Heat shock factor gains access to the yeast HSC82 promoter independently of other sequence-specific factors and antagonizes nucleosomal repression of basal and induced transcription

Alexander M. Erkine  
*Butler University*, aerkine@butler.edu  
C. C. Adams  
T. Diken  
D. S. Gross

Follow this and additional works at: [http://digitalcommons.butler.edu/cophs_papers](http://digitalcommons.butler.edu/cophs_papers)

Part of the [Cell Biology Commons](https://digitalcommons.butler.edu/cellbiology_commons), [Molecular Genetics Commons](https://digitalcommons.butler.edu/moleculargenetics_commons), and the [Pharmacy and Pharmaceutical Sciences Commons](https://digitalcommons.butler.edu/pharmacypharmaceuticalsciences_commons)

**Recommended Citation**

[http://digitalcommons.butler.edu/cophs_papers/139](http://digitalcommons.butler.edu/cophs_papers/139)

This Article is brought to you for free and open access by the College of Pharmacy & Health Sciences at Digital Commons @ Butler University. It has been accepted for inclusion in Scholarship and Professional Work – COPHS by an authorized administrator of Digital Commons @ Butler University. For more information, please contact omacisaa@butler.edu.
Heat Shock Factor Gains Access to the Yeast HSC82 Promoter Independently of Other Sequence-Specific Factors and Antagonizes Nucleosomal Repression of Basal and Induced Transcription

ALEXANDER M. ERKINE, CHRISTOPHER C. ADAMS, TUBA DIKEN, AND DAVID S. GROSS*

Department of Biochemistry and Molecular Biology, Louisiana State University
Medical Center, Shreveport, Louisiana 71130

Received 7 February 1996/Returned for modification 19 July 1996/Accepted 26 August 1996

Transcription in eukaryotic cells occurs in the context of chromatin. Binding of sequence-specific regulatory factors must contend with the presence of nucleosomes for establishment of a committed preinitiation complex. Here we demonstrate that the high-affinity binding site for heat shock transcription factor (HSF) is occupied independently of other cis-regulatory elements and is critically required for preventing nucleosomal assembly over the yeast HSC82 core promoter under both noninducing (basal) and inducing conditions. Chromosomal mutation of this sequence, termed HSE1, erases the HSF footprint and abolishes both transcription and in vivo occupancy of the TATA box. Moreover, it dramatically reduces promoter chromatin accessibility to DNase I and TAPl, as the nuclease-hypersensitive region is replaced by a localized nucleosome. By comparison, in situ mutagenesis of two other promoter elements engaged in stable protein-DNA interactions in vivo, the GRF2/REB1 site and the TATA box, despite reducing transcription three- to fivefold, does not compromise the nucleosome-free state of the promoter. The GRF2-binding factor appears to facilitate the binding of proteins to both HSE1 and TATA, as these sequences, while still occupied, are less protected from in vivo dimethyl sulfate methylation in a ΔGRF2 strain. Finally, deletion of a consensus upstream repressor sequence (URS1), positioned immediately upstream of the GRF2-HSE1 region and only weakly occupied in chromatin, has no expression phenotype, even under meiotic conditions. However, deletion of URS1, like mutation of GRF2, shifts the translational setting of an upstream nucleosomal array flanking the promoter region. Taken together, our results argue that HSF, independent of and dominant among sequence-specific factors binding to the HSC82 upstream region, antagonizes nucleosomal repression and creates an accessible chromatin structure conducive to preinitiation complex assembly and transcriptional activation.

The promoter regions of eukaryotic genes consist of multiple cis-acting elements that serve as binding sites for regulatory proteins. Two categories of cis-regulatory sequences have been described. The general downstream elements, exemplified by the TATA box and initiator element, specify transcription start site selection (10), while specialized upstream elements, target sites for a variety of activators and repressors, regulate the rate of transcription initiation (66). Since these elements must function in the context of chromatin, both the sequence-specific upstream regulatory proteins and components of the general transcriptional machinery must overcome the potential steric hindrance imposed by nucleosomes during the recognition of, and subsequent association with, a target promoter (reviewed in references 21, 27, 35, 71, and 72). Indeed, compaction of promoter DNA into nucleosomes prevents the initiation of transcription, both in vitro and in vivo (24, 34, 43, 62, 73). Therefore, cis-regulatory elements of transcriptionally poised genes usually reside within regions of chromatin that are hypersensitive to DNase I and, by inference, free of canonical nucleosomes (14, 26).

DNase I-hypersensitive regions provide “open windows” through which transcription factors and RNA polymerase holoenzyme gain access to the template DNA. What are the biochemical determinants of such accessible chromatin structures? The TATA-binding basal transcription factor, TFIID, may fulfill this function under certain circumstances, for it effectively potentiates promoter function when preincubated with a naked DNA template either during in vitro chromatin reconstitution (7, 73) or following injection into fertilized Xenopus eggs (57). Nonetheless, when histones are added to naked DNA simultaneously with TFIID, nucleosomal repression can be prevented only if an upstream activator protein is also included (74). Indeed, specialized upstream factors appear to normally underlie the genesis of accessible, nucleosome-free (or disrupted) regions at native promoters and enhancers (18, 20, 24, 30, 44, 67). That this activity is central to the normal function of upstream activators is suggested by the fact that physiological levels of transcriptional induction in reconstituted systems is contingent upon prior assembly of template DNA into stable, uniformly spaced nucleosomes (38, 53). However, largely unknown is the interrelationship of upstream factors in the remodeling process. Can a single, dominant upstream activator prompt the formation of a nucleosome-free region within native chromatin, or is a nuclease-hypersensitive site the product of multiple sequence-specific factors? Such factors may outcompete histones at the replication fork to establish a nucleosome-free region (exclusion model); alternatively, they may bind and displace (or disrupt) a preexisting nucleosome (displacement model) (reviewed in reference 72).

In the latter case, transcription factors may be assisted by ATP-dependent, multiprotein complexes such as SWI/SNF or NURF to reconfigure promoter chromatin structure (reviewed in references 31a and 55).
TABLE 1. Yeast strains used

