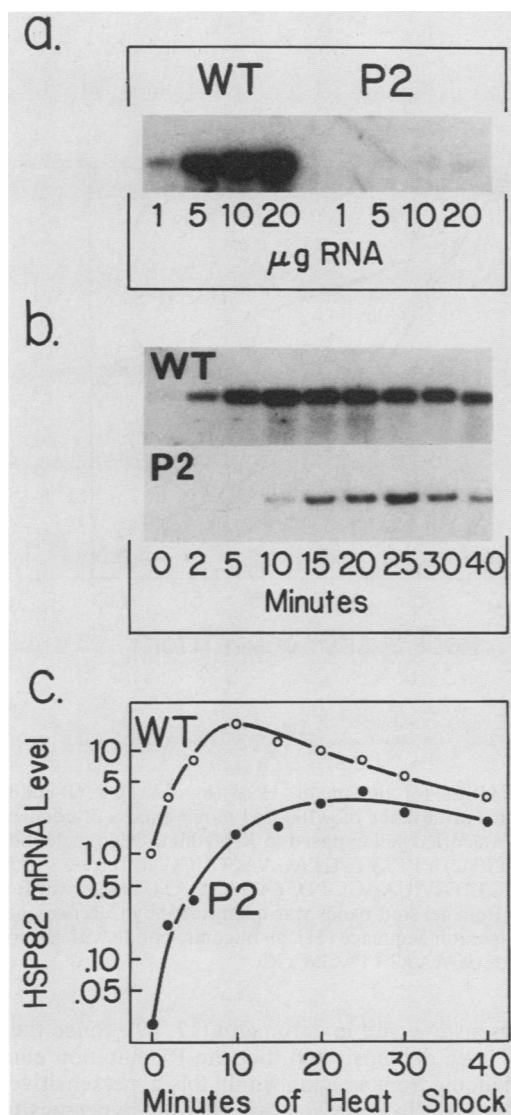


FIG. 3. Northern analysis of basal level transcription of the *HSP82* gene (a), kinetics of heat shock induction of WT and P2 *HSP82* genes (b), and (c) quantitation of the heat shock response. (a) The indicated amounts of total RNA, isolated from nonshocked exponential cultures of WT and P2 cells, were separated by electrophoresis on a formaldehyde-agarose gel, transferred to a membrane filter, and hybridized with a specific *HSP82* probe (probe 2 of Fig. 1b). (b) Cells grown at 30°C were shifted to 39°C for the indicated times. Equal amounts of RNA were analyzed as was done in panel a. (c) Panel b autoradiograms were scanned, and peak areas were determined by densitometry.

which subsequently have spontaneously lost *URA3* by undergoing recombination between duplicated adjacent sequences were isolated by the counterselection technique pioneered by Fink and co-workers (5). To identify cell clones that had correctly inserted the P2 mutation into the *HSP82* locus, we isolated DNA from WT and candidate P2 strains and performed genomic Southern analysis, using a probe complementary to sequences upstream of the mutated *XbaI* site (probe I, Fig. 1b). DNA isolated from the WT and P2 strains (Fig. 2b) subsequently used in the studies reported below exhibited identical-length, 2.9-kb *EcoRI* fragments diagnostic for the *HSP82* locus (see Fig. 1b for restriction

map). Importantly, however, the *XbaI* site located at -174 was no longer cleavable in DNA isolated from the P2 mutant, leading to an *XbaI* fragment 2.7 kb in length, instead of the 2.0-kb fragment seen for DNA isolated from WT cells (Fig. 2b). Therefore, the gene replacement was successful, since the appropriate *XbaI* site had been modified. This point was confirmed further by genomic sequencing (see Fig. 5).

