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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

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Heat shock genes are poised for rapid transcriptional activation in response to environmental stress. A universal structural characteristic of such genes is the presence of a nucleosome-free, DNase I hypersensitive promoter region. Here we investigate the structural and functional effects of mutating HSE1, the preferred heat shock factor (HSF) binding site upstream of the yeast *HSP82* gene. *In situ* deletion or substitution of this sequence reduces both basal and induced transcription by at least two orders of magnitude. Moreover, such mutations lead to a dramatic transition in chromatin structure: the DNase I hypersensitive region is replaced by two stable, sequence-positioned nucleosomes. One of these is centered over the mutated heat shock element, while the other—as revealed by DNase I genomic footprinting—is precisely positioned in a rotational sense over the TATA-initiation site. Overexpression of yeast HSF strongly suppresses the null phenotype of the induced *hsp82*-ΔHSE1 gene and re-establishes DNase I hypersensitivity over its promoter. Such suppression is mediated through sequence disposed immediately upstream of HSE1 and containing two low affinity heat shock elements. These data imply a critical role for HSF in displacing stably positioned nucleosomes in *Saccharomyces cerevisiae* and suggest that HSF transcriptionally activates *HSP82* at least partly through its ability to alleviate nucleosome repression of the core promoter.

Key words: DNase I hypersensitive sites/heat shock factor/nucleosomes/protein-DNA interactions/*Saccharomyces cerevisiae*/transcriptional regulation

Introduction

While most of the genomic DNA in a eukaryotic cell is packaged into nucleosomes, the upstream regulatory regions of active genes are generally assembled into chromatin structures hypersensitive to DNase I and free of typical nucleosomes (reviewed in Gross and Garrard, 1988; Elgin, 1988). A considerable body of evidence, from both *in vivo* and *in vitro* studies, indicates that nucleosomes can inhibit transcription initiation, either by hindering formation of preinitiation complexes at the TATA-initiation site or by preventing the binding of sequence-specific regulatory proteins at upstream activation sequences (UASs) or enhancers (reviewed in Grunstein, 1900a,b; Kornberg and Lorch, 1991; Felsenfeld, 1992) For example, promoter sequences assembled into a nucleosome *in vitro* cannot initiate transcription with either phage or mammalian RNA polymerase (Lorch *et al.*, 1987). However, when the TATA-binding factor, TFIID, is prebound to template DNA prior to nucleosome assembly, the potential for transcription is preserved (Workman and Roeder, 1987; Meisterernst *et al.*, 1990; Becker *et al.*, 1991). Compelling genetic evidence also exists supporting the notion that nucleosomes *per se* can regulate transcription. In the budding yeast *Saccharomyces cerevisiae*, altering the stoichiometry of the histone H2A-H2B and H3-H4 dimer sets alters the specificity of transcription initiation (Clark-Adams *et al.*, 1988), whereas nucleosome loss resulting from depletion of histone H4 leads to the induction of UAS-independent transcription (Han and Grunstein, 1988).

Two general mechanisms are employed by eukaryotic cells to ensure accessibility of regulatory sequences within promoter regions: either canonical nucleosomes are precisely positioned over such regions, or they are prevented from forming. In the promoter of the mouse mammary tumor virus (MMTV), access of the regulatory site is ensured by correct rotational alignment of the DNA helix on the surface of the nucleosome, allowing glucocorticoid receptor to bind, displace (or alter) the nucleosome, render accessible an adjacent NF1 binding site and activate transcription (Richard-Foy and Hager, 1987; Piña *et al.*, 1990). Similarly, positioning of nucleosomes along the promoter of the yeast *PHO5* gene exposes a PHO4 binding site in an accessible linker region in the uninduced state. Following induction, four positioned nucleosomes bracketing this UAS element are displaced, liberating stronger activation sequences and the TATA element (Almer *et al.*, 1986). Notably, when the displacement of nucleosomes is prevented, the gene cannot be transcriptionally activated (Straka and Horz, 1991).

Alternatively, nucleosome-free regions can be created by the DNA sequence itself or by boundary constraints imposed by proteins that block nucleosome formation. For example, polypurine·polypyrimidine sequences, often found in promoter regions, are known to repel histones *in vitro*; tracts longer than 80 bp cannot be assembled into nucleosomes (Kunkel and Martinson, 1981). In addition, GRF2, whose recognition site is associated with UASs, enhancers, centromeres and telomeres in yeast, creates a 230 bp nucleosome-free region upon binding DNA (Chasman *et al.*, 1990). A similar role in nucleosomal exclusion may be played by other abundant eukaryotic nuclear proteins, including Sp1 (Jongstra *et al.*, 1984), RAP1 (Devlin *et al.*, 1991), and ABF1 (Buchman and Kornberg, 1990). In the mouse β-major globin gene, a precisely positioned nucleosomal array spanning 4.5 kb is interrupted over the promoter and 5′ coding region by tightly bound proteins. This 700 bp nucleosome-free gap is found only in erythroid

cells, where the gene is either active or potentially active (Benezra *et al.*, 1986).

Heat shock genes are a class of genes whose upstream regulatory regions are marked by constitutive nuclease hypersensitive sites (Wu, 1980; reviewed in Gross and Garrard, 1988), a feature that may facilitate their rapid transcriptional response (Ashburner and Bonner, 1979). An important, as yet unanswered question is what establishes these nucleosome-free regions along the chromatin fiber. In *Drosophila,* at least three sequence-specific proteins may subserve this role. First, within the 5′ hypersensitive regions of *hsp26, hsp70,* and *hsp83,* sequences mapping to the TATA box are protected from nuclease digestion both prior to and following stress (Wu, 1984; Thomas and Elgin, 1988) indicating that TATA binding protein (TBP) may prevent the assembly of nucleosomes *in vivo* as it does *in vitro*. Second, crosslinking experiments with intact cells have indicated the presence of RNA polymerase II at the 5′ ends of the *hsp70* and *hsp26* genes both before and after heat shock (Gilmour and Lis, 1986; Rougvie and Lis, 1990), suggesting a potential role for this protein in excluding nucleosomes. Third, it has been shown that promoter sequence containing $(CT)_n \cdot (GA)_n$ repeats is essential for DNase I hypersensitive site formation within the *Drosophila hsp26* gene (Lu *et al.*, 1992), suggesting a potential role for the constitutively bound GAGA factor (Gilmour *et al.*, 1989; Thomas and Elgin, 1988) in displacing or altering nucleosomes *in vivo*. The human *hsp70* promoter is similarly characterized by the constitutive occupancy of several basal regulatory elements as assayed by *in vivo* footprinting (Abravaya *et al.*, 1991). A role for heat shock factor (HSF) in establishing nucleosome-free regions at the 5′ ends of heat shock genes of higher eukaryotes is unlikely, however, since metazoan HSF binds DNA only in response to heat shock (Wu, 1984; Sorger *et al.*, 1987; Larson *et al.*, 1988; Scharf *et al.*, 1990; Abravaya *et al.*, 1991). Indeed, neither human nor *Drosophila* HSF appears capable of binding to a nucleosomal template *in vitro* (Becker *et al.*, 1991; Taylor *et al.*, 1991).

Interestingly, HSF from *Saccharomyces cerevisiae* binds DNA both prior to and following heat shock (Sorger *et al.*, 1987; Jakobsen and Pelham, 1988; Gross *et al.*, 1990). This ability to bind DNA constitutively underlies HSF's role in directing basal-level transcription in yeast (McDaniel *et al.*, 1989; Park and Craig, 1989; Sorger, 1990). Here we address the role of yeast HSF (yHSF) in establishing the nucleosome-free, DNase I hypersensitive domain found at the 5′ end of the *HSP82* heat shock gene. We have found that deletion or substitution of HSE1, its preferred binding site within the upstream region, abolishes both transcription and DNase I hypersensitivity, and leads to the *de novo* appearance of stably positioned nucleosomes within the promoter and transcriptional unit. One of these nucleosomes is centered over the mutated heat shock element while the other is rotationally positioned over the TATA box and transcription initiation site. Overexpression of yHSF results in a dramatic, heat-shock-dependent re-establishment of DNase I hypersensitivity in *hsp82* alleles lacking HSE1, paralleled by a striking derepression of promoter function. Taken together, our data implicate yHSF as a critical determinant of nucleosome-free regions in *S.cerevisiae*, a function that may be central to its role as a transcriptional activator.

Results

In situ deletion or substitution of HSE1 severely reduces both basal and induced transcription

Within the *HSP82* upstream region, there are three sequences, termed HSEs 1–3, that exhibit significant homology to the heat shock element consensus (consisting of three or more tandem inverted repeats of *n*GAA*n*; Amin *et al.*, 1988; Xiao and Lis, 1988; see Figure 1A) and could potentially serve as binding sites for HSF. However, genomic footprinting has revealed that only the promoter-proximal heat shock element, HSE1, is detectably occupied in chromatin or in intact cells (McDaniel *et al.*, 1989; Gross *et al.*, 1990). Occupancy of HSE1 is constitutive, consistent with the notion that yeast HSF binds DNA in a stress-independent fashion (Sorger *et al.*, 1987; Jakobsen and Pelham, 1988; McDaniel *et al.*, 1989). To investigate the role that HSE1 and its associated protein complex play in regulating *HSP82*, we mutated this element *in situ* through a combination of oligonucleotide-directed mutagenesis and gene transplacement techniques. Successful transplacement of the wild-type allele was verified by Southern analysis alone or in combination with genomic sequencing (see below).

To assess the functional consequence of excising HSE1 and its flanking nucleotides from the *HSP82* promoter, we measured steady-state *HSP82* transcript levels in a haploid strain (termed **ΔHSE1**) bearing this chromosomal mutation. The 32 bp deleted in this strain, spanning −187 to −156 relative to the principal transcription start site of *HSP82*, corresponds to the region strongly protected in spheroplast lysates from enzymes and chemicals (S.F.Simmons, T.Diken and D.S.Gross, in preparation). As shown in Figure 2A and B, the 32 bp deletion effectively abolishes basal transcription and reduces induced expression two orders of magnitude following either an 11 min or 25 min heat shock. While this result suggests a dominant role for HSE1 in directing transcription of *HSP82*, the phenotype is considerably more severe than seen with point mutations of HSE1 (McDaniel *et al.*, 1989; Lee and Garrard, 1992; Gross *et al.*, in preparation). This may be the case if elements further upstream are functionally compromised by altered spacing. To rule out such a spacing effect, we substituted 32 bp of DNA sequence derived from the *PET56* structural gene—previously shown to lack promoter activity in *S.cerevisiae* (Struhl, 1985a)—for the region excised in **ΔHSE1**, creating a strain termed **ΔHSE1·**. As shown in Figure 2A and B, *hsp82* basal transcription is essentially abolished in the substitution strain. Following heat shock, detectable, albeit greatly reduced levels of expression are seen (diminished 60- to 100-fold relative to wild-type) (data summarized in Figure 2C). Therefore, as the phenotype of this mutant closely resembles that of **ΔHSE1**, we conclude that HSE1 is critically required for both basal and induced expression of *HSP82*.