<table>
<thead>
<tr>
<th>Yeast strain</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>W303-1B</td>
<td>MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trpl-1 1 ura3-1</td>
<td>40</td>
</tr>
<tr>
<td>CUD82</td>
<td>MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trpl-1 1 ura3-1 hisc82Δ::URA3</td>
<td>8</td>
</tr>
<tr>
<td>SLY101</td>
<td>MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trpl-1 1 ura3-1 cyh2^2</td>
<td>40</td>
</tr>
<tr>
<td>CRY102</td>
<td>SLY101; hisc82Δ::CYH2^2</td>
<td>This study</td>
</tr>
<tr>
<td>TAT100</td>
<td>CRY102; hisc82Δ::TATA</td>
<td>This study</td>
</tr>
<tr>
<td>TAT101</td>
<td>CRY102; hisc82Δ::TATA, ΔHSE1</td>
<td>This study</td>
</tr>
<tr>
<td>HSE100</td>
<td>CRY102; hisc82Δ::TATA, ΔHSE1, ΔURS1</td>
<td>This study</td>
</tr>
<tr>
<td>HSE101</td>
<td>W303-1B; hisc82Δ::HSE0</td>
<td>This study</td>
</tr>
<tr>
<td>HSE102</td>
<td>W303-1B; hisc82Δ::HSE1</td>
<td>This study</td>
</tr>
<tr>
<td>HSE103</td>
<td>W303-1B; hisc82Δ::HSE0, ΔHSE1, ΔURS1</td>
<td>This study</td>
</tr>
<tr>
<td>GRF200</td>
<td>CRY102; hisc82Δ::GRF2</td>
<td>This study</td>
</tr>
<tr>
<td>URS100</td>
<td>CRY102; hisc82Δ::URS1</td>
<td>This study</td>
</tr>
<tr>
<td>URS101</td>
<td>CRY102; hisc82Δ::HSE0, ΔURS1</td>
<td>This study</td>
</tr>
<tr>
<td>URS102</td>
<td>CRY102; hisc82Δ::HSE1, ΔURS1</td>
<td>This study</td>
</tr>
<tr>
<td>URS103</td>
<td>CRY102; hisc82Δ::HSE0, ΔHSE1, ΔURS1</td>
<td>This study</td>
</tr>
<tr>
<td>WT-HSC</td>
<td>MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trpl-1 1 ura3-1 hisc82Δ::URA3</td>
<td>This study</td>
</tr>
<tr>
<td>ΔURS1-HSC</td>
<td>MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trpl-1 1 ura3-1 hisc82Δ::URA3</td>
<td>This study</td>
</tr>
</tbody>
</table>

We are interested in understanding the role of chromatin in the heat shock transcription mechanism. Stress-induced activation of heat shock genes from organisms as diverse as yeast, tomato, fly, and human is achieved by the binding of heat shock factor (HSF) to its target DNA sequences, heat shock elements (HSEs) (reviewed in reference 42). A notable difference between heat shock gene activation in the budding yeast *Saccharomyces cerevisiae* and higher eukaryotes is that *S. cerevisiae* HSF (ScHSF) is constitutively bound to high-affinity HSEs within chromatin, whereas metazoan HSF binds to DNA only following heat shock (25, 29, 60). This difference underlies the unique role of ScHSF in directing basal (nonactivated) transcription (48, 52) and in maintaining the promoter region of at least one heat shock gene, *HSP82*, in a DNase I-hypersensitive, nucleosome-free state (24).

**Yeast HSC82** is a constitutively expressed heat shock gene whose level of expression is enhanced severalfold following exposure to chemical or thermal stress (1, 8). That this induction occurs at the level of transcription is suggested by measurements of both its own transcript (1, 15) as well as that of *lacZ* in strains bearing an episomal *HSC92-lacZ* fusion gene (25). *HSC82* harbors a broad, heat-shock-invariant DNase I-hypersensitive domain localized to its 5' end. Within this nucleosome-free zone are strong constitutive protein-DNA interactions that have been mapped at nucleotide resolution to the high-affinity HSE (HSE1), the consensus GRF2/REB1 site, and the TATA box (15). Here we investigate the structural and functional consequences of mutating these three sequences, as well as the promoter-proximal HSE (HSE0) and a consensus upstream repressor sequence (URS1). In situ mutagenesis of each of these five elements, individually and in combination, leads to the conclusion that the HSF-HSE1 complex not only plays a dominant role in remodeling promoter chromatin structure but can form independently of the other four sites. This observation provides a striking contrast to the *Drosophila hsp70* gene, where a specific in vivo architecture must be established by several independent factors to allow HSF access to the promoter (58).

**MATERIALS AND METHODS**

**Yeast strains, cell growth, and heat shock.** Strains of *S. cerevisiae* used in this study are listed in Table 1. Cells were grown in rich medium (1% yeast extract, 2% Bacto Peptone, 2% dextrose) to early log phase (2 × 10^7^ cells per ml; 50 Klett units), heat shocked (30-33°C temperature upshift for 15 min), and metabolically poisoned with 20 mM sodium azide as previously described (25).

**Sporulation.** Diploid strains were preadapted to respiratory metabolism by growth in presporulation medium (1% potassium acetate, 0.67% yeast nitrogen base, 5% phthalic acid [pH 5.5] supplemented with the appropriate auxotrophic requirements). At a cell density of ~10^9/ml, cells were harvested by centrifugation, washed with sterile water, and resuspended in buffered sporulation medium (1% potassium acetate, 0.1% yeast extract [pH 7.0] supplemented with the appropriate auxotrophic requirements). Cultures were maintained at 30°C with vigorous shaking. Aliquots (10 ml) were removed at the appropriate times following the shift to sporulation medium, and total RNA was isolated and evaluated as previously described (1).

**In vitro mutagenesis.** Oligonucleotide-directed mutagenesis was performed on a BamHI-KpnI *HSC82* fragment (spanning ~3140 to +321) cloned into pM13mp18 as described previously (24). To disrupt HSE1, point mutations were introduced into each of the three conserved nGAAn units; a fourth point mutation was also introduced into the nonconserved GAA at ~164. HSE0 was similarly disrupted by the introduction of four point mutations, while the GRF2 site was destroyed by a 6-bp substitution mutation. The HSE1 mutation destroys the ~174 XbaI site, the HSE0 mutation creates a novel HaelII site at ~134, and the GRF2 mutation creates a novel ClaI site at ~205. Such sites permitted a convenient screen for transformants harboring the desired *hsc82* allele (see below). To disrupt the URS1 element at ~226, the 11-bp region between ~228 and ~216 was deleted, whereas a 6-bp substitution mutation destroyed the TATA box at position ~98. All five mutations (see Fig. 2) were confirmed by dideoxy sequencing of the appropriate M13 constructs.

**Yeast strain construction.** Strain W303-1B (*HSC82*) was used as the recipient for gene displacement of *hsc82* alleles harboring mutations in either HSE0 or HSE1; the *hsc82Δ::CYH2* disruption strain, CRY102, was used as the recipient for construction of *hsc82* mutant strains bearing mutations in TATA, GRF2, or URS1 elements. In CRY102, an isogenic derivative of the *cry2* strain SLY101 (40), the *HSC82* BglII fragment, spanning ~409 to +95, was replaced with a 1.6-kb *PstI* fragment containing the *CYH2* gene, oriented in the opposite direction relative to *HSC82*. Inactivating mutations can be introduced into the *HSC82* gene due to the presence of *HSP92*, deletion of both *HSP90* genes is lethal (8). To permit two-step gene transplacement at the *HSC82* locus, the mutagenized BamHI-KpnI fragment was subcloned into pRS306, a *URA3*-containing yeast integrating vector (59). The resultant constructs were site directed to the *HSC82* locus by linearizing at the unique *EcoRI* site within *HSC82* at position ~615. Selection on Ura^− medium and counterselection on medium containing 1 mg of 5-fluoro-uracil acid per ml were done as previously described (40). In the case of the transplacement in CRY102, loss of the host *hsc82::CYH2* allele was initially screened by replica plating 5-fluoro-uracil acid-resistant clones onto rich medium containing cycloheximide.

In all cases, molecular verification of gene transplacement was achieved by Southern analysis; for selected alleles (*hsc82ΔHSE1, hisc82Δ::GRF2, and hisc82Δ::URS1*), verification was extended to the nucleotide level by genomic sequencing.

