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Dynamic Protein-DNA Architecture of a Yeast Heat Shock Promoter

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Here we present an in vivo footprinting analysis of the *Saccharomyces cerevisiae HSP82* promoter. Consistent with current models, we find that yeast heat shock factor (HSF) binds to strong heat shock elements (HSEs) in non-heat-shocked cells. Upon heat shock, however, additional binding of HSF becomes apparent at weak HSEs of the promoter as well. Recovery from heat shock results in a dramatic reduction in HSF binding at both strong and weak HSEs, consistent with a model in which HSF binding is subject to a negative feedback regulation by heat shock proteins. In vivo $KMnO_4$ footprinting reveals that the interaction of the TATA-binding protein (TBP) with this promoter is also modulated: heat shock slightly increases TBP binding to the promoter and this binding is reduced upon recovery from heat shock. $KMnO_4$ footprinting does not reveal a high density of polymerase at the promoter prior to heat shock, but a large open complex between the transcriptional start site and the TATA box is formed rapidly upon activation, similar to that observed in other yeast genes.

Heat shock genes are exquisitely sensitive to relatively small changes in temperature; an ~7°C increase can induce the transcription of some heat shock genes over 100-fold in a matter of minutes (27, 28). The protein-DNA architecture of *Drosophila* heat shock gene promoters has been studied in considerable detail, and they are found to associate with a number of proteins that facilitate their rapid transcriptional activation (9, 10, 32, 34, 39, 42, 43). Multiple GA repeats are found in most *Drosophila* heat shock promoters, where they are constitutively bound by the GAGA factor (8a, 11, 39). This interaction is likely to be important for preventing nucleosomal repression of the promoter (26, 40). Also associated with the uninduced promoter is the TATA-binding protein (TBP) and an RNA polymerase II elongationally paused ca. 20 to 40 bp into the gene (9, 10, 32, 33, 39, 42). Heat shock gene promoters in humans are likewise found to associate with proteins prior to activation; the *hsp70* promoter in non-heat-shocked HeLa cells appears to be bound with CTF, SP1, ATF, and TBP (2).

Transcriptional activation of heat shock genes in higher eukaryotes is triggered by the binding of heat shock factor (HSF) with heat shock elements (HSEs) of the promoter (29, 43, 44, 46). At least in *Drosophila melanogaster*, the binding of HSF to the promoter results in an increased rate of polymerase elongation from its paused configuration. Interestingly, polymerase density over the pause site is unchanged upon heat shock induction, suggesting that elongation of polymerase from the pause site is tightly coupled with the loading of polymerase onto the pause site (9, 27).

Yeast heat shock gene promoters are in some ways similar to those in *D. melanogaster* and humans; both have TATA boxes and are regulated through similar HSEs. In fact the *Drosophila hsp70* gene displays heat-induced transcription when transformed into yeast cells (4). As in *D. melanogaster*, TBP binds to the TATA box of heat shock genes in *Saccharomyces cerevisiae* both before and after induction (13, 14), but it is not clear whether a paused polymerase complex is also formed on the uninduced yeast gene. One apparent difference between *S. cerevisiae* and higher eukaryotes is that yeast HSF is believed to associate with HSEs equally in the absence and presence of heat shock; phosphorylation of the HSE-bound HSF has been suggested to be responsible for the regulation of heat shock genes in yeast cells (13, 14, 36). Indeed, the yeasts *S. cerevisiae* and *Kluyveromyces lactis* are the only eukaryotic organisms in which the DNA-binding activity of HSF is thought to be entirely unaffected by heat shock (15, 17, 36).

Here we studied the in vivo protein-DNA architecture on the yeast *HSP82* gene using both dimethyl sulfate (DMS) and $KMnO_4$. We found that although the in vivo DNA-binding activity of yeast HSF is high enough to bind HSEs prior to heat shock, temperature stress increases this DNA-binding activity. Recovery from heat shock results in the dissociation of HSF from HSEs, consistent with a feedback regulation of heat shock gene transcription (3). In vivo $KMnO_4$ footprinting reveals that heat shock increases DNA melting at the promoter; however, unlike in *D. melanogaster*, a high density of polymerase is not found at the promoter prior to heat shock. $KMnO_4$ also reveals that TBP occupancy of the TATA box changes under different growth conditions.

MATERIALS AND METHODS

DMS treatment of yeast cells. The yeast strain YPH102 was grown in yeast extract-peptone-glucose medium to an optical density of ~1.0. For heat-shocked samples, yeast cells were transferred to a flask prewarmed to 39°C for 15 min. Ten milliliters of culture was pelleted and resuspended in 150 μl fresh medium either at room temperature (~24°C) or at 39°C. This suspension was treated with 1 μl of DMS for 1 min. Fifty microliters was then withdrawn, and the reaction was terminated by dispersion in 550 μl of sorbitol stop buffer (0.9 M sorbitol, 0.1 M Tris-HCl [pH 8.0], 0.1 M EDTA, 40 mM β-mercaptoethanol). Spheroplast preparation and DNA isolation were performed as previously described (8). The sites of DMS modification were cleaved by treatment with 1 M piperidine for 30 min at 88°C. After piperidine treatment, the DNA was dried, resuspended in 50 μl of 0.3 M sodium acetate, ethanol precipitated, and washed with 70% ethanol. Usually, about one-quarter of this DNA was analyzed by ligation-mediated PCR (LMPCR; see below).

In vitro DNA binding of HSF. Full-length yeast HSF cloned in a baculovirus expression vector was produced in insect cells as previously described (7). It was found that a large fraction of the expressed HSF was in the medium. This HSF was purified by gradient elution from a heparin agarose column (eluting at ~0.5

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M NaCl in buffer A [7]) and concentrated with a Centricon-30 (Amicon) concentrator. HSF prepared in this manner was ~30% pure at a concentration of ~13 μg/ml.

End-labeled DNA containing the *HSP82* HSEs was prepared by PCR amplifying *Eco*RI-cut yeast genomic DNA with 4 pmol of primers UP0.2 and DP4 (see below), with one of the primers ^{32}P-end-labeled with T4 polynucleotide kinase (New England Biolabs). The amplified fragments were then gel purified, ethanol precipitated, and resuspended in 75 μl of H_2O.