Mutation of the TATA-proximal HSE selectively abolishes basal level transcription. To study the effect of the 2-bp substitution on *HSP82* gene expression, we performed Northern analysis on RNA samples isolated from control and heat-shock-induced WT and P2 isogenic strains. Figure 3a shows that basal level transcription is reduced at least 50-fold in the P2 mutant relative to that observed in WT cells. In spite of this, the mutated *HSP82* gene was still heat shock inducible (Fig. 3b). Quantitation indicates that the 2-bp change in the TATA-proximal HSE results in the conversion of a gene that was induced only 14-fold by heat shock to a gene that is induced at least 200-fold, reaching a maximal level only 3-fold lower than that of the WT (Fig. 3c). Therefore, minor base changes in the HSE closest to the TATA box create a novel promoter element.

The mutated HSE exhibits reduced affinity for factor binding in vitro. To determine whether binding of HSF might be affected by the mutation, we assayed protein-DNA interactions in vitro, using gel retardation. Figure 4a shows that a single retarded "HSF-HSE" complex is formed when a labeled 27-mer spanning the TATA-proximal HSE between -188 and -162 [YHSE(WT)] is incubated with extracts prepared from cells either before or after heat shock induction. Thus, in agreement with other investigators (36, 45, 47), even extracts of control cells have HSE binding activity. Also in agreement with previous results (45, 47), after prolonged electrophoresis we detected a lower mobility for the complex formed with heat-shocked cell extracts, consistent with the reported heat-shock-induced phosphorylation of the factor (data not shown). To verify these results, we performed competition experiments with a 34-mer containing *Drosophila* consensus sequences, designated DHSE, which possesses the identical core sequence used in the purification and cloning of the gene encoding the yeast heat shock factor (45-47) and for which yeast HSF binding has been functionally demonstrated in vivo (20, 46). Significantly, unlabeled DHSE effectively competes for complex formation, as does the WT yeast sequence, whereas a 27-mer corresponding to the P2 mutant is relatively ineffective [YHSE(P2)] (Fig. 4a). Furthermore, labeled DHSE generates a band with the same mobility as the YHSE(WT)-HSF complex and formation of this band is inhibited by WT, but not by the P2 oligomer (data not shown). The titration shown in Fig. 4b reveals that HSF has nearly equal affinity for DHSE and the *HSP82* WT sequence, while competition by P2 is weak and no greater than that observed with the unrelated *STE6* gene operator sequence (21). We conclude that minor base changes in the TATA-proximal HSE markedly affect the binding of HSF in vitro.

Guanines within the mutated HSE exhibit reduced protection to methylation by dimethyl sulfate in vivo. To demonstrate that the P2 mutation also affects binding of putative HSF in vivo, we used genomic sequencing to determine which guanine residues were protected from methylation by dimethyl sulfate within the major groove of DNA in living cells. We found that most guanines residing in the upstream region of the *HSP82* gene were highly accessible to the reagent (Fig. 5). Significantly, however, those located at -161 and -162 on the upper strand, only 10 bp away from