**Gene transplacement was assayed by Southern analysis using probe C1 (Fig. 1B).** In strains TAT100, TAT101, TAT102, URS100, URS101, URS102, and URS103, we confirmed that the 6.5-kb BamHI fragment characteristic of the *hsc82Δ::CYH2* allele of strain CRY102 was replaced by a 5.4-kb BamHI fragment (~1340 to +1400) and the 1.0-kb BamHI fragment characteristic of the *HSC82* allele (~1231 to ~173) was replaced by
RNA electrophoresis and Northern (RNA) blot hybridization. RNA samples were prepared from 10-mI aliquots of control and 15-min heat-shocked cultures, electrophoresed, and the blot hybridized to HSC82- and ACT1-specific probes as previously described (1). HSC82 RNA was detected by using either antisense RNA probe C3 or a synthetic oligonucleotide (65-mer) homologous to the 3'-untranslated region (spanning positions +2248 to +2184) (Fig. 1B). ACT1 RNA was detected using a 1.6-kb antisense RNA probe homologous to its coding region. Stringent hybridization conditions (45°C hybridization and wash for C3, 55°C hybridization and wash for the 65-mer) that eliminated cross-hybridization between HSC82 and its closely related homolog, HSF2, were used. HSC82 transcript levels (Table 2) were quantitated by laser densitometry of autoradiograms and by PhosphorImager analysis; they were internally normalized to ACT1 levels as described previously (40). Moreover, the residual background signal detectable in the hsc82Δ control strain, CRY102, was subtracted from each value (C3 hybridizations).

Nuclease digestion of chromatin, agarose DNA electrophoresis, and Southern blot hybridization. Cells from control and heat-shocked cultures were converted to spheroplasts as previously described (1). Washed spheroplasts were lysed and digested with DNease I, and the resultant DNA was purified, restricted with BamHI, electrophoresed, transferred to GeneScreen, and subjected to indirect end labeling using riboprobe C1 as before. Similarly, the micrococcal nuclease (MNase) indirect end-labeling analysis of isolated nuclei (Fig. 9) was conducted as previously described (15). For the MNase nucleosome-protected ladder analysis (Fig. 8), nuclei were isolated from 100-mI aliquots of early-log-phase cultures (10^7 cells per ml) and digested at 37°C for 10 min with 30, 60, 120, or 240 U of MNase (control) or 60 and 120 U of MNase (heat shocked); DNA cleavage sites for all indirect end-labeling experiments (Fig. 6, 7, and 9) was facilitated by the coelectrophoresis of landmark fragments, which were prepared by digesting wild-type (WT) genomic DNA with BamHI alone (+4100) or in combination with EcoRI (+370), or BamHI (+265, +553).

**FIG. 1.** HSC82 upstream sequence, sites of DNease I protection in nuclei and DMS protection/hyperreactivity in intact cells, and a physical map of the chromosomal locus. (A) The upstream sequence of HSC82, numbered relative to the principal transcription initiation site (8). Sequences bearing homology to consensus regulatory elements in S. cerevisiae are boxed. The heat shock elements HSE0 and HSE1 each exhibit a 10/12 match to conserved nucleotides of the HSF locus. (A) The upstream sequence of HSC82 study (Fig. 4 and 5) and from reference 15. (B) Physical map of the HSC82 region (Fig. 8 and 10/10 matches to conserved nucleotides of their respective consensus sequences (17). Note that HSE1 exhibits a 13/16 match to a recent refinement of the S. cerevisiae HSF consensus (consisting of tandem inverted repeats of AAGAA [22]), whereas HSE0 exhibits only an 11/16 match, consistent with the observed occupancy of HSE1 but not HSE0 (Fig. 4 and 5). Nucleotides protected from DNease I are indicated by filled bars; uncertainty in the extent of DNease I protection is indicated by open bars. Nucleotides constitutively protected from DMS are indicated by striped bars, those constitutively hyperreactive to DMS are indicated by arrowheads, and a nucleotide exhibiting heat shock-inducible hyperreactivity is marked with an open circle. (Note that for the lower strand, DMS analysis extends from -212 to -120 only, while the upstream limit of DNease I analysis is to position -215.) Footprinting data are derived from this study (Fig. 4 and 5) and from reference 15. (B) Physical map of the HSC82 locus on chromosome XII. Locations of naturally occurring TaqI sites within the sequenced region (1341 to +2257) are indicated by filled circles; a synthetic TaqI site, present only in the hsc82ΔGRF2 allele, is indicated by an open circle. Locations of the four hybridization probes used in this study are also indicated. C1 to C5 represent RNA probes, synthesized in vitro as previously described (15). 65-mer, upper-strand-specific probe used to detect HSC82 mRNA. B, BamHI; E, EcoRI; X, XbaI; Bc, BciI; Hf, HinfI.

a 6.5-kb XbaI fragment, while the 2.4-kb HaeIII fragment characteristic of the HSC82 allele (714 to +1686) was replaced by a 0.6-kb HaeIII fragment in strains HSE100 and HSE102 (2).

The diploid strains WT-HSC and ΔURS1-HSC were constructed by mating the MATα hsc82Δ::URA3 strain CUD82 (8) with either W303-1B (MATα HSC82*) or URS100 (MATα hsc82Δ::URS1). These constructions were confirmed by Southern analysis and, in the case of ΔURS1-HSC, by genomic sequencing. Complete genotypes of all strains used in this study are provided in Table 1.
reading frames. GST-ScHSF (judged intact by Coomassie blue staining and immunoblotting with an anti-ScHSF polyclonal antibody) was overproduced in *Escherichia coli* BL21 and purified essentially as described previously (22a) except that isolation conditions were scaled up for a 1-liter culture (typical yield was 100 mg). Binding reactions were conducted in 150 mM NaCl–1 mM CaCl$_2$–3 mM MgCl$_2$–20 mM Tris-HCl (pH 8.0)–0.5 mM EDTA–1 mM phenylmethylsulfonyl fluoride–100 mg of bovine serum albumin per ml as described previously (5).

RESULTS

In situ mutagenesis of the upstream region reveals critical roles for the HSE1, GRF2, and TATA elements in regulating *HSC82* transcription. To investigate the functional role of promoter elements stably occupied by protein in vivo, we constructed isogenic *hsc82* strains harboring mutations in the promoter-distal HSE (HSE1), the GRF2/REB1 motif, or the TATA box (*HSC82* promoter sequence and a summary of genomic footprinting data are presented in Fig. 1A; a physical map of the gene and locations of hybridization probes are provided in Fig. 1B). Moreover, two additional mutant strains were constructed: one in which the promoter-proximal HSE, HSE0, was disrupted, and another in which the consensus upstream repressor sequence, URS1, was deleted (Fig. 2). These latter two elements are not detectably protected from DNase I and are at best weakly protected from DMS (see below). As summarized in Table 2, a 4-bp substitution within HSE1 reduces *hsc82* transcript levels 25-fold in nonshocked cells and 100-fold in heat-shocked cells (strain HSE101). Similarly, a 6-bp substitution of the TATA motif reduces *HSC82* transcription; 5-fold irrespective of heat shock (strain TAT100), while a comparable mutation in the GRF2 site causes a 70% diminishment of basal transcription and an early 50% drop in induced transcription (strain GRF200). In contrast, a 4-bp mutation within HSE0 exhibits only a mild heat shock phenotype (having no impact on basal expression), despite this sequence exhibiting as strong a match to the HSF consensus sequence as HSE1 (strain HSE100; see the legend to Fig. 1). Strains harboring multiple promoter mutations were also made. Their phenotypes were as expected: HSE102, a strain harboring mutations in each HSE, and TAT101, a strain containing mutations in both HSE1 and TATA, each exhibit a