Binding reactions were performed by adding HSF in 6 μl of buffer A to ~25 fmol of end-labeled DNA in a solution containing 24 μl of 10% glycerol, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [pH 7.9]), 0.1 mg of bovine serum albumin per ml, 0.35 mg of poly(dI-dC) per liter, 1 mM EDTA, 0.1% Nonidet P-40, and 4 mM dithiothreitol. After the protein and DNA were incubated at room temperature for 30 min, DMS treatment was performed by adding 1 μl of DMS diluted 1:15 in ethanol. After reacting for 1 min at 39°C, reactions were stopped by adding 170 μl of ice-cold Tris-EDTA with 0.23 M β-mercaptoethanol. DNA was then adjusted to 0.3 M sodium acetate, ethanol precipitated, and washed with 70% ethanol. DNA was then dissolved in 100 μl of 1 M piperidine and incubated at 88°C for 30 min. Following piperidine treatment, DNA was dried under vacuum, resuspended in 75 μl of H_2O, transferred to a new tube, and dried again (transferring to a new tube greatly reduced piperidine-induced DNA smearing on the sequencing gel). DNA was resuspended in formamide loading buffer and run on a 5% polyacrylamide–8 M urea sequencing gel.

$KMnO_4$ treatment of *S. cerevisiae*. The yeast strain YPH102 was grown in yeast extract-peptone-glucose medium to an optical density of ~1.0. For heat-shocked samples, yeast cells were transferred to a flask prewarmed to 39°C for 15 min. Five milliliters of culture was pelleted and resuspended in 50 μl of fresh medium either at room temperature (~24°C) or at 39°C. This suspension was treated with 3.5 μl of 0.35 M $KMnO_4$ for 1 min. The reaction was stopped by adding 550 μl of sorbitol stop buffer. DNA was prepared and treated like the DMS-treated samples described above. G and T ladders were prepared as previously described (9, 22).

LMPCR and PCR primers. LMPCR was performed as previously described (8, 9). Primers to view DMS reactivity over the HSEs were as follows: the bottom strands were UP0.1 (GAACAGGAATAAAGCTTAATCGGAT) and UP0.2 (GGATTATTAACTCATACGCTTGTCAC), and the top strands were DP6 (GGTTGGTATTAAGATGAGAATTAACC) and DP7 (TAACCGCTCATA AAACCATGCGCGT). $KMnO_4$ modification of the promoter was observed with the following primers: the bottom strands were UP1 (TCTCATCTTAAT ACCAACCAGGTCC) and UP2 (GGTCCTTCCGCCACCCCCTAAAAC), and the top strands were DP1 (CCAATTGCTTTGGATCAGACAAAG) and DP2 (CAAAGATTTGTATCTAATTTTATCCAACGC). To view the bottom strand with primers DP1 and DP2, genomic DNA was cut with *Hin*dIII before the piperidine treatment. *Hin*dIII cut the closely related *HSC82* gene, thereby allowing amplification of the *HSP82* gene only. DP4, which was used to prepare the end-labeled DNA for the in vitro DNA-binding reactions, was ACAGCGG-GAAGAAATGAGGAGGTCA.

RESULTS

DMS footprinting of the *HSP82* HSEs. HSF DNA binding in *S. cerevisiae* and *K. lactis* is believed to be strictly constitutive (15, 17, 36). This conclusion has been drawn by measuring the in vitro DNA-binding activity of HSF isolated from heat-shocked and non-heat-shocked cells, in vivo footprinting, and in vivo HSE interference with *GAL4* binding (13, 14, 17, 36). However, since yeast HSF is the only HSF that appears to have a DNA-binding activity entirely unresponsive to heat shock, we decided to perform in vivo DMS footprinting on the *HSP82* promoter, paying close attention to any subtle changes in the level of HSF binding.

Figure 1 shows the HSE occupancy in yeast cells treated with DMS at room temperature (lanes NHS) and during heat shock (lanes HS). Also shown is the DMS modification pattern on purified DNA (lanes DNA). The *HSP82* promoter has three consensus, or near consensus, 5-bp AGAAN, HSF-binding repeats in HSE1 (Fig. 1) (7, 46). These repeats are predicted to bind HSF in two mutually exclusive configurations, which optimally associate with three contiguous 5-bp repeats in altering orientations but can recognize two appropriately positioned 5-bp units with lower affinity (30, 48). DMS protection is observed at G residues in HSE1 in non-heat-shocked cells, with more protection observed at positions −161, −162, and −171 than at −174. It therefore appears that in vivo, under non-heat

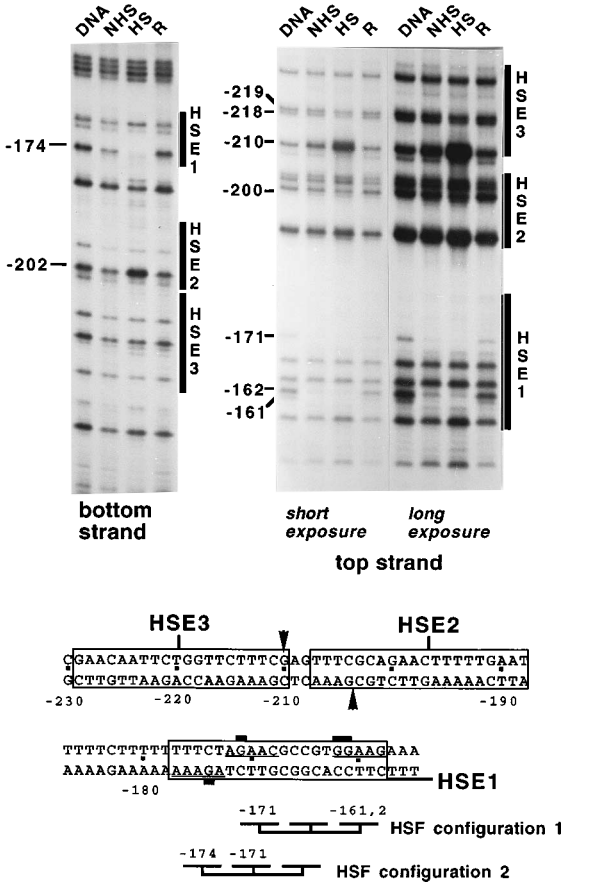

FIG. 1. In vivo DMS footprinting of the *HSP82* HSEs in non-heat-shocked and heat-shocked cells and heat-shocked cells allowed to recover. Cells were treated with DMS at room temperature (lanes NHS), at 39°C after a 15-min incubation at 39°C (lanes HS), and at room temperature after a 15-min incubation at 39°C and a 15-min room temperature recovery (lanes R). The DMS reactivity of purified genomic DNA is also shown (lanes DNA). DMS modification was detected by LMPCR with probes to detect either the top or bottom DNA strand. Two exposures of the top strand are shown to display clearly both the protection over HSE1 and the hyperreactivity at +210. The sequence of this region of the *HSP82* promoter is shown below the gels, with protected Gs indicated by bars and the hyperreactive Gs indicated by arrowheads. The two possible configurations with which the HSF trimer can strongly interact with HSE1 are shown. Configuration 1, with sites −161, −162, and −171 protected, appears to be preferred in non-heat-shocked cells. The numbering is relative to the transcriptional start site (6).