DNase I hypersensitivity over the core and upstream promoter regions is lost upon deletion or substitution of HSE1

To assess the outcome of the UAS mutations on the nucleoprotein structure of *HSP82*, we mapped sites of protein–DNA interaction within the upstream regulatory region and transcription unit. Spheroplasts were generated

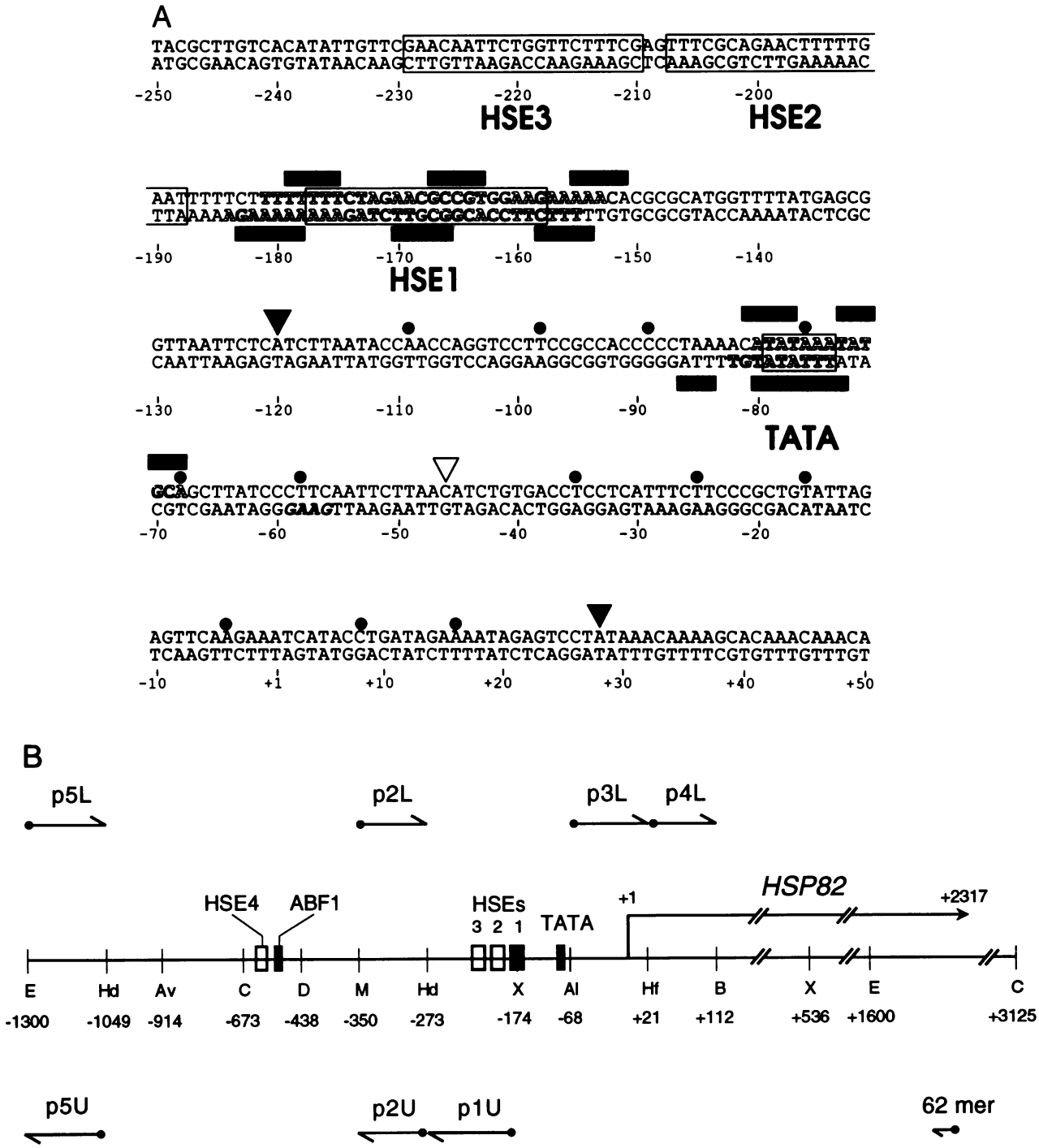
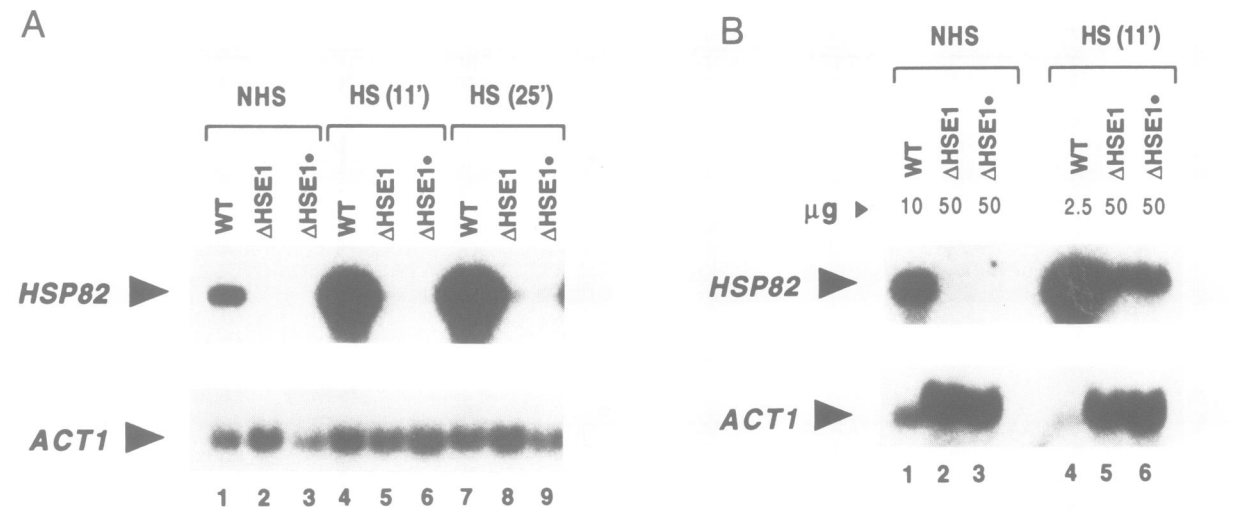


Fig. 1. *HSP82* upstream sequence, sites of chemical and enzymatic protection *in vivo*, and physical map of its chromosomal locus. (**A**) The upstream sequence is numbered relative to the principal transcription initiation site (+1) (Farrelly and Finkelstein, 1984). Elements bearing similarity to the heat shock consensus sequence (HSCS) are boxed; HSEs 1 to 3 exhibit 9/12, 9/12, and 8/12 matches, respectively, to conserved nucleotides of the HSCS (see Figure 2C). Also boxed is the TATA element, which exhibits a 6/6 match to the conserved TATAAA hexamer (Chen and Struhl, 1988). For the wild-type allele, guanines protected from DMS methylation in intact cells are indicated in bold, nucleotides protected from DNase I in spheroplast lysates are indicated by outlined letters, nucleotides diminished in their reactivity towards hydroxyl radical cleavage are delineated by black rectangles, and nucleotides hyperreactive to hydroxyl radical are indicated in bold italics (adapted from Gross *et al.*, 1990). For the *hsp82*-ΔHSE1 allele, upper strand nucleotides specifically cut by DNase I are indicated by filled circles; 5′ and 3′ extremities of the cleavage ladder are indicated by black triangles while its dyad axis is indicated by a white triangle (based on data presented in Figure 6B). (**B**) Physical map of the *HSP82* locus on chromosome XVI. The structural gene is indicated, as are putative binding sites for HSF (HSEs 1–4), TBP (TATA) and ABF1 (see text). Filled boxes represent regulatory sites demonstrated to bind sequence-specific binding proteins in intact cells or spheroplast lysates in the absence of overexpression (McDaniel *et al.*, 1989; Gross *et al.*, 1990; Lee and Gross, 1993; this paper). Also shown is the location of upper strand-specific (p1U, p2U, p5U) and lower strand-specific (p2L, p3L, p4L, p5L) RNA probes, synthesized *in vitro* as described by Gross *et al.* (1988). 62mer, upper-strand specific oligonucleotide used to detect *HSP82* mRNA (Adams and Gross, 1991). Restriction sites pertinent to this study are also depicted (Al, *Alu*I; Av, *Ava*I; B, *Bcl*I; C, *Cla*I; D, *Dra*I; E, *Eco*RI; Hd, *Hin*dIII; Hf, *Hin*fI; M, *Msp*I; X, *Xba*I).



C

STRAIN		HSP82 PROMOTER MUTATIONS		RELATIVE RNA LEVELS		
		HEAT SHOCK CONSENSUS NTTCNNGAANNTTCNNGAAN		NHS	HS (11')	HS (25')
WILD TYPE	-191	GAATTTTTCTTTTT TTTCTAGAACGCCGTGGAAG AAAAACACGCGCA	-145	100	1500	1500
ΔHSE1	-191	GAAT Δ AAACACGCGCA	-145	≤1	15	15
ΔHSE1•	-191	GAAT **GATCTCCTTTAGCTTCTCGACGTGGGCCTTTT** AAACACGCGCA	-145	≤1	15	25

Fig. 2. Transcriptional activity of wild-type and mutant alleles of *HSP82*. (**A**) Northern analysis of total cellular RNA (~15 μg per lane) isolated from early log phase cultures of strains W303-1B [wild-type (**WT**)], **ΔHSE1**, and **ΔHSE1•** cultivated under non-heat-shock (NHS) conditions (30°C) (lanes 1–3) or following a 39°C heat shock (HS) for either 11 min or 25 min (lanes 4–6 and 7–9, respectively). The membrane was sequentially hybridized with probes for *HSP82* and *ACT1*. (**B**) Northern analysis of total RNA isolated from **WT**, **ΔHSE1** and **ΔHSE1•** cells either prior to or following a 30°→39°C heat shock. To facilitate comparison of *HSP82* transcript levels, **WT** lanes were loaded with less RNA than mutant lanes as indicated. (**C**) Summary of relative *HSP82* transcript levels under non-shocked (NHS) and heat-shocked (HS) conditions for each of the three principal strains employed in this study. *HSP82* hybridization signals were quantified by laser densitometry and internally normalized to *ACT1*; each value represents the mean of at least three independent measurements. RNA levels for the mutant strains were quantified relative to those of the wild-type strain, which were assigned values of 100 and 1500 for non-shocked and heat-shocked states, respectively, based on a previous analysis (McDaniel *et al.*, 1989). *HSP82* transcript levels in strain SLY101, the isogenic *HSP82*$^+$ counterpart to strains **ΔHSE1** and **ΔHSE1•** (see Table I), are distinguishable from those of W303-1B (data not shown). The underlined sequence constitutes HSE1 (see Figure 1A); bold letters represent mutated nucleotides; outlined letters correspond to conserved nucleotides of the heat shock consensus (Xiao and Lis, 1988); Δ refers to a 32 bp deletion (−187 to −156, inclusive).

from both control and heat-shocked cells, lysed in a hypotonic buffer and mildly digested with DNase I. Double-strand cleavage sites were then mapped from an upstream position using indirect end-labelling. As shown in Figure 3, a pair of constitutive DNase I hypersensitive sites marks the promoter region of the wild-type allele, with centers of cleavage positioned ~260 and 100 bp upstream of the gene (lanes 2 and 6; indicated by filled and open circle, respectively), coinciding with the upstream and core promoter regions. These broad regions of hypersensitivity flank a strongly protected internal subdomain mapping to HSE1. Consistent with the severe functional phenotype, deletion or substitution of the heat shock element results in the loss of both hypersensitive sites (lanes 3 and 4) which are not restored upon heat shock (lanes 7 and 8). Accompanying the loss of DNase I hypersensitivity over the promoter is a dramatic increase in hypersensitivity further upstream (between −700 and −440). The contrast between cleavage profiles does not stem from different extents of digestion as lysates digested under very different conditions give similar results (see Figure 7B; also note similar intensity of the +1600 parental band for all chromatin samples in each experiment). While the significance of the −600 hypersensitive site is unclear, it should be noted that within this far upstream region lies a consensus ABF1 site and a potential HSF binding site (termed HSE4 in Figure 1B; see Figure 7B below).