![FIG. 2. Nucleotide substitutions and deletions introduced into *HSC82* promoter elements. Conserved nucleotides found within each regulatory sequence are boxed. Isogenic *hsc82* strains constructed in this study harbor one or more of these *cis*-acting mutations (Tables 1 and 2).](image)

| TABLE 2. *HSC82* mutant strains and summary of structural and functional phenotypes$^a$ |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Strain                          | Promoter mutation(s)                      | Normalized RNA level$^b$ | Effect on promoter accessibility$^c$ | Effect on nucleosome positioning$^d$ |
| W303-1B                         | None (HSC82$^{+}$)                       | 100                  | -               | -               |
| TAT100                          | Substituted TATA (ΔTATA)                  | 20                   | *               | -               |
| TAT101                          | ΔTATA + ΔHSE1                              | 2                    | ++              | -               |
| TAT102                          | ΔTATA + ΔHSE1 + ΔURS1                      | 1                    | <1              | ++              | ND               |
| HSE100                          | Substituted HSE0 (ΔHSE0)                  | 90                   | -               | -               |
| HSE101                          | Substituted HSE1 (ΔHSE1)                  | 4                    | 2               | ++              | -               |
| HSE102                          | ΔHSE0 + ΔHSE1                             | 2                    | <1              | ++              | -               |
| GRF200                          | Substituted GRF2 (ΔGRF2)                  | 30                   | +               | +               | -               |
| URS100                          | Deleted URS1 (ΔURS1)                      | 90                   | 210             | -               | +               |
| URS101                          | ΔURS1 + ΔHSE0                             | 100                  | 170             | -               | ND               |
| URS102                          | ΔURS1 + ΔHSE1                             | 2                    | 2               | ++              | +               |
| URS103                          | ΔURS1 + ΔHSE0 + ΔHSE1                    | 1                    | 1               | ++              | ND               |

$^a$ Values represent the means of at least two or three independent preparations.

$^b$ NHS, non-heat shocked; HS, heat shocked.

$^c$ WT frequency of DNase I or TaqI cleavage; * enhanced frequency of TaqI cleavage; ++, mild reduction in nuclease cleavage; +++, severe reduction in nuclease cleavage.

$^d$ WT location of 5' flanking nucleosomes; +, 10- to 20-bp translational shift in the position of the upstream nucleosomal array; ND, not determined.
FIG. 3. Deletion of URS1 does not block meiotic induction of HSC82. RNAs were extracted from aliquots of cultures of the diploid strains WT-HSC and ΔURS1-HSC at various times after transfer to nitrogen-deficient sporulation medium. Fifteen micrograms of purified RNA from each sample was separated on a 1.1% agarose-formaldehyde gel and transferred to nylon. HSC82, SS4A, HSP26, RPS8, and ACT1 transcripts were detected as previously described (1); in particular, HSC82 transcripts were detected by using probe C3. Lanes: (−), control cells grown in synthetic acetate (presporulation) medium at 30°C; (+), cells grown in presporulation medium and subjected to a 15-min heat shock at 39°C; 2 to 10, hours after transfer to nitrogen-deficient sporulation medium; 6−1, 6-h sample subjected to a 39°C heat shock for 15 min. Densitometric quantitation reveals that in both strains, HSC82 transcript levels (internally normalized to the ACT1 level) increase two- to threefold at 6 h, plateauing thereafter. Heat shock superimposed upon nitrogen starvation results in a further 10 to 30% increase in HSC82 RNA levels (lanes 8 and 16).

more severe phenotype than their singly mutated counterparts (Table 2). We conclude that while the HSE1, GRF2, and TATA sequences play critical regulatory roles under these growth conditions, HSE0 does not.

The URS1 element can be deleted without altering HSC82 expression during either mitotic or meiotic growth. Sequences bearing homology to the URS1 upstream repressor sequence are found 5′ of a number of regulated yeast genes, including the SS4A and HSP82 heat shock genes (52, 64). Genetic analyses have indicated that the URS1 motif, which is normally located between the upstream activation sequence (UAS) and the TATA element, functions as a repressor in mitotic cells (63). To test the function of the URS1 element at HSC82, we constructed isogenic strains bearing a deletion of this sequence alone and in combination with mutations in other promoter elements. URS1 is positioned 50 bp upstream of HSE1 (Fig. 1A) and is only weakly protected from DMS in vivo methylation (see below) despite exhibiting a 10/10 match to the consensus (47, 64). In logarithmically growing cells, there is no detectable phenotype associated with the URS1 deletion in any one of the four strains in which it was introduced (URS100 to URS103). This is most clearly seen in the absence of other cis-acting mutations (strain URS100), where HSC82 RNA levels are essentially indistinguishable from those in the WT strain, W303-1B (Table 2).

As the URS1 element has been shown to positively regulate meiosis-specific genes (9, 49, 64), we asked whether HSC82 was induced during sporulation and, if so, whether the URS1 deletion had any effect on this induction. To test this, we constructed two diploid strains that were heterozygous with respect to the hsc82 Δalleles, WT-HSC (HSC82::HSC82Δ::URA3) and ΔURS1-HSC (hsc82Δ::URA3/hsc82Δ::URA3) Northern analysis of WT-HSC cells reveals that the high constitutive level of HSC82 is induced severalfold following a shift to nitrogen-free medium, peaking between 6 and 10 h. This level of induction is comparable to what is seen during a brief heat shock (Fig. 3; compare lanes 5 to 7 with lane 2). Indeed, no further induction is seen when sporulating cells are subjected to a 15-min heat shock (lane 8). That this induction reflects an early meiotic rather than general starvation response is suggested by the fact that HSC82 is not induced in the WT haploid strain subjected to the identical regimen (data not shown). Surprisingly, an essentially identical expression pattern is seen in strain ΔURS1-HSC (lanes 9 to 16), ruling out a role for URS1 in regulating the meiotic induction of HSC82 transcription. Confirmation that these diploid strains were indeed sporulating was obtained by both cytological and molecular analyses. The fraction of cells in which two, three, or four spores were visible increased from 0% at 2 h to >50% at 10 h (>90% at 24 h). Similarly, rehybridizing the RNA samples with probes specific for other meiotically induced genes revealed that within 4 to 6 h after the shift to sporulation medium, both HSP26 and HSP82 were strongly induced (Fig. 3 and data not shown), as previously seen (36, 64). In contrast, SS4A was not measurably activated (although it was induced by heat shock), while the ribosomal protein gene RPS8 was strongly repressed early during sporulation (Fig. 3, lanes 3 and 11), as observed by others (70). Taken together, these data suggest that HSC82 is a meiotically induced gene whose activation can occur in the absence of URS1.