shock conditions, HSF prefers the 5-bp units that include residues −161, −162, and −171 (Fig. 1, configuration 1) to the 5-bp units that include sites −171 and −174 (Fig. 1, configuration 2). Upon heat shock, an increase in protection is observed at all G residues in HSE1 but is most dramatic at −174 on the bottom strand. The increased binding to −174 probably reflects the recruitment of an additional HSF trimer to HSE1, presumably through an interaction with a fourth, weaker 5-bp unit close by (presumably upstream of −174).

In addition to the small increase in HSF binding to HSE1, heat shock also induces a number of other changes in the DMS modification pattern (Fig. 1). The most striking heat-shock-induced change in reactivity occurs at the −210, top-strand G residue in HSE3, which becomes extremely hyperreactive. A slight increase in reactivity is also observed on the bottom strand at −202, and a modest protection appears on the top strand at −200 in HSE2 (although lane HS is slightly overloaded, protection at −200 becomes apparent when one com-

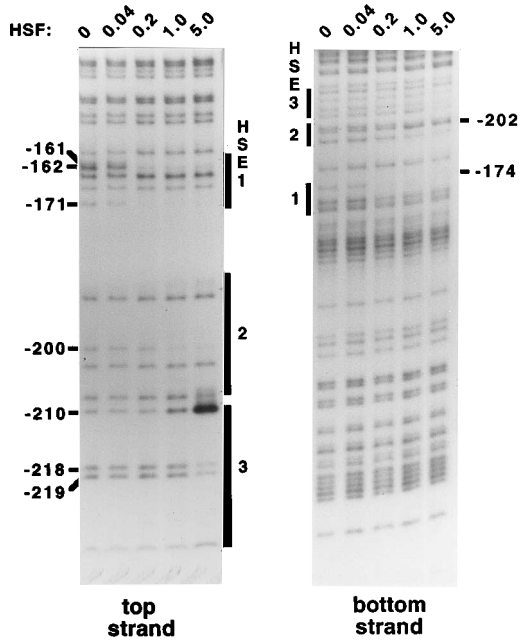

FIG. 2. Yeast HSF binding to *HSP82* HSEs displayed by in vitro DMS footprinting. Increasing concentrations (0-, 0.04-, 0.2-, 1-, and 5-μl equivalents) of purified, recombinant yeast HSF was included in a binding reaction with a portion of the *HSP82* promoter end labeled on either the bottom strand or top strand as indicated. After a 30-min preincubation with HSF, reaction mixtures were treated with DMS for 1 min. After sites of modification were cleaved with piperidine, products were displayed on 5% sequencing gels.

pares the ratio of reactivity at the −200 G with the next G residue upstream in lanes NHS and HS of Fig. 1). All these changes are observed in the in vitro DMS footprint with purified yeast HSF (see below and Fig. 2).

Because HSE2 and HSE3 are poor matches to the HSE consensus, we tested whether HSF could bind to these sites in vitro. Also, if a heat shock-induced increase in HSF activity is required for the binding of HSF to HSE2 and HSE3 in vivo, HSE1 would be expected to bind HSF at low concentrations, while HSE2 and HSE3 would bind only at high concentrations. For Fig. 2, recombinant yeast HSF was titrated onto the *HSP82* promoter with the binding assayed by DMS modification. At lower concentrations, HSF binds to HSE1, giving a protection pattern similar to that observed in vivo. Interestingly, the two potential HSF binding sites in HSE1 are bound with roughly equal affinities in vitro, indicating that the in vivo preference for sites −161, −162, and −171 may reflect binding constraints imposed on HSF by other promoter-bound proteins. At higher HSF concentrations, a DMS footprint over HSE2 and HSE3 is also observed; most notably, the hyperreactive sites at −210 (top strand) and −202 (bottom strand) and the protection at −200 (top strand) found in vivo appear. The changes at these positions in vivo can therefore be ascribed to an increase in the nuclear concentration of DNA-binding HSF triggered by heat shock. The protection at −200 is less apparent in vivo than it is in vitro. One possible explanation for this observation is that some cells in the culture may be inducing high levels of HSF binding to the upstream HSEs, while others may not. DMS modification of −200 in cells not responding as vigorously to the heat shock will obscure protection at this residue in cells that are binding HSF to this site. The hyperreactive sites formed in the cells responding vigorously to the heat shock, however, should still be apparent. We estimate that the increase in HSF DNA binding observed in vivo upon heat shock requires the concentration of DNA-binding HSF to increase ca. 10- to 25-fold.

Heat shock gene transcription is believed to be subject to feedback inhibition; reduction in HSP70 levels by deletion of the *SSA1* and *SSA2* genes results in constitutive heat shock gene expression in *S. cerevisiae* (3). Evidence implicating heat shock proteins in the regulation of HSF's DNA-binding activity, particularly in cells recovering from heat shock, has also been obtained (1, 31). We therefore determined if the level of HSF activity was reduced after a recovery from heat shock, when heat shock protein levels would be high. *S. cerevisiae* was heat shocked for 15 min and then allowed to recover at room temperature for 15 min. As shown in Fig. 1 (lanes R), recovery from heat shock results in an almost complete loss of HSF binding to all promoter HSEs. This is further evidence that the DNA-binding activity of HSF can be regulated in yeast cells, with this regulation being particularly important in cells recovering from heat shock.

Given that the level of DNA-binding activity of yeast HSF is lowest in recovered cells, we determined whether this lower activity was preserved in cellular extracts. If this lower activity is preserved in the extract, it would allow us to correlate HSF's multimeric state with its DNA-binding activity. However, the DNA binding activity was found to be the same in crude extracts prepared from non-heat-shocked, heat-shocked, and recovered cells (8a [data obtained with extracts prepared as described in reference 37]). Possibly, cellular lysis activates the DNA-binding activity of yeast HSF, making it difficult to determine in extracts how its multimeric state correlates with its DNA binding activity. Other alternatives are discussed below.