The nucleosome-free phenotype of the HSP82 promoter is lost upon deletion or substitution of HSE1

To address more directly whether nucleosomes assemble over the *hsp82* promoter in either mutant strain, we digested nuclei from non-shocked and heat-shocked cells with micrococcal nuclease (MNase), an enzyme that preferentially cleaves the DNA linking adjacent nucleosomes. DNA was purified, electrophoresed on a native 2% agarose gel and blotted on to a nylon membrane. To examine the nucleosomal organization of the UAS region (defined as the region spanning HSEs 1–3), the membrane was hybridized with probe p1U (see Figure 1B for probe map). As shown in Figure 4A, the wild-type gene is cleaved within this region into a heterodisperse array of fragments, both before (−)

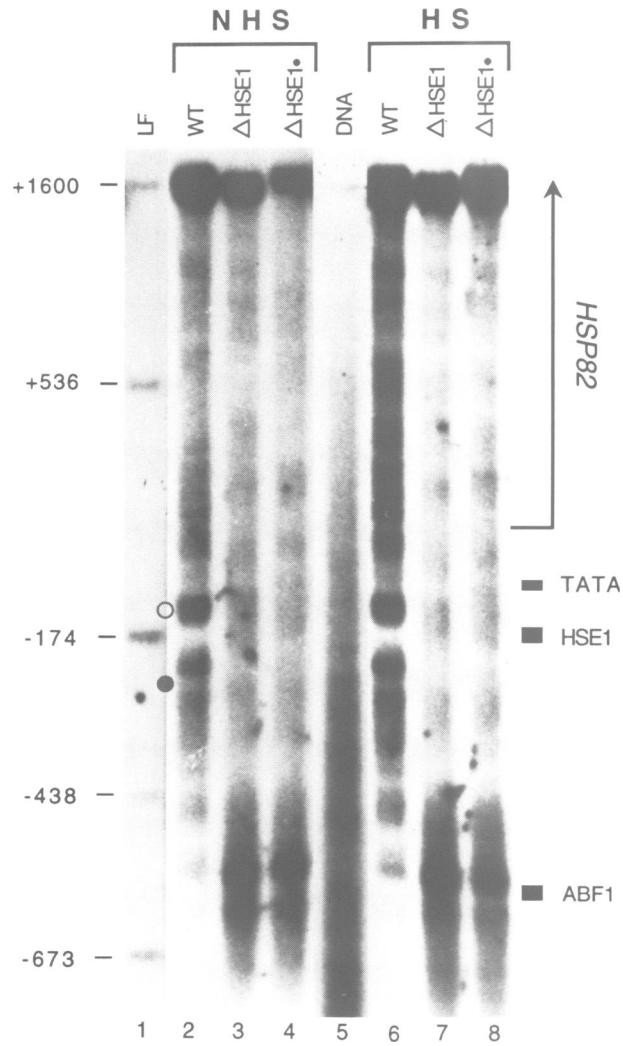


Fig. 3. DNase I chromatin footprints of wild-type and mutant alleles of *HSP82*. Spheroplast lysates from **WT**, **ΔHSE1**, and **ΔHSE1•** strains, non-shocked (NHS) and 15 min heat-shocked (HS) as indicated, were digested with 5×10^{-4} units DNase I/μg DNA for 80 min at 3°C. The DNA was purified, cleaved with *Eco*RI, electrophoresed on a 2% agarose gel, capillary blotted onto GeneScreen and indirectly end-labelled with probe p5L of Figure 1B. Naked genomic DNA (lane 5), purified from strain W303-1B, was digested with DNase I and processed similarly. Identification of pertinent landmark fragments (LF) is provided on the left. Locations of the two promoter-associated DNase I hypersensitive sites, centered 260 and 100 bp upstream of the *HSP82* structural gene, are indicated by the filled and open circle, respectively. Provided on the right are the locations and identities of protected sequences relative to the naked DNA control. Also depicted is the location of the *HSP82* transcriptional unit (arrow), mapped with respect to the underlying sequence. Samples represent non-contiguous lanes of the same gel; the composite shown here was aligned from uncut photographs and represents a single autoradiographic exposure. Similarly constructed composites are shown in Figures 4, 5, 6B and 7B.

and after (+) heat shock (lanes 1 and 2). This result, together with the DNase I hypersensitivity assay, confirms the absence of canonical nucleosomes over the *HSP82* promoter. In contrast, both mutated *hsp82* alleles are cleaved by MNase in a highly regular fashion to generate striking nucleosome-protected ladders irrespective of heat shock (lanes 5, 6, 9 and 10). Particularly noteworthy is the appearance of stable monomer- and dimer-length fragments, indicating the presence of nucleosomes within the upstream region of each allele. As a control for sample-to-sample variation in digestion, p1U was eluted and the membrane was rehybridized with a probe specific for a region of intergenic chromatin (IC) on chromosome XIII, unlinked to *HSP82* (see Materials and methods). Despite minor differences in the extent of digestion, clearly resolved nucleosomal ladders are present in all samples (Figure 4A, IC lanes). We conclude that the protein(s) binding to HSE1 prevents nucleosomal assembly of the upstream promoter in the wild-type allele; upon deletion or substitution of this sequence, typical nucleosomal structures are found within the UAS region.

To determine whether a comparable structural transition occurs over the transcription initiation site (Inr), MNase-digested samples from each strain were hybridized with probe p3L, spanning −68 to +21. In contrast to the heterodisperse array of fragments detected by the UAS-specific probe, p3L illuminates a discernable nucleosome-protected ladder in the wild-type allele ± heat shock, with a monomer fragment of ~170 bp. However, this cleavage product is clearly more diffuse than that generated within the control intergenic region (Figure 4B, compare lanes 1 and 2 with 3 and 4). Furthermore, mutation of HSE1 results in a distinctly sharper array of fragments, comparable to that seen at the intergenic locus (Figure 4B, compare lanes 5 and 6 with 7 and 8). We conclude that while the *HSP82* core promoter is cut into a nucleosome-sized fragment by MNase, substitution of the heat shock UAS with inert sequence results in an even more pronounced nucleosome phenotype within this region.

To investigate the possibility that the promoter-associated nucleosomes in strain **ΔHSE1•** are positioned with respect to the underlying DNA sequence, we mapped MNase cleavage sites within the upstream region using the indirect end-labelling technique as before. While the upstream region of the wild-type allele is cleaved in a pattern resembling that of naked DNA (Figure 5, compare lanes 2 and 3 with 1), prominent cleavages mapping to −290, −120 and +50 are seen within the *hsp82*-ΔHSE1• allele ± heat shock (lanes 4 and 5). In addition, chromatin-specific protections, centered at −375, −205 and −35, are readily discernible in **ΔHSE1•**. These results extend the foregoing analysis and suggest that upon regional deletion or substitution of HSE1, the nucleosome-free, DNase I hypersensitive region at the 5′ end of *HSP82* is replaced by two translationally positioned nucleosomes, termed **-I** and **-II**. The presence and location of these nucleosomes is strongly supported by DNase I mapping of the *hsp82*-ΔHSE1 allele, which reveals a very similar pattern of double-stranded cleavage and protection over the promoter [see Figure 7B (lanes 1 and 2) below]. Moreover, the presence of a phased nucleosome centered at −375 (**-III**) in both mutant and wild-type strains is suggested by the data in Figure 5 (lanes 2−5). Indeed, direct labelling of MNase cleavage products derived from the distal promoter confirms the presence of a nucleosome in strain **ΔHSE1•** [based on the presence of a sharp nucleosomal ladder detected by probe p2U (spanning −350 to −273)]. In contrast, the distal promoter region from the wild-type strain is cleaved into a heterodisperse array similar to that detected by the UAS-specific probe (data not shown), inconsistent with the presence of a stable nucleosome. Therefore, structural consequences of deleting or substituting HSE1 extend both upstream and downstream of the site of the lesion.

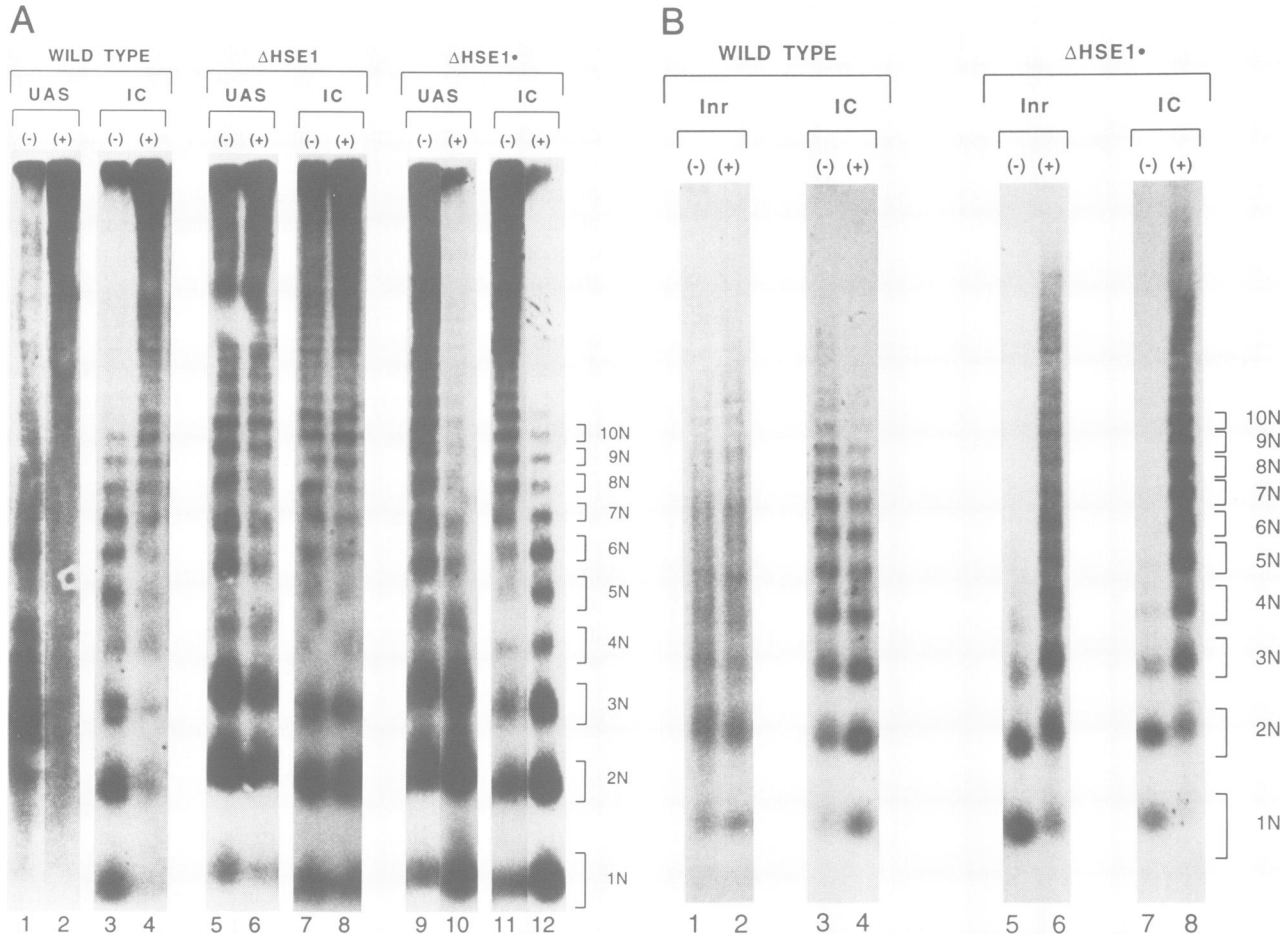


Fig. 4. Nucleosomal organization over the UAS and initiator (Inr) regions of wild-type and mutant *hsp82* alleles. **(A)** Nuclei, purified from control (−) and heat-shocked (+) W303-1B (wild-type), **ΔHSE1** and **ΔHSE1•** cells, were digested with 200 units of MNase per sample (~3×10^9 nuclei) for 25 min at 37°C. DNA was isolated, size-fractionated on a 2% agarose gel, capillary blotted onto a nylon membrane, and sequentially hybridized with an *HSP82* UAS-specific probe, p1U (UAS lanes), and probe C1, specific for a 730 bp region of intergenic chromatin (IC lanes; see Materials and methods). Heat shock was either 5 min (wild-type) or 15 min (**ΔHSE1**, **ΔHSE1•**). **(B)** Nuclei were isolated from wild-type and **ΔHSE1•** cells, not shocked (−) or 20 min heat-shocked (+) as indicated, and processed as above except the membrane was sequentially hybridized with the *HSP82* core promoter-specific probe p3L (Inr lanes) and probe C1 (IC lanes). In both panels, 1N−10N correspond to multiples of the nucleosome repeat length (~170 bp for UAS and Inr ladders, ~160 bp for IC ladders). MNase digestion of naked genomic DNA resulted in a random cleavage pattern in each region (data not shown).