Genomic footprinting indicates a role for the GRF2-binding factor in facilitating occupancy of HSE1 and TATA elements. To investigate the effect of mutating upstream regulatory elements on protein-DNA interactions, we mapped sites of DNase I cleavage and DMS modification within hsc82 promoter mutants at nucleotide resolution. Spheroplast lysates, generated from WT and three promoter mutant strains (GRF200, HSE101, and URS101), were digested with DNase I, and genomic DNA was purified and subjected to AMPEx. As illustrated in Fig. 4A, within the WT upstream promoter region, HSE1 is strongly protected from DNase I (irrespective of heat shock) and GRF2 is moderately protected, while URS1 and HSE0 are not detectably protected (compare lanes 3 and 4 with lane 2). As expected, DNase I protection is abolished at the mutated sites within the ∆GRF2 and ∆HSE1 strains. Interestingly, this assay also suggests that there is a diminished interaction at HSE1 in the ∆GRF2 mutant. This interpretation is based on the loss of protection at position −167, a site that is strongly cleaved in naked DNA and almost completely protected in WT chromatin (filled circle), as well as a reduction in flanking hyperreactivity. Notably, there is no comparable effect on the HSE1 footprint in the ∆URS1 ΔHSE0 double-mutant strain (lane 16), arguing that HSF binding to HSE1 is independent of both URS1 and HSE0 sites. Likewise, strong DNase I protection is retained at the GRF2 site in the ∆HSE1 strain (lanes 11 and 12), suggesting that the GRF2 factor can bind to the hsc82 promoter independently of HSF.

To confirm the GRF2 effect on HSE1 occupancy in intact cells, we used DMS in vivo footprinting. Cells were concentrated in rich medium and briefly reacted with DMS at either 23°C (control) or 39°C (heat shocked). Following purification of genomic DNA, sites of purine methylation were mapped by using AMPEx. Two sites of constitutive protection are evident within the HSC82′ upstream promoter: HSE1 and GRF2 (Fig. 5A, lanes 4 and 5, and Fig. 5B, lanes 4 and 5; protections denoted by striped bars). These two regions of protection are flanked by hyperreactive purine residues on the lower strand (arrowheads; data summarized in Fig. 1A). In the isogenic ∆GRF2 strain, in addition to the expected loss of protections and enhancements associated with the mutated site, reduced DMS protection is seen at each of the three consensus GAA modules within HSE1, at positions −159 and −169 on the upper strand and at position −172 on the lower strand; more-
over, the hyperreactivity mapping to the nearby −183 G residue is lost (lanes 9 and 10 in both panels). A quantitative analysis of the HSE1 region is shown in Fig. 5C, confirming that both major and minor groove protections (G and A residues, respectively) are reduced in the GRF2 mutant. Interestingly, loss of protection appears to be equally pronounced in control and heat-shocked cells. Taken together, the genomic footprinting data argue that the GRF2-binding protein assists and/or stabilizes the interaction of HSF within the HSC82 upstream region.

Dependence of HSF on the GRF2 element for stable binding raises the question of whether downstream factors, TATA-binding protein (TBP) in particular, are similarly dependent. As illustrated in Fig. 5D, the ΔGRF2 mutation also materially reduces the level of protection of adenosines within the TATA box to DMS relative to that seen in the WT promoter. DMS methylates adenosines on the N-3 moiety within the minor groove, the site of TBP binding (31). As above, this diminished level of protection is equally pronounced in nonshocked and heat-shocked cells and implies a further role for the GRF2-binding protein in the stabilization of the TBP-TATA interaction (see Discussion).

A 4-bp mutation in HSE1 abolishes in vivo occupancy of the TATA box. Given the profound expression phenotype associated with the ΔHSE1 lesion, we tested whether the HSE1 mutation would also affect the TBP-TATA interaction. We anticipated that this might be the case, in light of the above result with the ΔGRF2 strain as well as previous results implicating a central role for HSF in facilitating occupancy of the TATA box at HSP92 (24). Indeed, as illustrated by the scans in Fig. 5D, all minor groove interactions mapping to the TATA motif are abolished upon mutation of HSE1. Evidence presented below suggests that the TBP-TATA interaction is replaced by a stable nucleosome localized over the core promoter region. Notably, the reciprocal effect is not seen: a 6-bp substitution of the TATA element, despite significantly lowering transcript levels (Table 2), has no detectable effect on the occupancy of either HSE1 or GRF2 (Fig. 5C and data not shown). This finding argues that factors binding to these upstream sequences can do so independently of stably bound TBP.

The SchSF in vitro footprint strongly resembles its genomic counterpart. Given the central importance of the HSE1 protein complex in regulating both the expression and the structure of HSC82 (Table 2 and Fig. 6 to 8), we wished to determine whether HSF was solely responsible for the protection seen or whether factors in addition to HSF contribute to the footprint. To test this directly, we reacted a GST-SchSF fusion protein with a supercoiled template bearing the HSC82 upstream region. As illustrated in Fig. 4B, the footprint of the recombinant protein is essentially identical to the HSE1 genomic footprint, with respect to both the breadth of protection (spanning −180 to −150) and the presence of flanking hyperreactivity (Fig. 4A and reference 15). Notably, at an HSF concentration sufficient to strongly protect HSE1, no protection is seen within HSE0 (Fig. 4B). Interestingly, GST-SchSF binds HSE1 under both control and inducing temperatures, resembling the in vivo activity of the native factor but contrasting with the behavior of a mouse GST-HSF fusion protein which binds DNA only following a heat-induced conversion (23a). We conclude that the protection conferred by HSF can fully account for the HSE1 genomic footprint and that the absence of protection at HSE0 reflects the intrinsically low affinity of HSF for this site.

Mutation of HSE1 abolishes promoter-associated DNase I hypersensitivity and restricts accessibility to TaqI endonuclease. To investigate the effects of the promoter mutations on
local chromatin structure, we mapped regions of DNase I accessibility by indirect end labeling. As shown in Fig. 6, the broad DNase I-hypersensitive region spanning the WT promoter (lanes 1 and 2) is drastically reduced in intensity at the hsc82-DHSE1 allele (lanes 3 and 4). That the hyperreactive cleavages are chromatin specific has been confirmed by a pairwise comparison with a comparably digested deproteinized genomic DNA sample (data not shown; see also reference 15). This result suggests that a dramatic reduction in promoter accessibility accompanies the 4-bp mutation within HSE1. In contrast, a comparable 4-bp substitution mutation within HSE0 has virtually no effect on the DNase I cleavage profile of the gene (lanes 7 and 8). This result is consistent with measurements of steady-state HSC82 RNA levels (Table 2) which indicate that the HSE0 mutation has at best a mild phenotype, as well as genomic footprinting analyses of the wild-type allele which argue against the presence of stably bound protein at this site (Fig. 4 and 5) (15). Also consistent with the absence of an expression phenotype, robust nuclease hypersensitivity is retained in the URS1 deletion strain (lanes 5 and 6). Importantly, the chromatin samples derived from ΔHSE0, ΔURS1, and ΔHSE1 strains are digested to comparable degrees, yet promoter-associated DNase I hypersensitivity is clearly retained in the former two strains but nearly abolished in the latter (compare lanes 5 to 8 with lanes 3 and 4). Moreover, despite strongly affecting hsc82 transcription, mutations in either TATA or GRF2 do not detectably alter the promoter-associated DNase I-hypersensitive site (data not shown). We conclude that in contrast to the HSE1 substitution, mutations of the other four elements have relatively little impact on promoter chromatin structure, even when they are present in combination (data not shown; see Table 2 for a summary of phenotypes).