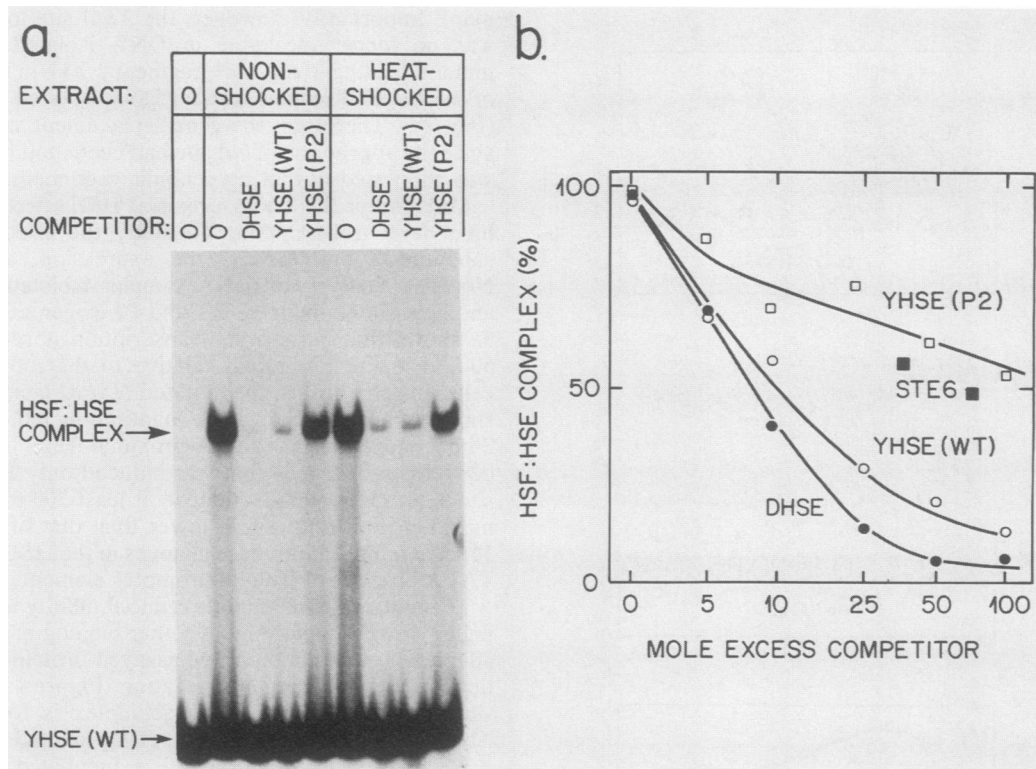


FIG. 4. In vitro binding of HSF to HSEs (a) and competition profile of HSEs for binding to HSF (b). (a) The ^{32}P -end-labeled, double-stranded probe was combined with extract and poly(dI-dC) in the presence or absence of a 100-fold molar excess of competitor, as indicated, and samples were electrophoresed in a 4% polyacrylamide gel, which was dried and exposed to X-ray film. Oligonucleotides used have the following sequences (only upper strands are shown): YHSE(WT), 5'TTTTCTTTTTTCTAGAACGCCGTG (probe 3 of Fig. 1b); YHSE(P2), 5'TTTTCTTTTTTGCTACAACGCCGTG; DHSE, 5'CTAGAAGCTTCTAGAAGCTTCTAGAAGATCCCCG. (b) Reactions were performed as in panel a, using extracts from control cells, and radioactivity from gel shift bands was quantitated by Cerenkov counting. Also shown are two points of competition (closed boxes) for the unrelated *STE6* operator sequence (21), an oligonucleotide with the following structure (only the upper strand is shown): STE6, 5'CATGTAATTACCTAATAGGGAAATTTACACGC.

the introduced mutations, were strongly protected in the WT, but not in the P2 mutant, either before or after heat shock. Therefore, protein-DNA contacts in living cells surrounding the TATA-proximal HSE are altered by nearby mutation.

The mutated HSE no longer exhibits a genomic footprint. To study further the effect of the P2 mutation on factor binding in living cells, we performed DNase I genomic footprinting experiments in spheroplast lysates. WT but not P2 cells exhibit a footprint over HSE1 and the immediate upstream sequences, both before and after heat shock induction (Fig. 6). Importantly, this footprint spans the region specifically protected from methylation in the WT cells. Thus, taken together with the methylation protection data, we conclude that mutation of the TATA-proximal HSE directly affects factor binding to the resident sequences in living cells. Further inspection of the footprinting data reveals that factor binding to the pertinent HSE induces hypersensitive nucleotide cleavage sites in the WT *HSP82* gene promoter which are characteristic of yeast HSF binding based on previous in vitro studies (53). In addition, in both WT and P2 cells, the TATA box region exhibits minor footprints both before and after heat shock induction whose fine structure appears to be strain specific (Fig. 6).