A rotationally positioned nucleosome over the TATA-initiation site in strain ΔHSE1

To assess the effect of the HSE1 deletion on protein−DNA interactions within the *HSP82* upstream region in greater detail, we performed DNase I genomic footprinting. Spheroplast lysates, obtained from control and heat shocked cells, were digested with DNase I as described above, the DNA purified, restricted with *Msp*I and *Bcl*I, electrophoresed on a denaturing sequencing gel, vacuum blotted to a nylon membrane and indirectly end-labelling with hybridization probes specific for either upper or lower strand (p2U and p2L, respectively). As depicted in Figure 6A, the wild-type gene exhibits strong protection over HSE1, weak protection over TATA, and elsewhere a cleavage pattern virtually identical to naked genomic DNA (lane 3 versus 2). This result is consistent with previous *in vivo* footprinting experiments indicating that irrespective of heat shock, two principal sites of sequence-specific interaction exist within this region of the *HSP82* promoter (McDaniel *et al.*, 1989; Gross *et al.*, 1990).

In marked contrast to the wild-type cleavage pattern, there is no detectable occupancy of either upstream or downstream elements in strain **ΔHSE1**. Moreover, there is no compensating occupancy in the upstream region to at least position −350 (Figure 6B, lanes 3 and 4 and data not shown). Instead, a striking chromatin-specific modulation in the cleavage pattern is seen in the **ΔHSE1** samples from both non-shocked and heat-shocked cells. Such modulation is not seen in the naked DNA, nor is it present in wild-type samples electrophoresed in parallel (Figure 6B, lanes 2, 5 and 6). Specifically, 15 hyperreactive cutting sites, spaced at regular 10 or 11 bp intervals from −120 and +28, are apparent in the upper strand DNase I genomic footprint of *hsp82*-ΔHSE1 (Figure 6B, triangles and filled circles; data summarized in Figure 1A). A similar cleavage ladder is seen for the lower strand (data not shown). As DNase I prefers to cut across the minor groove on the exposed face of the nucleosomal DNA helix (Simpson and Whitlock, 1976; Lutter, 1978), this cleavage profile indicates that nucleosome **-I**, translationally positioned in both substitution and deletion strains (Figure 5 and data not shown), is precisely positioned in a rotational sense as well. Thus, the periodicity of cuts

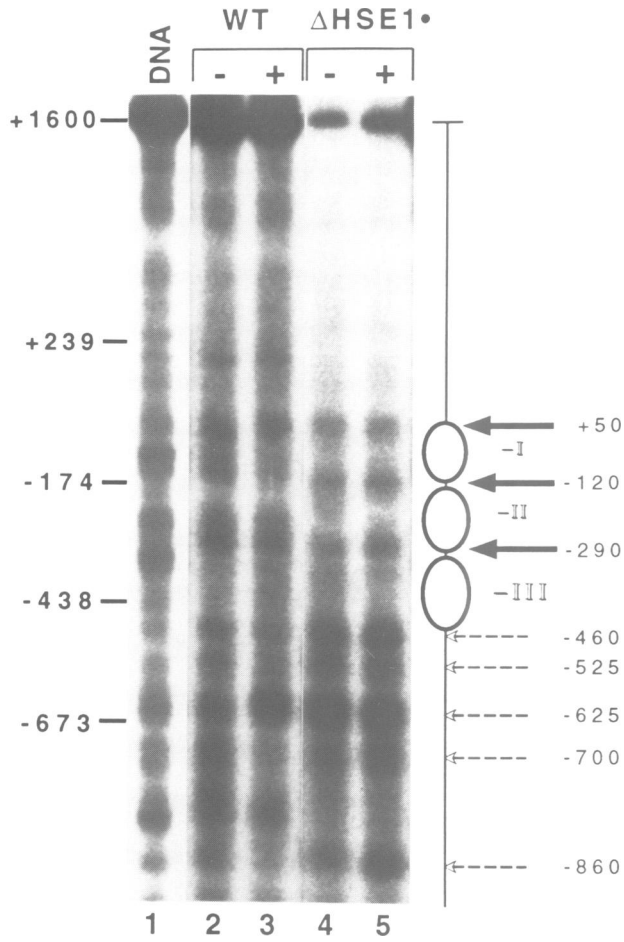


Fig. 5. MNase chromatin footprints of wild-type and **ΔHSE1**• alleles of *HSP82*. Nuclei, purified from control (−) and 15 min heat-shocked cells (+) of wild-type (**WT**) and **ΔHSE1**• strains, were digested with MNase as described in the legend to Figure 4. Following digestion, DNA was purified, restricted with *Eco*RI, size-fractionated on a 2% agarose gel and blotted onto a nylon membrane (lanes 2−4). A cloned *Eco*RI fragment of *HSP82* was purified and similarly digested (lane 1). *HSP82*-specific cleavages were visualized by indirect end-labelling with probe p5U and mapped using landmark fragments whose mobilities are indicated on the left. For strain **ΔHSE1**• (lanes 4 and 5), chromatin-specific cleavages are indicated in the diagram by bold arrows; these correspond to the linker regions of three sequence-positioned nucleosomes (ovals) designated **-I**, **-II** and **-III**. Broken arrows indicate the location of prominent cleavages in **ΔHSE1**• chromatin also seen in the naked control DNA (lane 1).

reflects the pitch of B-DNA on the surface of the histone octamer.

The average helical repeat length for DNA coiled around nucleosome **-I** (as deduced from the average distance between DNase I cuts), 10.5 bp/turn, is somewhat larger than that defined for a nucleosome reconstituted *in vitro* (Hayes *et al.*, 1990), but very similar to that reported for a nucleosome positioned adjacent to the α2 operator *in vivo* (Shimizu *et al.*, 1991). While sequences residing within or near the pseudodyad (position −46) are less frequently cleaved than those closer to the exterior (open triangle, Figure 6B), indicating a reduced accessibility of the nucleosomal interior to DNase I, three of the strongest cleavages map 20−40 bp from the pseudodyad, within the region of the TATA element (at −89, −76 and −68). The intensity and location of these cleavages suggests that while this element is at least partially accessible to its cognate factor, TBP, in spheroplast lysates, it is not strongly occupied. We conclude that in the absence of HSE1 and its flanking nucleotides, a rotationally positioned nucleosome assembles over the core promoter of *HSP82*, potentially blocking TBP−TATA interactions and impeding the formation of the preinitiation complex.

Overexpression of heat shock factor suppresses the mutant phenotype of the ΔHSE1 strains

The above analysis conclusively demonstrates that HSE1 is an essential determinant of the nucleosome-free state of the *HSP82* promoter. Moreover, it suggests a role for HSF in displacing or altering nucleosomes in this region, given that this protein has been implicated in binding HSE1 both *in vitro* and *in vivo* (McDaniel *et al.*, 1989; Gross *et al.*, 1990). However, the −187 to −156 region mutated in the **ΔHSE1** strains includes a poly(dT) sequence upstream and overlapping HSE1 (see Figure 1A) that could potentially serve as a binding site for a protein such as datin (Winter and Varshavsky, 1989). Similar sequences have been shown to activate yeast transcription both *in vivo* and *in vitro* (Struhl, 1985b; Lue *et al.*, 1989). To investigate more directly the role of heat shock factor in dictating the nucleosome-free region, we overexpressed yHSF in an attempt to suppress the effects of the chromosomal deletion. We reasoned that overexpression of the protein might result in its stable association with one or more low affinity HSF sites (e.g. HSE2 and HSE3) disposed upstream of HSE1. We therefore transformed strain **ΔHSE1** with *GAL1-HSF*, a chimeric gene in which the galactose-inducible *GAL1* promoter has been fused to the yeast *HSF* structural gene (Sorger and Pelham, 1988). When **ΔHSE1** cells bearing an episomal copy of *GAL1-HSF* were shifted from a non-inducing medium to one containing 0.5% galactose, intracellular HSF levels increased 15- to 30-fold over a 3.5 h period, as assessed by protein immunoblot analysis (data not shown). Strikingly, during this same period, heat-shock induced *HSP82* RNA levels increased nearly 20-fold (Figure 7A, lane 3 versus lane 1). The extent of derepression did not increase significantly with further incubation in galactose (lanes 4−7), reflecting a similar plateau in HSF levels. Non-induced *hsp82* expression remained undetectable throughout the 7.5 h timecourse (data not shown).

To ask if the suppression seen at the functional level was paralleled by any alteration of upstream chromatin structure, we performed a DNase I hypersensitivity assay. Spheroplast lysates were generated from cultures shifted to galactose for 0, 3.5 or 7.5 h and mildly digested with DNase I, and double-stranded cleavage sites were mapped using indirect end-labelling. In the absence of HSF overexpression, deletion of HSE1 leads to the loss of DNase I hypersensitivity over the *hsp82* promoter (Figure 7B, lanes 1 and 2), as seen earlier. However, in this experiment we electrophoresed DNA samples less extensively than in Figure 3, allowing a clearly resolved ladder of bands to be visualized. These bands map at ~170 bp intervals and span the gene from position −450 to at least +1400, indicating the presence of 11 positioned nucleosomes. Notably, the pattern is less distinct in the heat-shock induced **ΔHSE1** sample (lane 2), and is entirely absent in the wild-type sample (non-shocked; lane 7), consistent with the notion that transcriptional elongation results in the disruption of nucleosomes within the body of the *HSP82* gene (Lee and Garrard, 1991).