$KMnO_4$ reactivity in the yeast *HSP82* promoter. In order to determine if the yeast *HSP82* promoter supported an elongationally paused RNA polymerase II at its 5′ end like a *Drosophila* heat shock gene, the reactivity of the promoter to the single-strand, T-specific DNA-modifying reagent $KMnO_4$ was tested. $KMnO_4$ can detect DNA melting at the "transcription bubble" associated with an elongating polymerase that is paused (9, 19). Also, this experiment was of interest since it should reveal whether an enlarged open complex was formed on this gene like that found on the induced *GAL1* and *GAL10* genes (8).

Figure 3A shows the $KMnO_4$ reactivity pattern on the top and bottom strands in cells treated with $KMnO_4$ during heat shock (lanes HS), at room temperature (lanes NHS), or with purified DNA (lanes DNA). The most striking region of $KMnO_4$ hyperreactivity on the *HSP82* promoter occurs between the TATA box and the transcriptional start site upon heat shock induction (compare lanes NHS and HS in Fig. 3A). This region of hyperreactivity initiates 55 bp upstream of the transcription start site (~20 bp downstream of the TATA box), producing a region of transcription-induced melting similar to that found on the induced *GAL1* and *GAL10* promoters (8). What appears to be an extended polymerase open complex therefore forms on this heat shock promoter as it does on the *GAL* promoters examined previously. The differences in reactivity in this region observed between purified DNA and the DNA in non-heat-shocked cells (Fig. 3A, lanes DNA and NHS, respectively) occurs primarily at G residues and is discussed below.

Looking further into the gene, past the transcriptional start site, $KMnO_4$ hyperreactivity is observed on the bottom strand between ~30 and 60 bp downstream of the transcriptional start site in uninduced cells (Fig. 3A); the top strand is devoid of T residues in this region and is therefore uninformative (6). These hyperreactive sites are located where an elongationally

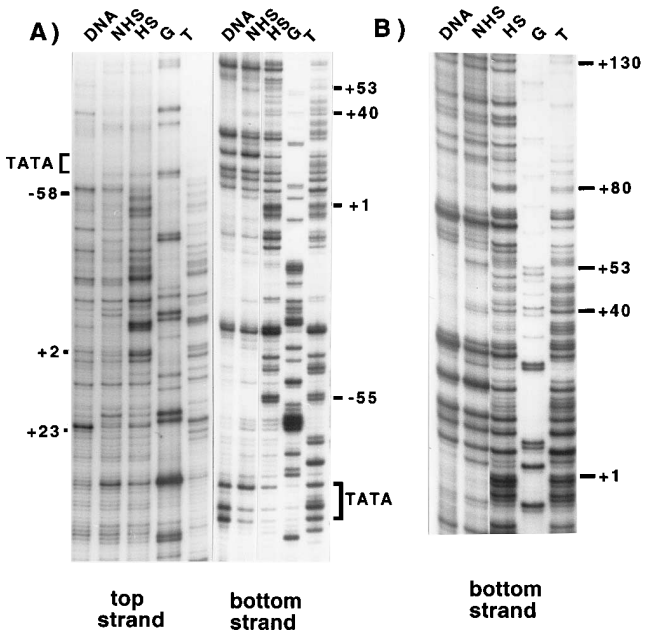

FIG. 3. (A) $KMnO_4$ reactivity of the *HSP82* promoter under heat shock or non-heat shock conditions. Cells were treated with $KMnO_4$ either at room temperature (lanes NHS) or at 39°C after a 15-min preincubation at 39°C (lanes HS). Genomic DNA was purified from mock-treated cells and subsequently treated with $KMnO_4$ in Tris-EDTA (lanes DNA). DNA modification was detected by LMPCR with probes to detect either the top or bottom DNA strand. (B) Longer electrophoretic run of the bottom strand to clearly show the +1 through +130 region. The G and T lanes are G and T sequence ladders, respectively.

paused polymerase would be expected to form on a *Drosophila* heat shock gene promoter (9, 32). However, comparison of the $KMnO_4$ hyperreactive sites with sequence ladders shows that only one of the three hyperreactive bands in the +30 to +60 region corresponds to a T residue; hyperreactivity at +53 and +39 localizes to G residues, whereas only one hyperreactive T is found at +40 (Fig. 3B). $KMnO_4$ does not react with guanosine efficiently (35), although others have detected such a reaction (5, 12). We suggest that the modification of these Gs is a secondary reaction that occurs when the $KMnO_4$ treatment is applied to yeast cells. This effect is not restricted to Gs at +39 to +53 but is observed at many locations along the gene even before induction (8a) and may not be a reliable indication of DNA melting. T hyperreactivity in the +30 to +60 region is therefore weak and restricted to the T at +40, even though this region is T rich. This is unlike the strong regions of T hyperreactivity on *Drosophila* heat shock promoters which are ~30 bp in length. It is therefore unlikely that the uninduced *HSP82* gene supports a high level of elongationally paused polymerase at a promoter-proximal position as the *Drosophila* heat shock promoters do, although it cannot be ruled out that the hyperreactive T at +40 is the result of fractional polymerase occupancy of the uninduced promoter.

Although the $KMnO_4$ hyperreactivity downstream of the transcriptional start site is weak prior to heat shock, hyperreactivity does appear in the +1 through +80 region of the gene after induction (Fig. 3B). This hyperreactivity occurs at T residues and could indicate that a *Drosophila*-like polymerase pause occurs in this region of the gene after induction. On *Drosophila* heat shock genes, a high density of polymerase persists over the pause site even after maximal induction (9).

In addition to detecting DNA melting, $KMnO_4$ can also detect TBP binding to the TATA box (9). T residues in double-stranded DNA can vary in their reactivity to $KMnO_4$, probably as a result of local distortions in the double helix. T residues in the TATA box are often quite reactive to $KMnO_4$, and the binding of TBP can substantially protect these residues. It is interesting that this protection is probably not due to a direct protection of the DNA from the solvent by TBP since TBP binds exclusively to the minor groove and $KMnO_4$ attacks T residues on the major groove side (22, 35, 38). Instead, the extreme compaction of the major groove by TBP probably results in the lower reactivity with $KMnO_4$ (20, 21). The T residues on the bottom strand of the *HSP82* TATA box are quite pronounced in purified, double-stranded DNA (Fig. 3A). $KMnO_4$ treatment of uninduced cells show some protection of the TATA box, indicating binding of TBP to the TATA box under non-heat shock conditions. This is similar to what has been found in the chromatin of lysed spheroplasts treated with DNase I (13, 14). We can, however, often detect a more complete protection of the TATA box in heat-shocked cells than in non-heat-shocked cells, suggesting that TBP binding is increased upon heat shock (Fig. 3A). The fraction of TATA boxes occupied by TBP was estimated by scanning densitometry and was found to increase from ~80 to ~100% upon heat shock. This small increase in TBP binding cannot account for the 15-fold increase in transcription effected by heat shock; the acceleration of a subsequent step in the transcription pathway must be largely responsible for *HSP82* activation (13, 14, 24).