To strengthen the foregoing conclusions, we examined whether the TCGA sequence at position −140, unique to the HSC82 upstream region and located within the promoter-associated DNase I-hypersensitive site, would be accessible to TaqI in isolated nuclei. Restriction enzyme cleavage is severely limited in nucleosomal DNA (3); thus, we reasoned that if the nucleosome-free (or nucleosome-disrupted) state of the WT promoter were preserved at the mutant hsc82 alleles, the promoter chromatin would remain accessible to TaqI. However, if this open chromatin configuration were replaced by a nucleosomal structure, TaqI accessibility would be reduced or lost. As anticipated, the −140 site is highly accessible in WT chromatin (Fig. 7A, lanes 2 to 5; Fig. 7B, lanes 3 to 6). In contrast to the DNase I hypersensitivity assay, however, the extent of TaqI cleavage appears to increase nearly twofold following heat shock (most clearly seen in Fig. 7A, lanes 4 versus 2 and 5 versus 3). Interestingly, the nine TCGA sites spanning the HSC82 transcription unit are relatively inaccessible to TaqI, particularly in nonshocked nuclei (compare WT samples with naked DNA [Fig. 7B, lanes 3 and 4 versus lanes 1 and 2]). This observation is consistent with previous observations indicating the presence of a canonical nucleosomal array over the coding region of this gene (15).

Strong TaqI cleavage is also apparent within the hsc82-ΔGRF2 promoter region (Fig. 7A, lanes 10 to 13). Note that this allele has two promoter-associated TCGA sites, the naturally occurring one at position −140 and a synthetic site at −205 (Fig. 1B and 2). The combined cleavage frequency of the two TaqI sites is slightly less than the frequency of cleavage of the single TaqI site in WT nuclei; following heat shock, they are essentially equivalent. Given that under these conditions TaqI is limiting (Fig. 7B, lanes 1 and 2; see Materials and Methods), such a comparison permits the conclusion that the ΔGRF2 mutation has only a modest effect on the accessibility of the promoter region to TaqI. Similarly, despite reducing HSC82 transcript levels ~80% (Table 2), substitution of the TATA box does not curtail accessibility of the promoter region to TaqI. Instead, cleavage of the −140 site is enhanced twofold in strain TAT100 compared to WT under both nonshock and heat-shocked conditions (Fig. 7B; compare lanes 7 to 10 with lanes 3 to 6). In marked contrast, the −140 TaqI site in strain HSE101 is only 15% (heat shock) to 35% (nonshock) as frequently cleaved as it is in WT nuclei (Fig. 7A; compare lanes 8 and 9 with lanes 4 and 5 and lanes 6 and 7 with lanes 2 and 3), indicating that a drastic reduction in promoter accessibility accompanies mutagenesis of HSE1, particularly following heat shock. This result is fully consistent with the reduction of
DNaseI hypersensitivity demonstrated above. Taken together, the DNase I hypersensitivity and TaqI accessibility assays argue that the HSE1 mutation severely restricts promoter accessibility, whereas comparable mutations in GRF2, TATA, HSE0, and URS1 do not.

A 4-bp mutation in HSE1 leads to a well-resolved nucleosome-protected ladder, while mutations in other promoter elements do not. The dramatic loss of DNase I hypersensitivity and TaqI accessibility accompanying the HSE1 mutation suggests that this lesion affects the nucleosome-free state of the hsc82 promoter region. To more directly address this possibility, we digested nuclei isolated from HSC82 and hsc82−ΔHSE1 cells with MNase, a sensitive probe of nucleosomal structure. Genomic DNA was purified, electrophoretically resolved, and blotted to nylon, and hsc82 promoter-specific fragments were visualized by hybridization to probe C2. As previously demonstrated (15), MNase excises a novel fragment of ~210 bp from the WT promoter region with or without heat shock (Fig. 8, lanes 1 to 6). In contrast, when a similar treatment is performed on nuclei isolated from strain HSE101, a stable, nucleosome-length fragment of ~170 bp is detected, as part of a larger, well-resolved ladder exhibiting a repeat length of 170 ± 5 bp (lanes 8 to 13). Notably, this difference between WT and mutant profiles is maintained irrespective of the level of digestion, arguing that the difference in monomer lengths is not an artifact of MNase exonuclease activity. To further validate the difference between the two strains, in an independent experiment we eluted probe C2 from the membrane and reprobed the DNA samples with C1, which is homologous to a region of intergenic chromatin centered approximately 1 kb upstream of HSC82 (Fig. 1B). We found that for each strain, the monomer-length fragment derived from intergenic chromatin exhibited precisely the same mobility as the monomer fragment excised from the mutated, but not WT, promoter (data not shown). This result is therefore consistent with the DNase I and TaqI experiments: all three indicate that a 4-bp mutation in HSE1 is associated with a striking structural phenotype. Similar analyses of hsc82 strains bearing promoter mutations in URS1, GRF2, HSE0, or TATA resulted in MNase ladders whose patterns were indistinguishable from the WT pattern (data not shown). We conclude that HSE1, unique among the regulatory elements tested, is critically required for maintaining the promoter region in a nucleosome-free (or nucleosome-disrupted) state.

FIG. 7. The ΔHSE1 mutation severely reduces accessibility of the hsc82 promoter to TaqI, while a comparable mutation in TATA increases it. (A) Nuclei were isolated from control and heat-shocked W303-1B (lanes 2 to 5), HSE101 (lanes 6 to 9), and GRF200 (lanes 10 to 13) cells and digested at 41°C with the indicated amount of TaqI per microgram of nuclear DNA for 20 min. DNA was purified, restricted with BamHI, electrophoresed, and blot hybridized with probe C1. Locations of naturally occurring TaqI sites (at positions +140, +679, +766, +1015, +1381, +1474, +1576, +1612, +1867, and +1969) are indicated with filled circles; the synthetic site at position −205, unique to the hsc82−ΔGRF2 allele, is indicated by an open circle. LF, landmark fragments. (B) Nuclei purified from W303-1B (lanes 3 to 6) and TATA01 (lanes 7 to 10) cells were digested with TaqI, and DNA was prepared as described above. Naked genomic WT DNA (lanes 1 and 2) was digested under identical conditions. Symbols are as for panel A.
Deletion of either URS1 or GRF2 shifts the location of nucleosomes positioned upstream of the hypersensitive site. Previous analysis of HSC82 chromatin structure has suggested the presence of an upstream nucleosomal array flanking the hypersensitive site (15). To investigate the potential role of regulatory sites within the HSC82 promoter in positioning this array, we mapped nucleosome positions in isogenic hsc82 strains by using MNase and indirect end-labeling techniques. Three prominent, chromatin-specific cleavage sites extending upstream from the promoter and spaced at ~170-bp intervals are seen in the WT samples irrespective of heat shock (Fig. 9A, lanes 2 and 3; Fig. 9B, lanes 2 and 4), consistent with the presence of two translationally positioned nucleosomes (symbolized by ovals). The dashed oval indicates the location of a less well positioned nucleosome. Cleavage site specifically protected in the ΔHSE1 strain. The locations of regulatory elements within the promoter (solid rectangles) are indicated. Positions of chromatin-specific cleavages are indicated on the left for WT and on the right for ΔURS1 (independently mapped by using landmark fragments electrophoresed on outside lanes [not shown]). (B) MNase cleavage sites in nuclei isolated from W303-1B (lanes 2 and 4) and GRF200 (lanes 3 and 5) cells, non-heat shocked (NHS) and heat shocked (HS) as indicated. The locations of chromatin-specific cleavages for the ΔGRF2 strain are indicated on the right; these have been confirmed in two independent experiments. LF, landmark fragments.