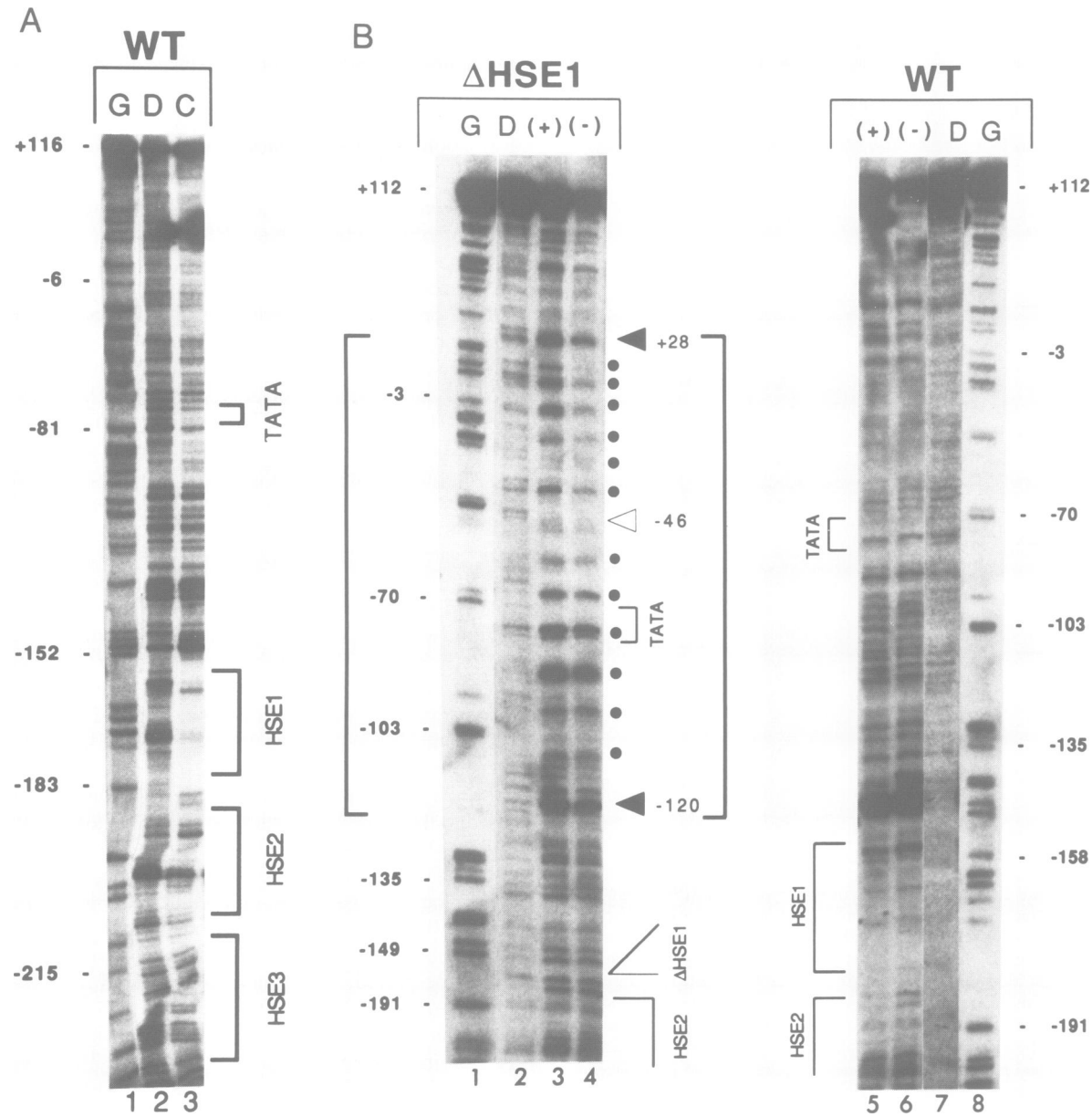


Fig. 6. Genomic footprinting of the *HSP82* upstream region in wild-type and **ΔHSE1** strains. (**A**) Spheroplast lysates, generated from non-shocked wild-type cells, were digested with DNase I at 37°C as described in Materials and methods. Genomic DNA was then purified, restricted with *BclI* and *Msp*I, size-fractionated on a 7.5% sequencing gel and vacuum blotted on to GeneScreen, and the lower strand was illuminated with probe p2L [chromatin (C), lane 3]. Deproteinized genomic DNA (D), restricted as above, was digested with DNase I and processed similarly (lane 2). G, guanine-specific sequencing ladder (lane 1). Locations of the TATA box and HSEs 1–3 are indicated on the right. (**B**) Spheroplast lysates, generated from control (−) and heat-shocked (+) cells of strains **ΔHSE1** (lanes 3 and 4) and W303-1B (**WT**, lanes 5 and 6) were digested with DNase I at 3°C and processed as described above except the upper strand was visualized by indirect end-labelling with probe p2U. Genomic DNA was purified from each strain, restricted with *BclI* and *Msp*I, and either digested with DNase I (lanes 2 and 7) or subjected to a guanosine-specific chemical cleavage reaction (lanes 1 and 8). The site of the −187 to −156 chromosomal deletion in strain **ΔHSE1** is indicated, as is the location of nucleosome **-I** (large bold brackets). Chromatin-specific cut sites spaced at 10 or 11 bp intervals (lanes 3 and 4) are indicated by dots. The extremes of the nucleosome core are delineated by black triangles while its pseudodyad is indicated by a white triangle. The intense cleavage mapping to position +112 in all samples represents the uncleaved *Msp*I–*BclI* parental fragment. The duration of the 39°C heat shock was 5 min for **WT** and 10 min for **ΔHSE1**. Rehybridization of **ΔHSE1** samples with a lower strand-specific probe (p4L of Figure 1B) confirms the presence of a strongly positioned nucleosome over the core promoter while ruling out the presence of an analogous structure further upstream (to position −350; data not shown).

Following galactose shift for 3.5–7.5 h, a progressive disruption of the DNase I cleavage profile is seen within the *hsp82* upstream region between −174 and −438 (Figure 7B, lanes 4 and 6). These alterations are more pronounced in heat-shock induced samples, consistent with the finding that only heat-shocked samples exhibit detectable transcription. Close inspection of the 7.5 h galactose-shifted sample reveals the presence of promoter-associated hypersensitivity similar to that seen in the wild-type allele (lane 6 versus lane 7). However, the hypersensitive site appears to be shifted upstream compared with the wild-type sample, with a region of internal protection mapping to HSEs 2 and 3 (between positions −190 and −220). An essentially identical result has been observed in strain **ΔHSE1** (data not shown). These

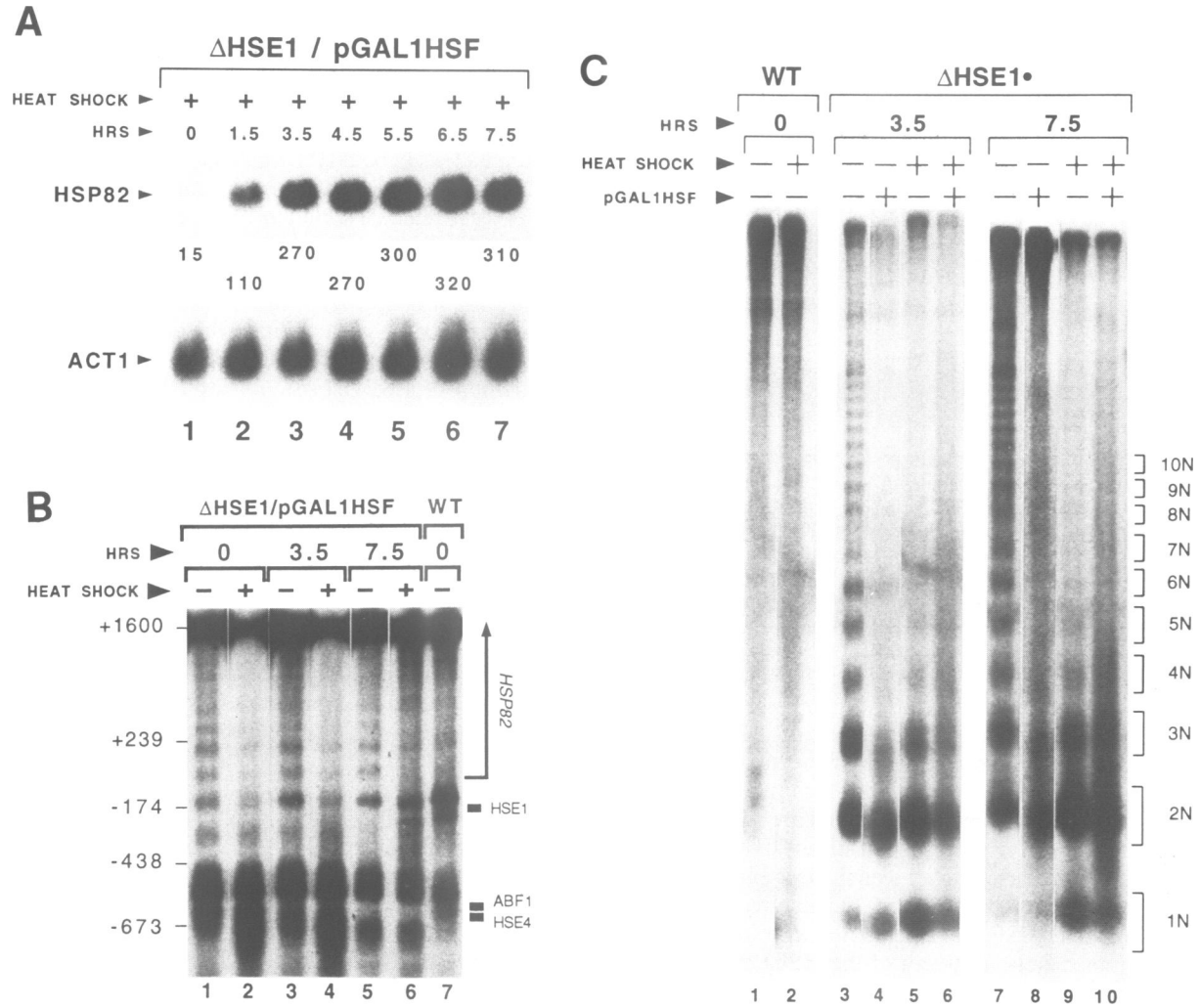


Fig. 7. Overexpression of yHSF suppresses the null phenotype of the *hsp82*-ΔHSE1 allele and reinstates promoter-associated DNase I hypersensitivity. (**A**) Northern analysis of pGAL1HSF-transformed **ΔHSE1** cells subjected to a 15 min, 30→39°C heat shock. Total cellular RNA was isolated from early log phase cells cultivated in synthetic medium containing 2% raffinose (lane 1) or following a shift to medium containing 1.5% raffinose/0.5% galactose for 1.5–7.5 h (lanes 2–7). Quantifications of relative *HSP82* RNA levels were done as described in the legend to Figure 2. (**B**) DNase I chromatin footprints of pGAL1HSF-transformed **ΔHSE1** cells non-shocked (−) or heat-shocked (+) as indicated. Cells were first grown at 30°C in 2% raffinose and then shifted to 1.5% raffinose/0.5% galactose. At the indicated times, aliquots were removed and either immediately converted to spheroplasts (−) or subjected to a 15 min 39°C heat shock prior to spheroplasting (+). Spheroplasts were lysed, then digested with 6×10^{-2} units of DNase I/μg DNA for 5 min at 37°C. The DNA was then processed as described in the legend to Figure 3 and samples were indirectly end-labelled with probe p5U (lanes 1–6). Lane 7, non-shocked, untransformed wild-type cells, grown in rich YPD medium, were converted to spheroplasts and handled similarly. The mobility of *HSP82* landmark fragments is provided on the left; the location of the transcription unit and pertinent upstream sequences is shown on the right. (**C**) MNase ladders of pGAL1HSF- (+) or YCp50-transformed (−) **ΔHSE1**• cells either not shocked (−) or heat-shocked for 15 min (+) as indicated. Cells were subjected to a 0.5% galactose shift as above. Nuclei were then isolated and digested with MNase, and the DNA was electrophoresed, blotted and hybridized to the UAS-specific probe, p1U, as described in the legend to Figure 4A.

results suggest that the sequence-positioned nucleosomes within the *hsp82* promoter of either mutant strain are either displaced or altered in the heat-shocked 7.5 h sample, and replaced by DNA-bound HSF. Also seen in the 7.5 h samples of either strain is a broad region of *de novo* protection mapping upstream of a consensus ABF1 site (lanes 5 and 6, and data not shown). Within this region lie seven non-consensus *n*GAA*n* modules between −654 and −608 (C.Szent-Gyorgyi, personal communication) that could potentially serve as a target for overexpressed HSF. The significance of the HSE4-associated footprint is presently unclear.

To confirm yHSF-mediated nucleosomal disruption more directly, we digested nuclei isolated from **ΔHSE1**• cells with MNase, and performed a nucleosome-protected ladder assay as described above. The results, shown in Figure 7C, clearly support this notion: cells transformed with pGAL1HSF, but not with vector alone, show marked galactose-dependent disruption of the nucleosome-protected ladder (UAS region probe). Notably, disruption is evident in both heat-shocked (lanes 6 and 10) and non-heat-shocked samples (lanes 4 and 8). A corresponding HSF-dependent alteration in the DNase I profile can be discerned within the −174 to −438 region in non-heat-shocked cells (Figure 7B, lane 5). Also as observed in the DNase I assay, the most complete suppression of the mutant phenotype is seen in the heat-shocked 7.5 h sample (lane 10). The integrity of the samples was assessed by rehybridizing them with an intragenic chromatin-specific probe as described above. In all cases, clearly resolved nucleosomal ladders were observed (data

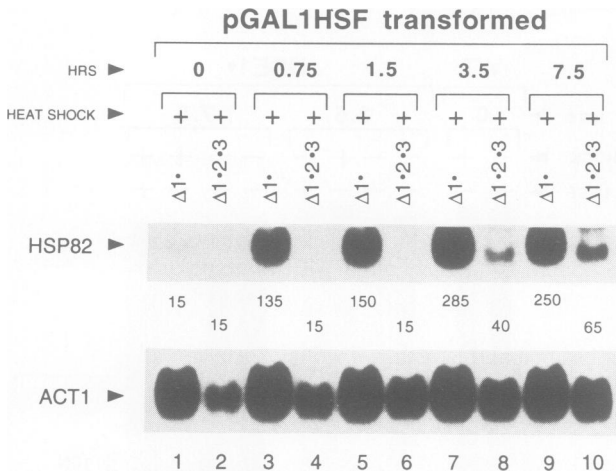


Fig. 8. Deletion of heat shock elements 2 and 3 virtually abolishes yHSF-mediated suppression of the *hsp82*-ΔHSE1· allele. Northern analysis of pGAL1HSF-transformed **ΔHSE1·** and **ΔHSE1·2·3** cells subjected to a 15 min, 30→39°C heat shock. Handling of samples and quantification of *HSP82* transcript levels were as in Figure 7A. Note that **ΔHSE1·2·3** samples, particularly in lanes 2 and 4, were somewhat underloaded (confirmed by ethidium bromide staining of 17S and 25S rRNAs).

not shown). We conclude that high levels of yHSF disrupt the stable nucleosomal structure over the mutant *hsp82* promoter region. Such alterations are more marked following transcriptional derepression, and are accompanied by a re-establishment of DNase I hypersensitivity strongly resembling the 5′ hypersensitivity seen in the wild-type allele.