Kinetics of *HSP82* activation and recovery. Since $KMnO_4$ treatment can be performed in 1 min, this reagent should be useful for following the kinetics with which different parts of the promoter become melted. In particular, we were interested in determining if the melting upstream of the transcriptional start site occurred before the melting in the transcribed region of the gene. Demonstrating that the melting upstream of the start site occurred first would lend support to the model that this melting was the result of a polymerase open complex and not due to DNA melting driven by negative supercoils formed behind elongating polymerases. Previous evidence for this model comes from the finding that, unlike regions of supercoil-driven melting, this region of in vivo melting is stable at lower temperatures (8).

Cells were grown at room temperature, spun down, and resuspended in medium prewarmed to 39°C. A 1-min $KMnO_4$ treatment was then initiated at 30 s or 2, 4, 8, or 12 min after transfer to the heat shock temperature. As shown in Fig. 4, DNA melting between the start site and the TATA box can be seen as early as 30 s after heat shock and reaches a maximal level after 2 min (note the labeled bands). In contrast, melting past the start site requires 8 min to reach its maximum (note the bands in the +15 region). The 5′-to-3′ movement of promoter melting is therefore consistent with a polymerase first associating with and melting DNA upstream of the start site and then moving into the body of the gene. The kinetics of promoter melting are similar to the kinetics of heat shock gene activation in *D. melanogaster*, as determined by UV cross-linking and nuclear transcriptional run-on assays, and is consistent with the rate of *HSP82* mRNA accumulation in *S. cerevisiae* (23, 28).

Figure 4 also shows the effect of heat shock recovery on $KMnO_4$ hyperreactivity. In this experiment, cells were heat shocked for 15 min, spun down, resuspended in room temperature medium, and treated with $KMnO_4$ after 30 s and 2, 4, 8, and 12 min of recovery. $KMnO_4$ reactivity indicates that the promoter melting decreases rapidly after the return to room temperature, with the hyperreactivity noticeably decreasing at the 30-s time point and no longer detectable after 8 min.

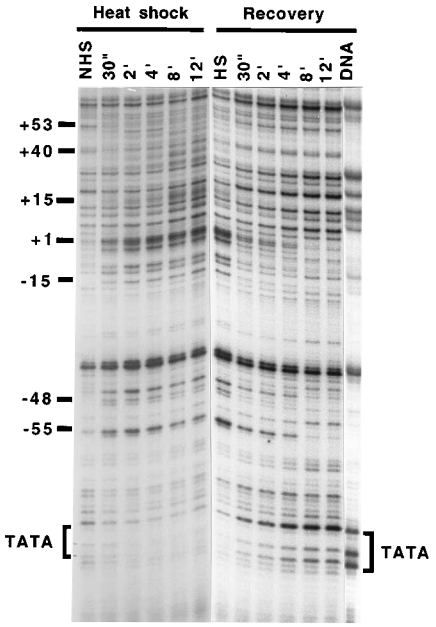

FIG. 4. Kinetic analysis of $KMnO_4$ reactivity changes accompanying *HSP82* activation and recovery. For the activation kinetics, cells grown at room temperature were pelleted and resuspended in either room temperature medium or medium prewarmed to 39°C. The cells resuspended in the room temperature medium were treated with $KMnO_4$ for 1 min immediately after resuspension (lane NHS). Cells resuspended at 39°C were treated for 1 min with $KMnO_4$, with this treatment initiated 0.5, 1, 2, 4, 8, or 12 min after resuspension at 39°C. For recovery kinetics, cells preincubated at 39°C for 15 min were pelleted and resuspended in either room temperature medium or medium prewarmed to 39°C. The cells resuspended in the 39°C medium were treated with $KMnO_4$ for 1 min immediately after resuspension (lane HS). Cells resuspended in the room temperature medium were treated for 1 min with $KMnO_4$, with this treatment initiated 0.5, 1, 2, 4, 8, or 12 min after resuspension at room temperature. Lane DNA, purified genomic DNA treated with $KMnO_4$.

Unlike the temporally ordered melting found upon activation, melting both upstream and downstream of the transcriptional start site fades concurrently in recovering cells.

Examination of the TATA box reactivity in recovered cells reveals a large decrease in protection, suggesting that inactivation is accompanied by TBP dissociation. Comparing results from the 12-min recovery with results from the purified-DNA lane shows that the TATA box appears largely unoccupied in recovered cells. Since only a small increase in TBP binding occurs upon heat shock, inactivation of the *HSP82* gene in cells recovering from heat shock does not appear to be a simple reversal of events that occur upon heat shock. Instead, a distinct, inactive form of the promoter that is largely devoid of both TBP and HSF is found in recovered cells.

DISCUSSION

In this study the in vivo protein-DNA architecture of the yeast *HSP82* gene was analyzed at high resolution. We were particularly interested in determining how three key participants in *HSP82* transcription, HSF, RNA polymerase II, and TBP, interacted with the promoter. All three of these proteins were found to interact with the *HSP82* promoter in a dynamic fashion. The findings of these studies are summarized schematically in Fig. 5.

Dynamic interactions of HSF with the *HSP82* promoter. We find that although HSF does associate with promoters before heat shock, heat shock leads to a notable increase in the binding of HSF to the *HSP82* HSEs. This increased binding is observed to a small extent on stronger HSEs and, to a much larger extent, weaker HSEs of this promoter. By comparing the extent of binding in vivo with that found in in vitro footprinting reactions, we estimate that the HSF DNA-binding activity increases ca. 10- to 25-fold upon heat shock in *S. cerevisiae*. This increase is smaller than the ~100-fold increases estimated to occur in higher eukaryotes. The high level of DNA-binding activity of HSF prior to heat shock probably made the heat-shock-induced increase in HSF binding difficult to detect in previous in vivo and in vitro studies (13, 14, 36). We also found that cells recently recovered from heat shock have a substantially lower level of DNA-binding HSF than non-heat-shocked cells. Our results therefore demonstrate that the DNA-binding activity of HSF is regulated in *S. cerevisiae*.