The constitutive DNase I-hypersensitive site within the *HSP82* gene promoter persists in the P2 mutant. Like other heat-shock-inducible genes (16), the promoter region of the yeast *HSP82* gene is organized into a constitutive DNase

I-hypersensitive site in chromatin (17, 49). Since the above studies have demonstrated that the P2 mutation eliminates factor binding from a region within this hypersensitive site, it was of interest to determine whether the hypersensitive site persisted in the mutant. To address this question, nuclei prepared from control and heat-shocked cells were digested with DNase I and cleavage sites were mapped by indirect end labeling (55). Both WT and P2 cells exhibited a constitutive DNase I-hypersensitive site within the promoter region and in a region further upstream centered at about -600 (Fig. 7). However, careful inspection revealed that WT but not P2 cells exhibit a constitutive footprint over HSE1 within the promoter-associated hypersensitive site, in agreement with the above genomic footprinting data, indicating that constitutive binding of factor is abolished by the minor base changes in the pertinent HSE. We conclude that the hypersensitive site persists in the P2 mutant, even though the TATA-proximal HSE remains unoccupied. A more detailed in vivo footprinting analysis of the WT *HSP82* gene will be presented elsewhere (Gross et al., submitted).

DISCUSSION

An HSE is required for basal level expression of the yeast *HSP82* gene. It has been proposed previously that HSEs in yeast cells may direct basal-level expression in addition to mediating heat shock induction (44, 47). Indeed, it has been shown that an HSE from the yeast *HSP70* gene can confer

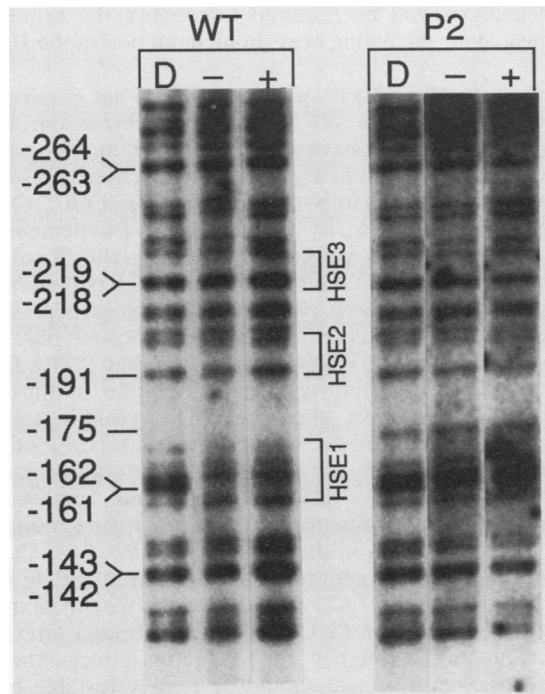


FIG. 5. Methylation protection analysis of HSE regions of the *HSP82* gene in living cells. Control (-) and 5-min heat-shocked (+) WT or P2 cells (as indicated) were incubated for either 7.5 (-) or 22.5 (+) min at 0°C with 0.5% dimethyl sulfate; naked DNA (D) was incubated for 0.33 min. Samples were cleaved with *Bcl*I, electrophoresed, blotted, and indirectly end labeled by hybridization with a probe complementary to the upper strand between +21 and +112 (probe 4 of Fig. 1b). The genomic sequence demonstrates in the P2 mutant that guanine was inserted at -175 and deleted at -171. When P2 cells were heat shocked for 20 min, identical results were obtained. HSE1, -2, and -3 depict regions corresponding to the sequences shown in Fig. 1a.

high basal as well as heat-inducible expression to an extra-chromosomal reporter gene (36, 44). The results of our study, however, demonstrate for the first time that a heat-shock-inducible gene in its native chromosomal environment requires an HSE for basal-level expression. A simple 2-bp substitution in the TATA-proximal HSE leads to at least a 50-fold reduction in basal-level expression of the *HSP82* gene (Fig. 3).