Finally, to rule out a non-specific, global effect mediated by overexpressed yHSF, we constructed a strain in which the 42 bp immediately upstream of the HSE1 mutation in strain **ΔHSE1·** were deleted (spanning −229 to −188). This strain, lacking all three TATA-proximal heat shock elements and termed **ΔHSE1·2·3**, was transformed with the episomal *GAL1-HSF* gene as above. Following galactose-induced overexpression of yHSF, *hsp82* transcript levels in heat-shocked **ΔHSE1·2·3** cells are dramatically reduced relative to those seen in **ΔHSE1·** cells (Figure 8), particularly in the less extensively suppressed samples (lanes 3−6). This result is consistent with the notion that the sequence lying immediately upstream of the mutated heat shock element in the **ΔHSE1** strains mediates the effects of overexpressed yHSF. Also arguing for specificity is the fact that expression of the non-heat shock gene *ACT1* is unaffected throughout the 7.5 h galactose shift (Figure 7A and 8). In addition, HSF-mediated suppression of the *hsp82*-ΔHSE1 alleles does not occur by generally increasing *HSP82* transcription, since induced transcript levels of the wild-type gene are unaffected by HSF overexpression (data not shown).

Discussion

Deletion or substitution of HSE1 abolishes HSP82 promoter function and leads to a dramatic remodeling of its chromatin structure

In this study we show that *in situ* deletion or substitution of HSE1, the preferred HSF binding site upstream of *HSP82* (Figure 6A; McDaniel *et al.*, 1989; Gross *et al.*, 1990), reduces both basal and induced levels of transcription by at least two orders of magnitude. Moreover, such mutations result in a profound reconfiguring of the chromatin structure of *HSP82*. At the gene's 5′ end, the nuclease hypersensitive region spanning ~340 bp is replaced by sequence-positioned nucleosomes centered over both upstream and core promoter regions. Importantly, this effect on chromatin structure is *not* a consequence of transcriptional inactivation. Double point mutations in both HSE1 and the TATA box essentially inactivate *hsp82*; nevertheless, prominent DNase I hypersensitivity is retained within the mutated promoter (Lee and Garrard, 1992). Therefore, the upstream structural changes that we describe here stem directly from mutating HSE1, and are not a secondary effect of promoter inactivation.

Four lines of evidence indicate that a dramatic transition in chromatin structure occurs upon either deletion or substitution of HSE1. First, DNase I hypersensitive sites both upstream and downstream of HSE1 are abolished in each **ΔHSE1** strain. Second, MNase cleaves the upstream promoter region of either mutant *hsp82* allele, but not of wild-type, into stable, nucleosome-sized (~170 bp) DNA fragments. Third, indirect end-labelling of either MNase- or DNase I-generated DNA fragments reveals the *de novo* appearance of two translationally positioned nucleosomes at the 5′ end of the gene, one centered over the UAS and the other over the TATA-initiation site. Fourth and most striking, DNase I genomic footprinting of strain **ΔHSE1** reveals that the nucleosome mapping to TATA-initiation site (nucleosome **-I**) is in fact rotationally positioned with respect to the DNA helix. Notably, the nucleosome (**-II**) packaging the UAS region (site of the chromosomal deletion) is not itself rotationally positioned.

The base pair precision with which nucleosome **-I** is positioned resembles the precise positioning of nucleosomes downstream of the α2 operator in genes subject to α2 repression in *S.cerevisiae* (Shimizu *et al.*, 1991); such positioning requires interactions between the α2−MCM1 complex bound to the operator and residues in the amino-terminus of histone H4 within the nucleosome core (Roth *et al.*, 1992). In contrast, nucleosome **-I** appears to form spontaneously in the absence of HSE1. Significantly, it is assembled over a region of the promoter that is unaltered from the wild-type state, and may be the consequence of positioning determinants inherent in the underlying DNA sequence (Shrader and Crothers, 1989). Indeed, the region between −135 and −55 bears the highest concentration of nucleosome-positioning motifs—defined as $(A/T)_3nn(C/G)_3nn$—of any within the 4 kb *HSP82* locus (C.Szent-Gyorgyi, personal communication). Similar sequences are effective in rotationally positioning nucleosomes *in vitro* (Shrader and Crothers, 1989; Taylor *et al.*, 1991). Thus, in *HSP82*, the factor(s) bound to HSE1 must actively prevent the assembly of a strongly positioned, stable nucleosome over the core promoter *in vivo*.

Interestingly, there appears to be some form of nucleosome structure over the transcription initiation region in the wild-type promoter, based on the presence of an MNase-protected fragment of mononucleosome length (Figure 4B). This structure does not appear to be stable, since its presence is not detected by indirect end-labelling of either DNase I or MNase cleavage products (Figures 3, 5 and 7B). (Note that nucleosome **-I** in the **ΔHSE1** strains is readily detected in the same assays.) It is nonetheless conceivable that in the *HSP82* allele, histone binding to the core promoter is in a quasi-equilibrium with components of the general transcriptional machinery whereby the strongly preferred state is the open, DNase I hypersensitive structure.

Comparable competition between upstream regulatory factors and histones for binding to the UAS region does not appear to occur; by all criteria, the UAS appears to be constitutively nucleosome-free. Structural models of the *HSP82* and *hsp82*-ΔHSE1· promoters, consistent with chromatin and genomic footprinting results presented here and elsewhere (Gross *et al.*, 1990), are presented in Figure 9.

Of relevance to this study is the observation that while a double point mutation within HSE1 (termed **P2**) abolishes *hsp82* basal transcription, it has relatively little effect on the level of heat shock-induced transcription, presence of 5′ DNase I hypersensitivity (McDaniel *et al.*, 1989; Lee and Garrard, 1992), or pattern of MNase cleavage (M.Gao and D.S.Gross, unpublished observations). This is in spite of the fact that protein–DNA interactions at the mutated element are undetectable both *in vitro* and *in vivo* (McDaniel *et al.*, 1989). Thus, the more extensive HSE1 mutations described in this study—spanning the entire DNase I footprinted region (McDaniel *et al.*, 1989; Gross *et al.*, 1990; this paper)—cause a considerably more severe phenotype than the HSE1 double point mutation. Whether this reflects the retention of weak HSF–HSE1 interactions within the *hsp82*-P2 upstream region and/or the presence of additional factors in *hsp82*-P2 whose binding is abolished in the *hsp82*-ΔHSE1 alleles is currently being investigated. Whatever the basis, it is noteworthy that overexpression of yHSF in the ΔHSE1 strains effectively suppresses the phenotypic difference between the two alleles.

HSF is implicated in establishing the nucleosome-free, DNase I hypersensitive region upstream of HSP82

Previous studies have suggested that TBP is capable of potentiating eukaryotic promoters for transcription in the context of chromatin. DNase I protection analyses of *Drosophila* chromatin indicate that in contrast to HSEs, the TATA boxes of heat shock genes remain occupied both prior to and following heat shock (Wu, 1984; Thomas and Elgin, 1988). Moreover, incubation of promoter DNA with TFIID (or recombinant TBP) before, but not after, nucleosomal assembly preserves the transcriptional competence of both viral and chromosomal genes (Workman and Roeder, 1987; Meisterernst *et al.*, 1990; Becker *et al.*, 1991). The structural experiments reported here, in contrast, clearly indicate that retention of the TATA box is not sufficient to allow a potentiated chromatin structure to assemble within the upstream regulatory region of *HSP82*. Rather, preservation of HSE1 is necessary for establishment of the nucleosome-free region over the promoter. These observations thus suggest a critical role for DNA-bound HSF in displacing or excluding nucleosomes *in vivo*, an idea strengthened by the overexpression experiments (see below). Therefore, it appears that TBP, in the absence of stably bound HSF, is unable to prevent nucleosomal assembly over the promoter. The relative unimportance of the TATA element in establishing the DNase I hypersensitive site is further indicated by the essentially normal chromatin structure of an *hsp82* strain bearing a double point mutation in the conserved TATAAA motif (Lee and Garrard, 1992).

Several additional observations strengthen the notion that HSF plays a critical role in establishing nucleosome-free, DNase I hypersensitive regions in *S.cerevisiae*. First, overexpression of yHSF suppresses the essentially null *hsp82*

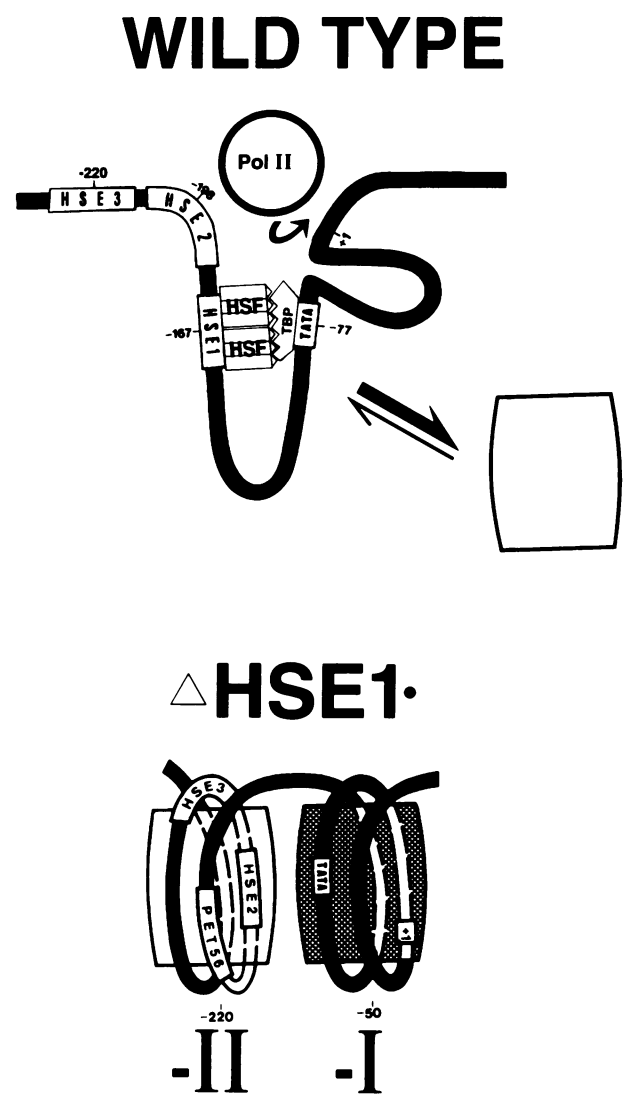


Fig. 9. Proposed protein–DNA interactions within the *HSP82* promoter region of wild-type and **ΔHSE1·** strains. Hypothetical nucleoprotein structures are based on DMS, hydroxyl radical and DNase I genomic footprinting data for the wild-type allele (Gross *et al.*, 1990) and DNase I genomic footprinting data for the **ΔHSE1·** allele (this study). In particular, evidence has been obtained suggesting that in the wild-type allele, HSF preferentially binds HSE1 in chromatin (±heat shock) as either one or two complexes, that HSF and TBP bind on the same face of the DNA helix, and that helical distortion exists 15 bp downstream of the TATA box (depicted here as a locus of DNA looping) (Gross *et al.*, 1990). Other features of the illustration are oversimplified; e.g. the fraction of wild-type promoters containing a preinitiation complex (indicated here as 'Pol II') has not been determined. Moreover, an unstable nucleosome may exist over the core promoter of the wild-type allele (depicted here as a histone octamer in quasi-equilibrium with downstream factors) (see text). In the *hsp82*-ΔHSE1· allele, the above protein–DNA interactions are replaced by two translationally positioned nucleosomes, designated **-I** and **-II**, localized over the downstream and upstream promoter regions, respectively. Nucleosome **-I** is extremely stable, being rotationally positioned with respect to the underlying DNA helix (indicated by shading). A third sequence-positioned nucleosome (**-III**), located in the distal promoter region (centered at position −375), is also present (not shown). Architecture of the *hsp82*-ΔHSE1· allele is based on data presented in Figures 3–7.