The activation of HSF DNA-binding activity in higher eukaryotes depends on the trimerization of monomeric HSF subunits upon heat shock (41). This trimerization depends upon the interaction of leucine zippers between three HSF monomers. We suspect that a similar trimerization of monomers is responsible for the modulation of HSF DNA-binding activity in *S. cerevisiae*. In the case of *S. cerevisiae*, however, we envision a relatively high concentration of trimer before heat shock. We cannot, however, rule out other mechanisms by which the DNA-binding activity might be increased in vivo. For example, heat shock may localize more HSF to the nucleus or alter chromatin structure to modulate DNA binding. An alternative that we have investigated is one in which heat shock induces HSF to bind DNA cooperatively. We have not, however, been able to detect any changes in the cooperative binding potential of HSF in crude extracts prepared from cells at different growth conditions; in all cases yeast HSF appears to bind HSEs independently (8a). Additional evidence that the activation of

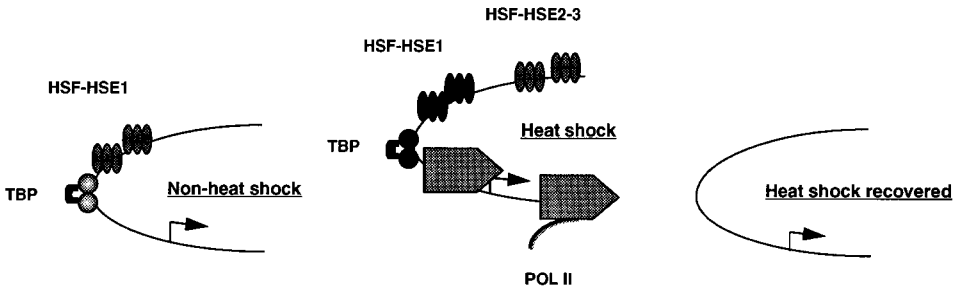

FIG. 5. Summary of changes on the *HSP82* promoter before, during, and after recovery from heat shock. Fractional occupation of the TATA box and HSE1 is indicated by shaded symbols over these sequence elements; the increased protection observed upon heat shock is represented by solid black symbols over these elements. After recovery most of the HSF and TBP have dissociated from the promoter. The wide arrowheads designate RNA polymerase II (POL II) in two distinct forms: open complexes upstream of the start site (arrow) and paused elongation complexes downstream of the start site.

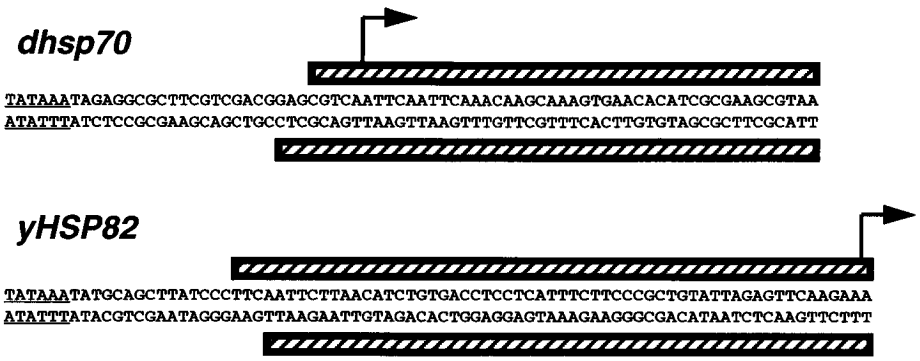

FIG. 6. Comparison of $KMnO_4$ hyperreactive regions on the *Drosophila hsp70* promoter (*dhsp70*) and the yeast *HSP82* promoter (*yHSP82*) after heat shock induction. The TATA boxes of these promoters (underlined) are aligned. The regions of $KMnO_4$ hyperreactivity are indicated by the hatched bars, and the major transcriptional start sites are indicated by the arrows.

yeast HSF DNA binding occurs through a mechanism like that in higher eukaryotes comes from experiments with the anti-inflammatory drug sodium salicylate. We find that, as in human cells, sodium salicylate stimulates the DNA-binding activity of yeast HSF (8a, 18).

The mechanism by which the trimerization of HSF is regulated is not entirely clear. Heat shock proteins themselves are thought to play a role in the HSF monomer-to-trimer transition in higher eukaryotes, and considerable attention has been paid to HSP70's potential role in this process. In human cell extracts, HSP70 can prevent the in vitro activation of HSF (1). However, this protein cannot be the sole negative regulator since it cannot by itself monomerize trimers. It has recently been reported that increased levels of heat shock proteins in *Drosophila* and rat cells do not affect the activation of HSF DNA binding but rather function to inactivate HSF binding in cells recovering from heat shock (31). Our finding that the DNA-binding activity of yeast HSF is lowest in recovered cells is consistent with the heat shock proteins being pivotal in the inactivation of HSF in yeast cells as well.

HSF trimerization to a DNA-binding form is not by itself sufficient for heat shock gene activation; a second modification step, possibly HSF phosphorylation, is also required (18, 36). It is therefore conceivable that heat shock gene transcription in *S. cerevisiae* could be differentially regulated through the modulation of both the HSF DNA-binding activity and its modification state. Promoters with weaker HSEs would be sensitive to both the concentration of DNA-binding HSF and its modification state, whereas promoters with strong HSEs would be limited primarily by HSF's modification state.

Dynamic interactions of polymerase with the *HSP82* promoter. An important feature of *Drosophila* heat shock promoters is the elongationally paused polymerase that forms ca. 20 to 50 bp downstream of the transcriptional start site (9, 10, 32, 33). The DNA melting associated with this elongationally paused polymerase is clearly revealed by $KMnO_4$ hyperreactivity in this region of the promoter (9). Using $KMnO_4$, we examined the *HSP82* promoter for evidence of an elongationally paused RNA polymerase II complex forming under non-heat shock conditions. This probing did not reveal a region of strong $KMnO_4$ hyperreactive T residues like that found on a *Drosophila* heat shock gene promoter. A single T was, however, found to be weakly hyperreactive at +40 on the noninduced *HSP82* promoter. It is possible that polymerase pausing on the *HSP82* gene is transient, generating, on average, less than one paused polymerase per gene. Alternatively, pausing may be occurring over a wide range of sites, reducing the hyperreactivity detected by $KMnO_4$. Conclusively demonstrating a lack of polymerase pausing on the uninduced *HSP82* gene will require further study.