**Fig. 9.** Mutations within URS1 or GRF2, but not HSE1, alter the location of MNase cleavages upstream of the hsc82 promoter. (A) MNase-cut sites in nuclei isolated from control (−) and heat-shocked (+) strains W303-1B (lanes 2 and 3), HSE101 (lanes 4 and 5), URS100 (lanes 6 and 7), and URS102 (lanes 8 and 9) were mapped by indirect end labeling using probe C1 following DNA purification and restriction with BamHI. MNase-digested WT genomic DNA (D) is shown in lane 1. Boldface arrows represent regions exhibiting enhanced MNase cleavage; these sites likely correspond to the DNA linking adjacent sequence-positioned nucleosomes (symbolized by ovals). The dashed oval indicates the location of a less well positioned nucleosome. Filled circle, cleavage site specifically protected in the ΔHSE1 strain. The HSE1 mutation abolishes HSF binding (Fig. 4A), abrogates promoter expression of different hsc82 alleles under native conditions. Our principal finding is that HSF, independently of other sequence-specific factors, creates an open chromatin configuration within the HSC82 promoter region. This conclusion is based on three lines of evidence. First, a 4-bp substitution of HSE1, which abolishes HSF binding (Fig. 4A), abrogates promoter-associated DNase I hypersensitivity (Fig. 6) and severely reduces TaqI accessibility (Fig. 7A). Second, the HSE1 mutation transforms the MNase cleavage profile into a ladder of discrete monoo- and oligonucleosome-length fragments. In contrast, the WT ladder is more heterodisperse and contains a nonnucleosomal, ~210-bp monomer-length fragment (Fig. 8 and reference 15). Third, mapping of MNase cleavage sites (Fig. 9A) indicates that the ~40 site, sharply cleaved in WT chromatin, is protected in nuclei purified from the ΔHSE1 strain. Taken together, these data suggest that the HSE1 mutation occludes promoter chromatin structure. Finally, an hsc82 allele containing both ΔHSE1 and ΔURS1 mutations shows a pronounced upstream shift in MNase cleavage sites, virtually identical to those seen in the ΔURS1 strain (Fig. 9A, lanes 8 and 9). We conclude that the HSE1, GRF2, and URS1 elements contribute independently to the chromatin structure of the HSC82 upstream region. While HSE1 influences primarily the core promoter region, URS1 and GRF2 exert their influence predominantly over the distal promoter and intergenic regions.

**DISCUSSION**

HSF creates a nucleosome-free region over the HSC82 core promoter independently of other sequence-specific factors. We have performed a genetic dissection of transcriptional control elements found within the HSC82 promoter region. All of our experiments were conducted in vivo on integrated transgenes, which allowed us to compare the structures and levels of expression of different hsc82 alleles under native conditions. Our principal finding is that HSF, independently of other sequence-specific factors, creates an open chromatin configuration within the HSC82 promoter region. This conclusion is based on three lines of evidence. First, a 4-bp substitution of HSE1, which abolishes HSF binding (Fig. 4A), abrogates promoter-associated DNase I hypersensitivity (Fig. 6) and severely reduces TaqI accessibility (Fig. 7A). Second, the HSE1 mutation transforms the MNase cleavage profile into a ladder of discrete monoo- and oligonucleosome-length fragments. In contrast, the WT ladder is more heterodisperse and contains a nonnucleosomal, ~210-bp monomer-length fragment (Fig. 8 and reference 15). Third, mapping of MNase cleavage sites (Fig. 9A) indicates that the ~40 site, sharply cleaved in WT chromatin, is protected in nuclei purified from the ΔHSE1 strain.
together, these data are consistent with the presence of a localized nucleosome over the hsc82-ΔHSE1 promoter. That this nucleosome is not stably positioned is indicated by the absence of chromatin-specific DNase I or MNase cleavages mapping to the mutated promoter region (Fig. 6 and 9A). This occluded nucleoprotein structure represents a dramatic contrast to the accessible, nucleosome-free state of the WT promoter, which is characterized by the presence of stably bound TBP (Fig. 5D). Structural models for the HSC82* and hsc82-ΔHSE1 upstream regions are provided in Fig. 10.

Notably, mutations in any one of four other regulatory elements upstream of HSC82 have no comparable effect on promoter architecture. Indeed, HSE1 remains occupied and DNase I hypersensitivity remains essentially unaltered in hsc82
alleles bearing mutations in GRF2, URS1, HSE0, or TATA (Fig. 4 and 5; but see below). These results contrast with those of a recent study of the chicken β-globin gene enhancer, which found that hypersensitivity was determined by multiple factors which additively increased accessibility (9a). The findings reported here also extend previous observations suggesting that SchSF is capable of binding nucleosomal DNA in vivo (24, 54), for they demonstrate that it can do so independently of other sequence-specific factors. This observation also provides a striking contrast to the behavior of Drosophila HSF, whose in vivo access to the hsp70 promoter is critically dependent on DNA elements adjacent to the HSEs (58). In particular, displacement of nucleosomes by GAGA factor appears to be an essential prerequisite for HSF binding to Drosophila heat shock promoters (44, 46, 58). Similar remodeling of chromatin may be required for promoter occupancy by human HSF, given its inability to bind nucleosomal DNA in vitro (65), and Xenopus HSF, given its impaired ability to bind microinjected hsp70 promoter DNA templates lacking Y-box elements (37). Importantly, our data do not rule out a role for global activators such as the SWI/SNF or NURF complexes (28, 31a, 55) in facilitating ScHsf-directed chromatin remodeling.

HSF stimulates transcription through its ability to alleviate nucleosomal repression. In addition to its dramatic impact on promoter architecture, the ΔHSE1 mutation essentially abolishes expression of the gene, reducing basal transcription 25-fold and induced transcription >100-fold (Table 2). Three lines of evidence argue that this inactivation is due to active nucleosomal repression. First, the TBP footprint is erased (Fig. 5D), suggesting that the occluding structure in hsc82-ΔHSE1 blocks TBP access to the promoter, thereby preventing efficient transcriptional initiation. Second, the GRF2 site remains strongly occupied (Fig. 4A), implying the presence of stably bound Grf2p (see below) within the mutant promoter. Third, it has been previously shown that a GRF2 site can function as a UAS in an episomal context (where chromatin may be less stable than seen here), activating transcription to an extent comparable to that of other weak activators (12). Thus, the nucleosome present within the hsc82-ΔHSE1 core promoter may block the ability of Grf2p, and other DNA-bound factors, to stimulate transcription. We conclude that HSF functions as an antirepressor at HSC82, antagonizing nucleosomal repression of both basal and induced transcription.

The stably bound TFIIID complex limits core promoter accessibility. The twofold increase in Taq1 cleavage at hsc82-ΔTATA (Fig. 7B) is intriguing and implies that the TFIIID complex itself limits the accessibility of the promoter DNA to sequence-specific proteins. This is an unprecedented yet unsurprising observation, given the size of the yeast TFIIID complex (56) and the proximity of the TATA box (position -98) to the Taq1 site (position -140). Retention of an open promoter configuration at hsc82-ΔTATA argues against a significant structural role for the TATA box, consistent with comparable analyses of the Drosophila hsp26 heat shock gene (45) and the yeast HSP82, SUC2, and PHO5 genes (19, 28, 39).