phenotype of either deletion or substitution strain, and reinstates DNase I hypersensitivity at the 5′ end of each mutant allele. This dramatic, HSF-dependent transition in *hsp82* chromatin structure is also detected using MNase,

which clearly shows evidence for nucleosomal disruption over the UAS in the non-heat-shocked state (ruling out the possibility that this upstream chromatin change is a consequence of transcriptional induction). Second, we have observed a similar loss of DNase I hypersensitivity 5′ of the constitutively expressed *HSC82* heat shock gene following *in situ* mutagenesis of its principal HSE. Notably, four point substitutions, altering only *n*GAA*n* modules within the HSE, are sufficient to elicit this striking phenotypic change (C.C.Adams and D.S.Gross, unpublished results). Third, Costlow *et al.* (1985) found that the native pattern of DNase I hypersensitivity 5′ of the *Drosophila hsp70* gene is preserved when this gene is integrated into the yeast genome. Upstream deletion to −74 has no effect on hypersensitivity, whereas deletion to −43 abolishes the parental pattern. As the latter deletion disrupts the strong HSE upstream of *hsp70* (site I), loss of HSF binding may underlie this finding as well.

How might HSF establish a nucleosome-free region over the *HSP82* promoter? In theory, it could work through either of two mechanisms, exclusion or displacment (Felsenfeld, 1992; Workman and Buchman, 1993). In a nucleosome exclusion scenario, HSF gains access to its binding sites immediately following DNA replication, when they are transiently free of nucleosomes. Subsequent nucleosome assembly is then blocked, directly or indirectly, by HSF's DNA binding domain and/or transcriptional activator domains. In a nucleosome displacement scenario, HSF directly binds a nucleosome and then destabilizes or displaces it in the absence of DNA replication. Neither mechanism is formally ruled out by our results. The fact that remodeling of the upstream chromatin structure of *hsp82*-ΔHSE1 requires a galactose shift of >3.5 h in duration (sufficient time for at least one cell division) is consistent with an exclusion mechanism. However, since establishment of DNase I hypersensitivity occurs exclusively within the 15 min, 30−39°C heat shock (Figure 7B, compare lanes 4 and 6 with 3 and 5), it is clear that profound alterations in upstream chromatin structure occur in a relatively short time frame, suggesting that displacement, particularly of the downstream nucleosome, accompanies transcriptional activation. Also consistent with a displacement mechanism is the observation that transcriptional suppression of the *hsp82*-ΔHSE1 allele is not blocked by a prior arrest of cells in G1 (B.Stentz and D.S.Gross, unpublished results), indicating that overexpressed HSF can mediate its functional effects in the absence of transit through S phase. Although further experiments are necessary to distinguish rigorously between these two mechanisms, the available evidence would seem to favor displacement, at least of nucleosome **-I**.

In this regard, yHSF resembles PHO4 and GAL4, which have been shown to disrupt stable nucleosomes over the downstream promoters of the *PHO5* and *GAL1* genes, respectively (Fascher *et al.*, 1990; Schmid *et al.*, 1992; Axelrod *et al.*, 1993). Eviction of promoter-associated nucleosomes is probably a common mechanism by which transcriptional activators operate in eukaryotes (Grunstein, 1990b; Adams and Workman, 1993). Indeed, promoter-binding proteins have been shown to facilitate the formation of preinitiation complexes on reconstituted nucleosomal templates (Workman *et al.*, 1990; Laybourn and Kadonaga, 1991; Lorch *et al.*, 1992). A role for activator domains in preserving promoter function during nucleosomal assembly has been suggested by *in vitro* studies (Workman *et al.*, 1991; Croston *et al.*, 1992), consistent with observations that transactivation functions of estrogen receptor derivatives and GAL4 facilitate disruption of chromatin structure *in vivo* (Pham *et al.*, 1991; Axelrod *et al.*, 1993). Likewise, we have presented evidence that the *hsp82*-ΔHSE1 phenotype suppression requires heat shock, implicating a role for the transcriptional activation domains of yHSF (Sorger, 1990; Nieto-Sotelo *et al.*, 1990) in nucleosome disruption or displacement. Whether prebound yHSF directly antagonizes histone binding to the core promoter or does do indirectly—by recruiting other components of the transcriptional apparatus—is not addressed in our experiments. We suggest that DNA-binding proteins which serve dual structural and functional roles—such as PHO4, GAL4, yHSF and steroid hormone receptors (Perlmann and Wrange, 1988; Piña *et al.*, 1990; Archer *et al.*, 1991)—be designated *POWER* (promoter open window entry regulator) factors.

Implications for heat shock factor function in yeast

Our data indicate that yHSF, in addition to functioning as a conventional transcriptional activator, plays a critical role in *S.cerevisiae* in establishing nucleosome-free regions. It is intriguing that even in the absence of its preferred binding site, yHSF is capable of disrupting the stable nucleosomal structure over either **ΔHSE1** promoter. These effects appear to be mediated through HSEs 2 and 3, since chromosomal deletion of this region, coupled with a 32 bp substitution of HSE1, greatly reduces the extent of suppression. As HSEs 2 and 3 map to the center of positioned nucleosome **-II** (see Figure 9), it would seem possible, as discussed above, that yHSF binds nucleosomal DNA *in vivo*, at least under conditions of overexpression.

How can these results be reconciled with those of Kingston and co-workers, which demonstrated that heat-shock activated human HSF (hHSF) is incapable of binding even high affinity HSEs (consisting of as many as six perfect *n*GAA*n* units) assembled into nucleosomes *in vitro* (Taylor *et al.*, 1991)? It is possible that the different outcomes reflect a functional divergence between yeast and human HSFs. For example, there exists no significant amino acid sequence homology outside of the DNA binding and amino-terminal trimerization domains (Rabindran *et al.*, 1991; Schuetz *et al.*, 1991). The greater size of yHSF (~60% larger than hHSF1) further suggests the possibility that the yeast factor possesses novel function(s), particularly in its activation domains, that may account for the difference between our results and those of Taylor *et al.* (1991). A second possibility is that yeast and human HSFs have the same function, but that yHSF is facilitated in its binding to nucleosome **-II** within the *hsp82*-ΔHSE1 promoter by one or more components of the preinitiation complex. Indeed, hHSF binding to a nucleosomal template is strongly facilitated by the prior binding of yeast TFIID (Taylor *et al.*, 1991). Third, as histone acetylation has recently been shown to facilitate TFIIIA binding to a reconstituted nucleosome (Lee *et al.*, 1993), a similar requirement may apply to yHSF. Yeast histones exhibit high levels of endogenous acetylation (Nelson, 1982) while core histones from amphibian erythrocytes or human HeLa cells, employed in the study of Taylor *et al.* (1991), are considerably less acetylated (van

Holde, 1989). Fourth, yHSF may recruit other factors, such as products of the *SWI* and *SNF* genes (reviewed in Winston and Carlson, 1992), to assist it in nucleosome binding and/or displacement. Finally, there may in fact be no difference: yHSF may disrupt nucleosome **-II** by an exclusion rather than displacement mechanism as discussed above. Future experiments will be required to distinguish between these and other possibilities.

In conclusion, we have provided evidence that HSF regulates chromatin structure in *S. cerevisiae*. Within the *HSP82* promoter, HSF not only releases the underlying nucleosome (**-II**), but also disrupts the adjacent, stably bound nucleosome centered over the core promoter (**-I**). That a rotationally positioned nucleosome can spontaneously form over the TATA-initiation site of *hsp82* in the absence of HSE1 is striking. This phenomenon is unlikely to be novel to *hsp82* and may reflect a general tendency of yeast core promoter DNA sequences to direct the assembly of positioned nucleosomes in the absence of upstream regulatory factors. It also points to the necessity of POWER activators that can both antagonize the repressive effect of histones (through disruption or displacement of nucleosomes) and stimulate transcriptional activation (by catalyzing the rate-limiting step(s) in the formation of the preinitiation complex). Yeast HSF therefore presents a striking contrast to *Drosophila* HSF, which binds DNA only following heat shock and appears to play virtually no role in establishing the chromatin structure of the *hsp26* heat shock gene (Lu *et al.*, 1993). There, $(CT)_n$ elements are necessary (but not sufficient) for establishing the DNase I hypersensitive region (Lu *et al.*, 1992). It would thus appear possible that yHSF embodies the functions of both GAGA factor and *Drosophila* HSF. Whether this capability is intrinsic to yHSF *per se*, or is a property of heteromeric complexes that it may form *in vivo*, is currently being investigated.

Materials and methods

Materials

Oligonucleotides were purchased from Oligos Etc. Inc. Enzymes were from Promega, New England Biolabs or United States Biochemical Corporation, except lyticase (Sigma), oxalyticase (Enzogenetics; Corvallis, OR), micrococcal nuclease (Pharmacia LKB) and DNase I (DPRF grade; Organon Teknika, Malvern, PA). Nylon blotting membranes were from either Bio-Rad (Zeta-Probe) or DuPont (GeneScreen). Radionucleotides were acquired from New England Nuclear or ICN. *Saccharomyces cerevisiae* strains used in this study are listed in Table I.

Table I. Yeast strains used in this study

Strain	Genotype	Source
W303-1B	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1*	R.Rothstein
SLY101	*MATα, ade2, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3, cyh2^r*	Lee and Gross (1993)
SLY102	Isogenic to SLY101; *hsp82Δ::CYH2^2*	Lee and Gross (1993)
ΔHSE1	Isogenic to SLY101; *hsp82*-ΔHSE1	This study
ΔHSE1·	Isogenic to SLY101; *hsp82*-ΔHSE1·	This study
ΔHSE1·2·3	Isogenic to SLY101; *hsp82*-ΔHSE1·2·3	This study

In vitro mutagenesis

Oligonucleotide-directed mutagenesis was performed essentially according to Kunkel *et al.* (1987). A 2.9 kb *Eco*RI *HSP82* fragment, spanning −1300 to +1600 relative to the transcription start site (+1) (Farrelly and Finkelstein, 1984), was subcloned in its inverse orientation into M13mp18, creating a construct termed KEM101. Mutant oligonucleotides were annealed to uracil-containing KEM101 (propagated in CJ236 [*dut*$^-$, *ung*$^-$]) and subsequently subjected to extension and ligation. The resultant double-stranded product was then transformed into JM109 (*dut*$^+$, *ung*$^+$) to permit selection against the wild-type strand. The mutated fragment, confirmed by dideoxy sequencing, was subcloned into the unique *Eco*RI site of the yeast integrating vector, p102 (Lee and Gross, 1993). The resulting construct was then transformed into yeast strain SLY102 to mediate gene transplacement at the *HSP82* locus as described below.

Yeast strain construction

The *hsp82Δ::CYH2^s* disruption strain, SLY102, was used as the recipient for gene transplacement. In this strain, the *HSP82 Xba*I fragment, spanning −174 to +536 (Figure 1B), has been replaced with a 1.6 kb *Pst*I fragment containing the *CYH2^S* gene (Lee and Gross, 1993). Inactivating mutations can be introduced into the *HSP82* promoter since *hsp82* null mutants are viable due to the presence of the constitutively expressed *HCS82* gene, whose protein product is 97% homologous to HSP82 (Borkovich *et al.*, 1989). To construct isogenic *hsp82* promoter mutants, SLY102 was transformed with *Ava*I-linearized derivatives of p102 (*Ava*I cuts uniquely at position −914 of the *HSP82*$^+$ allele) as previously described (Lee and Gross, 1993). Ura$^+$ transformants were recovered, and cells that spontaneously excised plasmid and duplicated genomic sequences by homologous recombination were selected on medium containing 5-fluoroorotic acid. Clones that replaced the *hsp82Δ::CYH2^s* allele with the desired promoter mutation were screened on medium containing cycloheximide.