A function for yeast HSF in basal-level transcription. A function for yeast HSF in basal-level transcription would resolve the puzzle of why in yeasts (20, 36, 45, 47), but not in other eucaryotes (22, 25, 50, 56, 59), HSF binds to HSEs before heat shock induction. Our results strongly imply that HSF binding to the TATA-proximal HSE of the yeast *HSP82* gene is responsible for triggering basal-level transcription. It seems unlikely that a basal transcription factor other than HSF independently binds to a site common to, or overlapping, the proximal HSE. The WT but not the P2 sequence of this region formed a single protein-DNA complex in vitro in the gel mobility shift experiments, whose formation was inhibited by a consensus HSF binding site (Fig. 4). Furthermore, in vivo studies revealed identical major groove methylation protection and very similar DNase I footprints within this region, both before and after heat shock induction in WT cells (Fig. 5-7). Recently, however, Park and Craig (36) have found an upstream repression sequence that overlaps an HSE in a yeast *HSP70* gene. It is

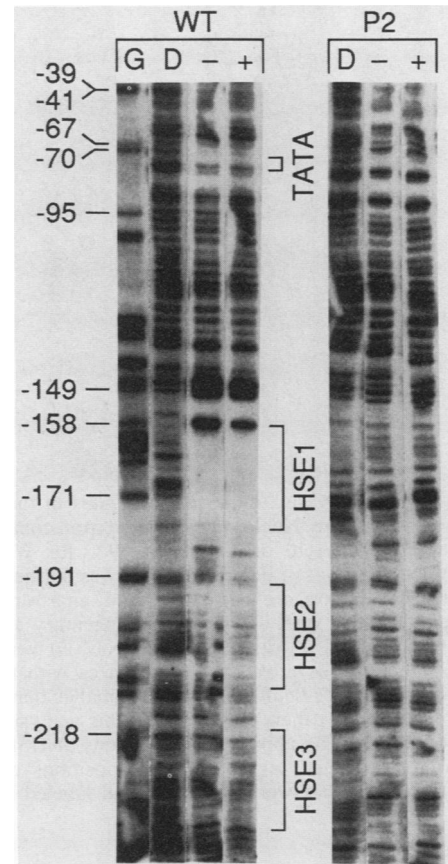


FIG. 6. DNase I genomic footprinting of the promoter region of *HSP82* in spheroplast lysates of WT and P2 cells. Control (-) and 5-min heat-shocked (+) cells were converted to spheroplasts and gently lysed in hypotonic buffer. Following digestion with DNase I, DNA was isolated, cleaved with *Msp*I and *Bcl*I, electrophoresed, blotted, and indirectly end labeled by hybridization with an RNA probe complementary to the upper strand between -350 and -273 (probe 5 of Fig. 1b). Naked DNA control samples (D), isolated from both WT and P2 cells, were similarly digested with DNase I and processed for indirect end labeling. Lane G depicts the Maxam-Gilbert cleavage reaction of guanines. The HSE and TATA regions indicated correspond to those sequences shown in Fig. 1a.

possible that a similar negative element resides in the *HSP82* gene since there is some sequence similarity between this upstream repression sequence and the sequences surrounding the TATA-proximal HSE studied here.

The TATA-proximal HSE of the yeast *HSP82* gene is necessary but not sufficient to direct basal-level expression. Putative HSF binding to the TATA-proximal HSE must direct basal-level expression in conjunction with other transcription factors, since we have found that a 2-bp substitution within the TATA box of the *HSP82* gene reduces basal-level and heat-shock-induced transcription 5- and 18-fold, respectively, when integrated in the native chromosomal locus (unpublished results). In addition, polypyrimidine tracts within the promoter have sequence similarity with those found in upstream regions of many constitutively expressed yeast genes (48), including *HSP70* (44). These sequences may also participate in basal-level transcription in association with yet other DNA-binding proteins (29, 48, 54). In other experiments patterned after those of Craig and co-workers (36, 44), we have inserted the 27-mer containing the TATA-proximal HSE of the yeast *HSP82* gene into a