An interesting feature of the polymerase pausing on *Drosophila* heat shock genes is that polymerase density at the pause site remains high after heat shock (9, 27). This finding suggests that a slow step in early elongation persists after gene activation. Although $KMnO_4$ hyperreactivity downstream of the yeast *HSP82* transcriptional start site is not intense in non-heat-shocked cells, activation results in numerous T residues in the +1 through +80 region becoming hyperreactive. This melting may be indicative of elongational pausing occurring after heat shock activation of the yeast *HSP82* gene, much like that seen on an induced *Drosophila* heat shock gene (9, 27). It is therefore possible that elongational pausing is an important feature of the yeast *HSP82* promoter but that prior to heat shock activation, transcriptional initiation is slow relative to the elongation from the pause site. After heat shock, initiation rates might increase in *S. cerevisiae*, resulting in the accumulation of elongationally paused polymerases on the *HSP82* gene.

An alternative view of how the melting on the yeast *HSP82* promoter might relate to the melting on *Drosophila* heat shock promoters is seen when the TATA boxes of the yeast *HSP82* promoter and the *Drosophila hsp70* promoter are aligned (Fig. 6). This perspective reveals that the region of promoter melting found upstream of the transcriptional start site of the yeast *HSP82* gene colocalizes with the melting found largely downstream of the start site on the activated *Drosophila hsp70* gene. One interpretation, therefore, is that on both promoters, polymerase has a long dwell time at this position after activation, regardless of whether it has initiated transcription. In the case of elongational pause on the *Drosophila hsp70* gene, we have speculated that polymerase is restrained at the promoter by contacts maintained with basal transcription factors assembled at the TATA box (45, 47). A similar restraint may be responsible for the high density of polymerase found close to the TATA box on the yeast *HSP82* promoter as well.

Although Fig. 6 shows only the promoter-proximal melting found on the *Drosophila hsp70* gene, a similar region of melting is found on other heat shock and non-heat shock genes in *D. melanogaster* as well. Polymerase therefore passes through a slow, possibly rate-limiting step early in elongation on many genes in *D. melanogaster*. Likewise, a similar region of melting is formed on the non-heat-inducible yeast *GAL1* and *GAL10* genes as well. It is therefore possible that a long polymerase

dwell time at the promoter is a common rate-limiting step in transcription in *S. cerevisiae* as it is in *D. melanogaster*.

Dynamic interactions of TBP with the *HSP82* promoter. Mutational analysis and genomic footprinting studies performed by Gross and colleagues have demonstrated that one role for promoter-bound HSF is to stabilize the interaction of TBP with the promoter (13). It was suggested that HSF may act in part as an antirepressor, preventing nucleosome binding to the TATA box (13). In *S. cerevisiae*, HSF might play a role similar to that of the GAGA factor, which associates with *Drosophila* heat shock promoters and has been found to prevent nucleosome binding to these promoters in vitro and in vivo (26, 40). A direct interaction between TBP and HSF might also help to stabilize TBP binding to the promoter, a mechanism by which other transcriptional activators are believed to function (16). Our results support a role for HSF in stabilizing the binding of TBP with the TATA box. We find that the DNA binding of HSF correlates with that of TBP; both are increased slightly upon heat shock and both largely dissociate upon recovery.

Upon heat shock, a ~20% increase in TBP association with the promoter is observed. This small increase in TBP binding cannot account for the observed 15-fold increase in heat-shock-activated transcription of the *HSP82* gene (13, 14, 23, 24). The stimulation of a subsequent step in the transcription pathway, perhaps the recruitment of TFIIB or the polymerase, must therefore be responsible for the bulk of the activation observed upon heat shock (25). However, upon recovery from heat shock, a large decrease in TBP binding is observed. This loss of TBP binding might be responsible for the reduction of transcription in recovered cells. HSF might therefore be serving two distinct functions on the *HSP82* promoter: facilitating the binding of TBP to the promoter and, after heat shock, accelerating a subsequent step in the transcription pathway.

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REFERENCES

1. **Abravaya, K., M. P. Meyers, S. P. Murphy, and R. I. Morimoto.** 1992. The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. Genes Dev. **6:**1153–1164.
2. **Abravaya, K., B. Phillips, and R. I. Morimoto.** 1991. Heat shock-induced interactions of heat shock transcription factor and the human hsp70 promoter examined by in vivo footprinting. Mol. Cell. Biol. **11:**586–592.
3. **Boorstein, W. R., and E. A. Craig.** 1990. Transcriptional regulation of *SSA3*, an HSP70 gene from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**3262–3267.
4. **deBanzie, J. S., L. Sinclair, and J. T. Lis.** 1986. Expression of the major heat shock gene of *Drosophila melanogaster* in *Saccharomyces cerevisiae*. Nucleic Acids Res. **14:**3587–3601.
5. **Duncan, R., L. Bazar, G. Michelotti, T. Tomonga, H. Krutzsch, M. Avigan, and D. Levens.** 1994. A sequence-specific, single strand DNA binding protein activates the far upstream element of *c-myc* and defines a new DNA-binding motif. Genes Dev. **8:**465–480.
6. **Farrelly, F. W., and D. B. Finkelstein.** 1984. Complete sequence of the heat shock-inducible *HSP90* gene of Saccharomyces cerevisiae. J. Biol. Chem. **359:**5745–5751.
7. **Fernandes, M., H. Xiao, and J. T. Lis.** 1994. Fine structure analyses of the *Drosophila* and *Saccharomyces* heat shock factor-heat shock element interactions. Nucleic Acids Res. **22:**167–173.
8. **Giardina, C., and J. T. Lis.** 1993. DNA melting on yeast RNA polymerase II promoters. Science **261:**759–762.

8a. **Giardina, C., and J. T. Lis.** Unpublished data.