GRF2 stabilizes in vivo protein-DNA interactions at HSE1 and TATA. Earlier mapping experiments suggested that the GRF2-binding protein (Grf2p/Reblp) creates a nucleosome-free zone of ~230 bp within the UASc of G4-L1, since deletion of the GRF2 site led to nucleosome encroachment over the mutated sequence (20). Stable occupancy of the GRF2 site in the hsc82-ΔHSE1 allele (Fig. 4A) argues that in contrast, Grf2p is incapable of preventing nucleosome formation over the hsc82 core promoter. This is most clearly shown by the Taq1 accessibility assay (Fig. 7A), which indicates that sequence within 60 bp of GRF2 is strongly occluded upon mutation of HSE1. Therefore, the ability of Grf2p ability to remodel chromatin structure appears to be context dependent. The alternative interpretation that a protein unrelated to Grf2p is actually bound to this site is unlikely for two reasons. First, the GRF2 element represents a perfect 8/8 match of conserved nucleotides to the Grf2p consensus (12, 41). Second, the pattern of guanine protection is virtually identical to that seen in an in vitro methylation interference assay with a related GRF2 site and partially purified Grf2p (11). Interestingly, the GRF2-binding factor may exert a mild repulsive effect on the adjacent upstream nucleosome, given that MNase cleavages are shifted ~20 bp downstream in the ΔGRF2 strain (Fig. 9B). A structural model of the hsc82-ΔGRF2 allele is presented in Fig. 10.

In addition to its mild effect on nucleosomal positioning, the GRF2 mutation affects protein-DNA interactions at other regulatory elements. The ΔGRF2 lesion weakens both enzymatic and chemical protection at HSE1 (Fig. 4A and 5A to C) and reduces DMS protection over the TATA box (Fig. 5D). These weakened interactions are not due to a fortuitous secondary mutation. Using genomic sequencing, we have confirmed the upstream sequence of the hsc82-ΔGRF2 allele between the URS1 and TATA elements (Fig. 5 and data not shown). Interestingly, mutation of either HSE1 or TATA has no detectable effect on GRF2 occupancy, arguing that such facilitation of binding is not reciprocal and therefore not reflective of true cooperativity. A very similar behavior has recently been described for the abundant DNA-binding protein RAP1, which appears to facilitate the in vivo binding of an adjacent activator at TFIIf in a nonreciprocal fashion (13a). Also noteworthy is the fact that HSE1 occupancy at either HSC82- or hsc82-ΔGRF2 is largely unaffected by heat shock (Fig. 5C). This is unexpected, given the previously observed enhancement of HSF binding to HSP82 accompanying heat shock (16, 23). While the basis for this difference is unclear, the presence of several functional HSEs at HSP82 may facilitate cooperative interactions between induced HSF trimers that are not possible at HSC82.

How might the GRF2 factor facilitate the binding of HSF to chromatin? Given the abundance of Grf2p (1,000 to 2,000 molecules per diploid cell [12]) and the fact that its binding sites are found within a large number of polymerase II and polymerase III regulatory regions, we suggest that one of its roles is to destabilize local histone-DNA interactions until relatively scarce gene-specific factors such as HSF are available for binding. Such an activity may contribute to both expression and structural phenotypes of the AGRF2 mutation. For example, the attenuated TBP-TATA interaction might be a consequence of the destabilized HSF-HSE1 complex, as discussed above. We have previously demonstrated that one role for promoter-bound HSF is to stabilize the interaction of TBP with the core promoter of the closely related HSP82 gene (24). Taken together with our previous results, the ΔGRF2 and ΔHSE1 phenotypes (Fig. 5D) provide compelling support for the notion that a critical physiological function of upstream activators is to increase recruitment of TBP to the promoter (13, 32, 33, 75).

Deletion of URS1 uncouples chromatin structure from gene activity. The absence of any detectable role for the URS1 element in regulating HSC82 transcription (Fig. 3) is surprising, given its perfect match to the extended consensus (64) and its reported activity as either a mitotic repressor (52, 63) or a meiotic activator (9, 64). The absence of URS1 activity may therefore reflect an unfavorable sequence context (47), or it may be attributable to its uncharacteristic position within the HSC82 promoter (upstream of the UAS), or both. A less
perfect but more favorably positioned URS1 motif is active as both a repressor and an HSE-dependent activator at HSPl2 (64). It is interesting that URS1 is more strongly occupied in the hsc82-ΔHSE1 allele than in HSC82+ (Fig. 4A and data not shown), implying that stably bound HSF antagonizes the association of protein(s) with URS1.

Consistent with its negligible role in regulating transcription, URS1 exerts no effect on the nucleoprotein architecture of the HSC82 core promoter. Interestingly, however, the sequence appears to actively participate in organizing the phasing frame of the upstream nucleosomal array. Specifically, the ΔURS1 mutation causes nucleosomes to shift at least 10 to 20 bp upstream, as well as become less precisely positioned with mutation, which is characterized by a translational shift toward the promoter. It would therefore appear that the combination of a weakly bound URS1 complex and tightly bound GRF2 factor (seen at both HSC82+ and hsc82-ΔHSE1 alleles [Fig. 4A]) precisely positions the adjacent nucleosomal array, since mutation of one regulatory element or the other both shifts and diffuses its translational setting.

A central structural role for HSF in S. cerevisiae. In this study, we tested the hypothesis that cis-regulatory elements within the HSC82 promoter regulate transcription, at least in part, through their ability to generate an accessible chromatin structure. As discussed above, only HSE1 mediates its effects through creation of an accessible, nucleosome-free promoter structure. This result, in conjunction with previous work demonstrating the ability of SchHF to disrupt or displace nucleosomes at the hsp82-ΔHSE1 promoter (24), suggests that a critical physiological role of SchHSF is to remodel the chromatin structure of yeast core promoters. This function clearly contrasts with that played by the GRF2-binding protein, which cannot alleviate nucleosomal repression at either hsc82-ΔHSE1 or GAL1, despite its ability to constitutively bind DNA in vivo (Fig. 4A and data not shown). It is notable that GAL4, and not Grf2p, is required for disruption of the TATA-associated nucleosome during transcriptional induction of GAL1 (6). The sharp contrast between GAL4 and SchHF on the one hand and Grf2p on the other is intriguing and fortifies the notion that only a subset of transcription factors are capable of creating an accessible, nucleosome-free chromatin structure at the core promoter, site of formation of the preinitiation complex. We have previously suggested that proteins that are capable of reconfiguring nucleosomal structure by designated POWER (promoter open window entry regulator) factors (24). What distinguishes these POWER activators, such as SchHSF, GAL4, steroid hormone receptors, and GAGA factor (4, 6, 51, 67–69), from other sequence-specific activators, such as Grf2p, NFI, Drosophila HSF, and especially TBP (this study and references 4, 24, and 58), is an interesting and as yet unanswered question.

ACKNOWLEDGMENTS
A. M. Erkine and C. C. Adams contributed equally to this work.

We thank Mike Hampsey and Chris Szent-Gyorgyi for critical reading of the manuscript, Ned Seckinger and Kate Dayton for technical assistance, and Seewoo Lee, David Finkelstein, and Sue Lindquist for reagents.

This study was supported by grants from the National Institute of General Medical Sciences (GM45842) and the Center for Excellence in Cancer Research at LSUMC to D.S.G.

REFERENCES