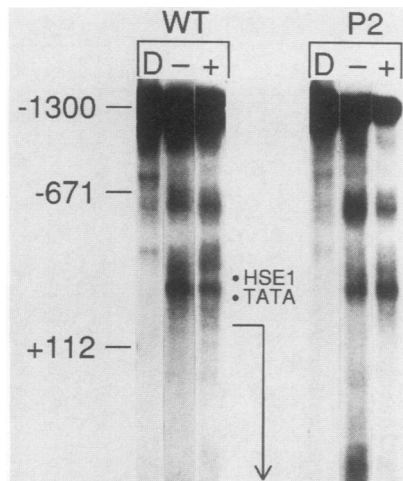


FIG. 7. Mapping DNase I-hypersensitive sites in the upstream region of the *HSP82* gene. Nuclei were isolated from control (-) and heat-shocked (+) cells (10 or 15 min at 39°C for WT and P2, respectively). After DNase I digestion, DNA was purified, digested with *EcoRI*, and electrophoretically separated, and transfers were indirectly end labeled with a probe complementary to +843 to +1598 (probe 6 of Fig. 1b). Naked DNA controls (D) were similarly processed. Although this probe cross-hybridizes with the *HSC82* gene, the parent *HSC82 EcoRI* fragment is smaller than any fragment shown on the gel, thereby permitting this analysis (see also references 6 and 49). The approximate positions of HSE1 and the TATA box are indicated, as well as the positions of selected restriction fragments that were indirectly end labeled as internal standards.

CYC1-lacZ fusion gene containing a defective promoter and have found that the sequence can direct high basal as well as heat-inducible expression (data not shown). The biological significance of this result is difficult to evaluate, however, since with this approach we were not able to demonstrate readily an effect of the P2 mutation on reduction of basal or heat-induced transcription (data not shown). Clearly, there is no substitute for site-directed integration in evaluating the true biological significance of a given mutation.

Mechanism of heat shock induction of the *HSP82* gene. The mechanism responsible for heat shock induction of the *HSP82* gene remains somewhat puzzling. Earlier studies have shown that the 273-bp region immediately upstream from the transcription start site is capable of conferring basal and heat-shock-induced transcription in a variety of yeast plasmid constructs (12, 13). Although sequence analysis of this upstream region reveals an array of TTC or GAA half-sites spanning five helical turns (Fig. 1b), *in vivo* footprinting data demonstrate that most of these sites are not stoichiometrically occupied. In WT but not P2 cells, only the TATA-proximal HSE exhibits protected guanines or a DNase I footprint, either before or after heat shock induction (Fig. 5-7). One possibility is that this single HSE may serve a dual role in both basal and heat-inducible transcription, depending on the phosphorylation state of bound HSF (25, 45, 47). Transient or fractional binding within the cell population of HSF to the mutated HSE may explain the threefold reduction in the maximal heat shock response exhibited by P2 cells relative to WT cells (Fig. 3), along with the absence of *in vivo* footprints within the region (Fig. 5-7). Alternatively, other HSEs could transiently or fractionally bind HSF in the P2 cell population. Clearly, further muta-

tional analysis will be required to identify the sequences responsible for mediating heat shock induction of the *HSP82* gene.

Occupancy of the TATA-proximal HSE is not required for nuclease-hypersensitive site formation in chromatin. Heat-shock-inducible genes have a chromatin structure poised for transcriptional induction, as revealed by the hypersensitivity of their promoter regions to nuclease digestion (16). Although the mechanism by which DNase I-hypersensitive sites are formed is not yet known, studies in the *Drosophila* system reveal that TATA factor (56) and RNA polymerase II (41) are associated with promoter regions of heat shock genes prior to induction; HSF becomes associated only after heat shock (57, 59). It has been proposed that TATA factor may be primarily responsible for generation of these 5'-nuclease-hypersensitive sites (56). The yeast system is clearly different, since HSF is associated with HSEs prior to heat shock (20, 36, 45, 47; see below). Our studies demonstrate that constitutive HSF binding is not required for hypersensitive site formation in the *HSP82* gene promoter, since the site persists in the chromatin of P2 cells. Careful inspection of the genomic footprints shown in Fig. 6, however, reveals that the TATA box of the *HSP82* gene exhibits a footprint in WT and P2 cells, both before and after heat shock induction. Whether TATA factor induces the nuclease-hypersensitive site should be readily testable by an experimental scenario similar to that utilized for the HSE analysis. Also remaining to be determined is whether RNA polymerase II is associated with the 5' regions in uninduced yeast genes and the role of other factors, such as poly(dA) · (dT) binding proteins (54), on hypersensitive site formation.