9. **Giardina, C., M. Perez-Riba, and J. T. Lis.** 1992. Promoter melting and TFIID complexes on *Drosophila* genes in vivo. Genes Dev. **6:**2190–2200.
10. **Gilmour, D. S., and J. T. Lis.** 1986. RNA polymerase II interacts with the promoter region of the noninduced *hsp70* gene in *Drosophila melanogaster* cells. Mol. Cell. Biol. **6:**3984–3989.
11. **Gilmour, D. S., G. H. Thomas, and S. C. R. Elgin.** 1989. *Drosophila* nuclear proteins bind to regions of alternating C and T residues in gene promoters. Science **245:**1487–1490.
12. **Gilson, E., M. Roberge, R. Giraldo, D. Rhodes, and S. Gasser.** 1993. Distortion of the DNA double helix by RAP1 at silencers and multiple telomeric DNA binding sites. J. Mol. Biol. **231:**293–310.
13. **Gross, D. S., C. C. Adams, S. Lee, and B. Stentz.** 1993. A critical role for heat shock transcription factor in establishing a nucleosome-free region over the TATA-initiation site of the yeast *HSP82* heat shock gene. EMBO J. **12:**3931–3945.
14. **Gross, D. S., K. E. English, K. W. Collins, and S. Lee.** 1990. Genomic footprinting of the yeast *HSP82* promoter reveals marked distortion of the DNA helix and constitutive occupancy of heat shock and TATA elements. J. Mol. Biol. **216:**611–631.
15. **Hoj, A., and B. K. Jakobsen.** 1994. A short element required for turning off heat shock transcription factor: evidence that phosphorylation enhances deactivation. EMBO J. **13:**2617–2624.
16. **Ingles, C. J., M. Shales, W. D. Cress, S. J. Triezenberg, and J. Greenblatt.** 1991. Reduced binding of TFIID to transcriptionally compromised mutants of VP16. Nature (London) **351:**588–590.
17. **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.
18. **Jurivich, D. A., L. Sistonen, R. A. Kroes, and R. I. Morimoto.** 1992. Effect of sodium salicylate on the human heat shock response. Science **255:**1243–1245.
19. **Kainz, M., and J. Roberts.** 1992. Structure of transcription elongation complexes in vivo. Science **255:**838–841.
20. **Kim, J. L., D. B. Nikolov, and S. K. Burley.** 1993. Co-crystal structure of TBP recognizing the minor groove of a TATA element. Nature (London) **365:**520–527.
21. **Kim, Y., J. H. Geiger, S. Hahn, and P. B. Sigler.** 1993. Crystal structure of a yeast TBP/TATA-box complex. Nature (London) **365:**512–520.
22. **Lee, D. K., M. Horikoshi, and R. G. Roeder.** 1991. Interaction of TFIID in the minor groove of the TATA element. Cell **67:**1241–1250.
23. **Lee, M.-S., and W. T. Garrard.** 1991. Transcription-induced nucleosome 'splitting': an underlying structure for DNase I sensitive chromatin. EMBO J. **10:**607–615.
24. **Lee, M.-S., and W. T. Garrard.** 1992. Uncoupling gene activity from chromatin structure: promoter mutations can inactivate transcription of the yeast *HSP82* gene without eliminating nucleosome-free regions. Proc. Natl. Acad. Sci. USA **89:**9166–9170.
25. **Lin, Y. S., and M. R. Green.** 1991. Mechanism of action of an acidic transcriptional activator in vitro. Cell **64:**971–982.
26. **Lu, Q., L. L. Wallrath, B. D. Allan, R. L. Glaser, J. T. Lis, and S. C. R. Elgin.** 1992. Promoter sequence containing $(CT)_n \cdot (GA)_n$ repeats is critical for the formation of the DNase I hypersensitive sites in the *Drosophila hsp26* gene. J. Mol. Biol. **225:**985–998.
27. **O'Brien, T., and J. T. Lis.** 1991. RNA polymerase II pauses at the 5′ end of the transcriptionally induced *Drosophila hsp70* gene. Mol. Cell. Biol. **11:**5285–5290.
28. **O'Brien, T., and J. T. Lis.** 1993. Rapid changes in *Drosophila* transcription after an instantaneous heat shock. Mol. Cell. Biol. **13:**3456–3463.
29. **Pelham, H. R. B.** 1982. A regulatory upstream promoter element in the *Drosophila hsp70* heat-shock gene. Cell **30:**517–528.
30. **Perisic, O., H. Xiao, and J. T. Lis.** 1989. Stable binding of *Drosophila* heat shock factor to head-to-head or tail-to-tail repeats of a 5 bp recognition unit. Cell **59:**797–806.
31. **Rabindran, S. K., J. Wisniewski, L. Li, G. C. Li, and C. Wu.** 1994. Interaction between heat shock factor and hsp70 is insufficient to suppress induction of DNA-binding activity in vivo. Mol. Cell. Biol. **14:**6552–6560.
32. **Rasmussen, E. B., and J. T. Lis.** 1993. In vivo transcriptional pausing and cap formation on three *Drosophila* heat shock genes. Proc. Natl. Acad. Sci. USA **90:**7923–7927.
33. **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.
34. **Rougvie, A. E., and J. T. Lis.** 1990. Postinitiation transcriptional control in *Drosophila melanogaster*. Mol. Cell. Biol. **10:**6041–6045.
35. **Rubin, C. M., and C. W. Schmid.** 1980. Pyrimidine-specific chemical reactions useful for DNA sequencing. Nucleic Acids Res. **8:**4613–4619.
36. **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.
37. **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.
38. **Starr, D. B., and D. K. Hawley.** 1991. TFIID binds in the minor groove of the TATA box. Cell **67:**1231–1240.
39. **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.

40. **Tsukiyama, T., P. B. Becker, and C. Wu.** 1994. ATP-dependent nucleosome disruption at a heat shock promoter mediated by binding of GAGA transcription factor. Nature (London) **367:**525–532.
41. **Westwood, J. T., J. Clos, and C. Wu.** 1991. Stress induced oligomerization and chromosomal relocalization of heat-shock factor. Nature (London) **353:** 822–827.
42. **Wu, C.** 1984. Two protein-binding sites in chromatin implicated in the activation of heat-shock genes. Nature (London) **309:**229–234.
43. **Wu, C.** 1985. An exonuclease protection assay reveals heat-shock element and TATA box DNA-binding proteins in crude nuclear extracts. Nature (London) **317:**82–87.
44. **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.
45. **Xiao, H., J. D. Friesen, and J. T. Lis.** 1994. A highly conserved domain of RNA polymerase II shares a functional element with acidic activation domains of upstream transcription factors. Mol. Cell. Biol. **14:**7507–7516.
46. **Xiao, H., and J. T. Lis.** 1988. Germline transformation used to define key features of heat-shock response elements. Science **239:**1139–1142.
47. **Xiao, H., J. T. Lis, H. Xiao, J. Greenblatt, and J. D. Friesen.** 1994. The upstream activator CTF/NF1 and RNA polymerase II share a common element involved in transcriptional activation. Nucleic Acids Res. **22:**1966–1973.
48. **Xiao, H., O. Perisic, and J. T. Lis.** 1991. Cooperative binding of *Drosophila* heat shock factor to arrays of a conserved 5 bp unit. Cell **64:**585–593.