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LITERATURE CITED

- Amin, J., J. Ananthan, and R. Voellmy. 1988. Key features of heat shock regulatory elements. *Mol. Cell. Biol.* 8:3761-3769.
- Bardwell, J. C. A., and E. A. Craig. 1987. Eukaryotic Mr 83,000 heat shock protein has a homologue in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 84:5177-5181.
- Bardwell, J. C. A., and E. A. Craig. 1988. Ancient heat shock gene is dispensible. *J. Bacteriol.* 7:2977-2983.
- 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.
- Boeke, J. D., J. Trueheart, G. Natsoulis, and G. R. Fink. 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol.* 154:164-175.
- 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.

7. **Chen, W., and K. Struhl.** 1988. Saturation mutagenesis of a yeast *his3* "TATA element": genetic evidence for a specific TATA-binding protein. *Proc. Natl. Acad. Sci. USA* **85**:2691-2695.
8. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. USA* **81**:1991-1995.
9. **Cohen, R. S., and M. Meselson.** 1988. Periodic interaction of heat shock transcriptional elements. *Nature (London)* **332**: 856-858.
10. **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.
11. **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.
12. **Finkelstein, D. B., and S. Strausberg.** 1983. Identification and expression of a cloned yeast heat shock gene. *J. Biol. Chem.* **258**:1908-1913.
13. **Finkelstein, D. B., and S. Strausberg.** 1983. Heat-shock-regulated production of *Escherichia coli* β -galactosidase in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **3**:1625-1633.
14. **Finkelstein, D. B., S. Strausberg, and L. McAlister.** 1982. Alterations of transcription during heat shock of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **257**:8405-8411.
15. **Gross, D. S., K. W. Collins, E. M. Hernandez, and W. T. Garrard.** 1988. Vacuum blotting: a simple method for transferring DNA from sequencing gels to nylon membranes. *Gene* **74**:347-356.
16. **Gross, D. S., and W. T. Garrard.** 1988. Nuclease hypersensitive sites in chromatin. *Annu. Rev. Biochem.* **57**:159-197.
17. **Gross, D. S., C. Szent-Gyorgyi, and W. T. Garrard.** 1986. Yeast cell biology. *UCLA Symp. Mol. Cell. Biol. New Ser.* **33**: 345-366.
18. **Hoffman, C. S., and F. Winston.** 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* **57**:267-272.
19. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **53**:163-168.
20. **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.
21. **Johnson, A. D., and I. Herskowitz.** 1985. A repressor (MAT α 2 product) and its operator control expression of a set of cell type specific genes in yeast. *Cell* **42**:237-247.
22. **Kingston, R. E., T. J. Schuetz, and Z. Larin.** 1987. Heat-inducible human factor that binds to a human hsp70 promoter. *Mol. Cell. Biol.* **7**:1530-1534.
23. **Koyasu, S., E. Nishida, T. Kadowaki, F. Matsuzaki, K. Lida, 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. Lindquist.** 1984. Changing patterns of gene expression during sporulation in yeast. *Proc. Natl. Acad. Sci. USA* **81**:7323-7327.
25. **Larson, J. S., T. J. Schuetz, and R. E. Kingston.** 1988. Activation *in vitro* of sequence-specific DNA binding by a human regulatory factor. *Nature (London)* **335**:372-375.
26. **Lasky, R. A.** 1980. The use of intensifying screens or organic scintillators for visualizing radioactive molecules by electrophoresis. *Methods Enzymol.* **6**:363-371.
27. **Lindquist, S.** 1986. The heat-shock response. *Annu. Rev. Biochem.* **55**:1151-1191.
28. **Lindquist, S., and E. A. Craig.** 1988. The heat shock proteins. *Annu. Rev. Genet.* **22**:631-677.
29. **Lue, N. F., A. R. Buchman, and R. D. Kornberg.** 1989. Activation of yeast RNA polymerase II transcription by a thymidine-rich upstream element *in vitro*. *Proc. Natl. Acad. Sci. USA* **86**:486-490.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. *Molecular cloning: a laboratory manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**:499-560.
32. **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.
33. **McAlister, L., S. Strausberg, A. Kulaga, and D. B. Finkelstein.** 1979. Altered patterns of protein synthesis induced by heat-shock of yeast. *Curr. Genet.* **1**:63-74.
34. **Mosser, D. D., N. G. Theodorakis, and R. I. Morimoto.** 1988. Coordinate changes in heat shock element-binding activity and hsp70 gene transcription rates in human cells. *Mol. Cell. Biol.* **8**:4736-4744.
35. **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.
36. **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.
37. **Parker, C. S., and J. Topol.** 1984. A *Drosophila* RNA polymerase II transcription factor contains a promoter-region-specific DNA-binding activity. *Cell* **36**:357-369.
38. **Pelham, H.** 1985. Activation of heat shock genes in eucaryotes. *Trends Genet.* **1**:31-35.
39. **Reed, K. C., and D. A. Mann.** 1985. Rapid transfer of DNA from agarose gels to nylon membranes. *Nucleic Acids Res.* **13**: 7207-7221.
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-2 α kinase has sequence similarity with the 90-kilodalton heat shock protein. *Biochemistry* **26**:6583-6587.
41. **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.
42. **Sanchez, E. R., S. Meshinchi, W. Tienrungraj, 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.
43. **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.
44. **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.
45. **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.
46. **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.
47. **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.
48. **Struhl, K.** 1985. Naturally occurring poly(dA-dT) sequences are upstream promoter elements for constitutive transcription in yeast. *Proc. Natl. Acad. Sci. USA* **82**:8419-8423.
49. **Szent-Gyorgyi, C., D. B. Finkelstein, and W. T. Garrard.** 1987. Sharp boundaries demarcate the chromatin structure of a yeast heat shock gene. *J. Mol. Biol.* **193**:71-80.
50. **Thomas, G. H., and S. C. R. Elgin.** 1988. Protein/DNA architecture of the DNase I hypersensitive region of the *Drosophila* hsp26 promoter. *EMBO J.* **7**:2191-2201.
51. **Tuite, M. F., P. Bossier, and I. T. Fitch.** 1988. A highly conserved sequence in yeast heat shock gene promoters. *Nucleic Acids Res.* **16**:11845.
52. **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.

53. Wiederrecht, G., D. J. Shuey, W. A. Kibbe, and C. S. Parker. 1987. The *Saccharomyces* and *Drosophila* heat shock transcription factors are identical in size and DNA binding properties. *Cell* **48**:507-515.
54. Winter, E., and A. Varshavsky. 1989. A DNA binding protein that recognizes oligo(dA) · oligo(dT) tracts. *EMBO J.* **8**:1867-1877.
55. Wu, C. 1980. The 5'-ends of *Drosophila* heat shock genes in chromatin are hypersensitive to DNase I. *Nature (London)* **286**:854-860.
56. Wu, C. 1984. Two protein binding sites in chromatin implicated in the activation of heat shock genes. *Nature (London)* **309**: 229-234.
57. Wu, C., S. Wilson, B. Walker, I. Dawid, T. Paisley, V. Zimarino, and H. Ueda. 1987. Purification and properties of *Drosophila* heat shock activator protein. *Science* **238**:1247-1253.
58. Xiao, H., and J. Lis. 1988. Germline transformation used to define key features of heat shock response elements. *Science* **239**:1139-1142.
59. Zimarino, Z., and C. Wu. 1987. Induction of sequence-specific binding of *Drosophila* heat shock activator protein without protein synthesis. *Nature (London)* **327**:727-730.
60. Zoller, M. 1985. Manual for advanced cloning course. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.