The structure of each *hsp82* mutant allele was verified by genomic Southern analysis. Briefly, DNA was purified from overnight YPD cultures using a rapid glass bead lysis technique (Hoffman and Winston, 1987), digested with *Xba*I and *Cla*I, electrophoresed on a 1.5% agarose gel and blotted to Zeta-Probe by alkaline capillary transfer (Reed and Mann, 1985). DNA was covalently crosslinked to the nylon membrane (Stratolinker model 1800 set at 120 000 μJ) and then subjected to hybridization with probe p1U. Such an analysis confirmed replacement of the 4.4 kb *hsp82Δ::CYH2^s* fragment with a 1208 bp fragment in strain **ΔHSE1·**, an 1176 bp fragment in strain **ΔHSE1** and an 1166 bp fragment in strain **ΔHSE1·2·3** (data not shown). Moreover, the chromosomal lesion in strain **ΔHSE1** was verified at the nucleotide level by genomic sequencing (Figure 6B).

Cultivation conditions

Untransformed strains were cultivated at 30°C in rich growth medium (YPD) to a final density of $2.5-5\times10^7$ cells/ml [corresponding to a Klett reading (red filter) of 60−130]. In a typical heat shock experiment, the culture was shifted to 39°C by rapid mixing with an equivalent volume of medium prewarmed to 51°C; cells were then maintained in a 39°C shaking waterbath for either 11 min or 25 min. Heat shock was terminated by chilling the cells on ice and adding sodium azide to a final concentration of 20 mM. This concentration of azide effectively terminates heat shock-induced transcription of *HSP82* within seconds (Lee and Garrard, 1991).

Strains transformed with either a *GAL1-HSF* fusion gene borne on the centromeric *URA3*-containing plasmid, pGAL1HSF (Sorger and Pelham, 1988; generously provided by P.K.Sorger) or vector alone (YCp50) were inoculated in synthetic complete medium lacking uracil and containing 2% raffinose. Cultures were grown at 30°C to early logarithmic phase, harvested and resuspended in synthetic medium containing 1.5% raffinose/0.5% galactose, and grown for an additional 1.5−7.5 h at the same temperature. Cells were pregrown in a non-catabolite repressing carbon source to permit rapid induction of the *GAL1* promoter (strong induction within 10−20 min of galactose shift; Adams, 1972). For protein and RNA extractions, 10 ml aliquots were removed at regular intervals following the shift to galactose-containing medium and sodium azide was either added immediately (control) or subsequent to a 15 min, 30−39°C heat shock. For DNase I and MNase digestions of chromatin, 50 ml aliquots were used and handled similarly.

Protein isolation and immunoblot analysis

Whole cell extracts were prepared from the *hsp82* strains **ΔHSE1** and **ΔHSE1·** essentially as described by Harlow and Lane (1988). In an effort to minimize proteolysis, the lysis buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris−HCl, pH 8) was fortified with protease inhibitors (1 PMSF, 2 mM benzamidine, 2 mM *N*-ethylmaleimide, 1 mM EDTA, 2 μg/ml pepstatin, 2 μg/ml antipain, 0.6 μg/ml chymostatin and 7.2 μg/ml E-64). Two hundred micrograms of total cellular protein,

quantified by the Bio-Rad protein assay (using BSA as a standard), were dissolved in SDS sample buffer containing the same protease inhibitors. Samples were electrophoresed on a 10% SDS–polyacrylamide gel at 23°C and then electroblotted to a nitrocellulose membrane. Immobilized proteins were visualized by staining with India ink, then the blot was incubated with rabbit antiserum specific to yeast HSF (kindly provided by P.K.Sorger) at a dilution of 1:500. Specifically bound antibody was detected using ^{125}I-labelled protein A as described by Towbin *et al.* (1979). Preflashed XAR5 film (Kodak) was exposed to the membrane at −70°C using a DuPont Cronex Lightning plus intensifying screen and the resultant autoradiogram quantified by laser densitometry (LKB Bromma UltraScan). Quantifications cited in the text are based on multiple exposures taken within the linear response range of the film.

Northern blot analysis

Total RNA was extracted from control and heat-shocked cells, electrophoresed, vacuum-blotted to GeneScreen and hybridized to gene-specific probes as previously described (Adams and Gross, 1991). *HSP82* transcripts were detected using an oligonucleotide probe complementary to the 3′ untranslated region [spanning positions +2226 to +2287 relative to the cap site (62mer of Figure 1B)]. Under the stringent conditions employed (hybridization and wash both at 45°C), this probe does not detectably cross-hybridize to the closely related *HCS82* transcript (Borkovich *et al.*, 1989), based on the absence of signal in the *hsp82*Δ strain SLY102 ± heat shock (data not shown). Following exposure on film, blots were rehybridized with a 1.6 kb antisense RNA probe specific for *ACT1* as previously described (Adams and Gross, 1991). Hybridization signals were quantified by laser densitometry as above.

DNase I chromatin mapping

To map sites within the *HSP82* upstream region sensitive to DNase I double strand cleavage, yeast cells were harvested from early logarithmic cultures grown and heat-shocked as described above. Following addition of sodium azide, cells were converted to spheroplasts at 30°C using oxalyticase in the presence of 1 mM PMSF; spheroplasts were then lysed by hypotonic shock, suspended in digestion buffer (DB) (10 mM HEPES pH 7.2, 0.5 mM $MgCl_2$, 0.05 mM $CaCl_2$, 1 mM PMSF, 2 mM *N*-ethylmaleimide, 2 mM benzamidine, 0.6 μg/ml chymostatin, 2.0 μg/ml pepstatin, 2.0 μg/ml antipain, 2.0 μg/ml leupeptin, 1.1 μg/ml phosphoramidon, 1.7 μg/ml aprotinin and 7.2 μg/ml E-64) and digested with DNase I as described in the legends to Figures 3 and 7B. DNA samples were deproteinized, restricted with *Eco*RI, electrophoresed on a neutral 2% agarose gel, capillary blotted to GeneScreen and indirectly end-labelled with probe p5L as described by Lee and Gross (1993). Naked genomic DNA, restricted with *Eco*RI, was digested with 1×10^{-5} U DNase I/μg DNA for 10 min at 37°C. Nuclease cleavage sites were mapped by electrophoresing the chromatin samples in parallel with landmark restriction fragments obtained by digesting genomic DNA with *Eco*RI alone (+1600) plus *Cla*I (−673), *Dra*I (−438), *Xba*I (−174, +536) or *Bgl*II (+239).

Micrococcal nuclease chromatin mapping and nucleosome-protected ladder analyses

Early log *S.cerevisiae* cultures (500 ml) were split into two aliquots, one of which was heat shocked for 15–20 min at 39°C. Heat shocks were terminated by the addition of sodium azide, then cells were converted to spheroplasts as above. Crude nuclei were purified essentially according to Szent-Gyorgyi *et al.* (1987) and gently suspended in 200 μl of DB. Suspended nuclei were divided into 100 μl aliquots [3×10^9 nuclei [~75 μg DNA) each], prewarmed at 37°C for 5 min and digested with either 200 or 400 units MNase/sample for 25 min at 37°C. Digestions were terminated through the addition of 1 vol of TES (50 mM Tris–HCl, pH 8, 5 mM EDTA, 50 mM NaCl). For digestion of cloned DNA as a control, a gel-purified *Eco*RI fragment encompassing the *HSP82* upstream region was incubated at 37°C for 5 min in DB (without protease inhibitors) with 2×10^{-2} to 2×10^{-1} units MNase/μg DNA. For the nucleosome-protected ladder assay, DNA was deproteinized, size-fractionated on a neutral 2% agarose gel and then blotted and UV-crosslinked to Zeta-Probe as described above. Immobilized DNA was hybridized and stringently washed at 53°C using RNA probes homologous to the *HSP82* upstream region (probes p1U, p2U and p3L of Figure 1B) as previously described (Gross *et al.*, 1990). To control for sample-to-sample variation in MNase digestion, each *HSP82*-specific probe was eluted (by incubating the membrane in 0.05 M NaOH at 37°C for 60 min) and the blot rehybridized with a probe homologous to a region of intergenic chromatin on chromosome XIII (spanning −1340 to −610 relative to the transcription startsite of *HCS82*; C.C.Adams and D.S.Gross, submitted). Nucleosome repeat length calculations were based on the mobility of end-labelled *Hae*III-ΦX174 DNA fragments electrophoresed in parallel. To permit mapping of MNase cleavage sites by indirect end-labelling with probe p5U, chromosomal DNA was purified, digested with *Eco*RI, electrophoresed and blotted in the same manner. Sizing was based on parallel electrophoresis of *Eco*RI landmark restriction fragments as described above.

DNase I genomic footprinting

Spheroplasts were prepared from control and 5–10 min 39°C heat-shocked cultures as above. Pellets were washed once in modified DB (made 40 mM in HEPES, pH 7.2, 1 mM in $MgCl_2$, and 0.1 mM in $CaCl_2$), employing 1 ml per 10^9 spheroplasts, then suspended to a density of $\sim5\times10^9$ spheroplasts per ml. Samples were digested at 37°C for 10 min (3×10^{-4} units/μg DNA); at 23°C for 10 min ($1-3\times10^{-2}$ units/μg DNA); or in an attempt to minimize protein degradation and exchange, at 3°C for 80 min ($0.3-1.5\times10^{-1}$ units/μg DNA). Essentially identical results were found in each case (data not shown). Digestions were terminated through addition of EDTA to 10 mM, and the DNA was deproteinized and digested with a combination of *Bcl*I and *Msp*I. Naked control genomic DNA, restricted similarly, was digested with 5.0–12.5 U/μg DNA at 37°C for 10 min. Guanine-specific sequencing ladders were generated by treating *Bcl*I and *Msp*I-digested naked genomic DNA with 0.5% dimethyl sulfate for 1 min at 0°C, and processed as described by Maxam and Gilbert (1980). DNAs were electrophoresed on 7.5% sequencing gels (50:1 acrylamide: *bis*), vacuum blotted to GeneScreen, covalently crosslinked and hybridized to strand-specific RNA probes (p2U and p2L) as previously described (Gross *et al.*, 1988, 1990; McDaniel *et al.*, 1989). Mapping of DNase I cleavage sites was done as described by Gross *et al.* (1990).

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References

Abravaya,K., Phillips,B. and Morimoto,R.I. (1991) *Mol. Cell. Biol.*, **11**, 586–592.
Adams,B.G. (1972) *J. Bacteriol.*, **111**, 308–315.
Adams,C.C. and Gross,D.S. (1991) *J. Bacteriol.*, **173**, 7429–7435.
Adams,C.C. and Workman,J.L. (1993) *Cell*, **72**, 305–308.
Almer,A., Rudolph,A., Hinnen,A. and Horz,W. (1986) *EMBO J.*, **5**, 2689–2696.
Amin,J., Ananthan,J. and Voellmy,R. (1988) *Mol. Cell. Biol.*, **8**, 3761–3769.
Archer,T.K., Cordingley,M.G., Wolford,R.G. and Hager,G.L. (1991) *Mol. Cell. Biol.*, **11**, 688–698.
Ashburner,M. and Bonner,J.J. (1979) *Cell*, **17**, 241–254.
Axelrod,J.D., Reagan,M.S. and Majors,J. (1993) *Genes Dev.*, **7**, 857–869.
Becker,P.B., Rabindran,S.K. and Wu,C. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 4109–4113.
Benezra,R., Cantor,C.R. and Axel,R. (1986) *Cell*, **44**, 697–704.
Borkovich,K.A., Farrelly,F.W., Finkelstein,D.B., Taulien,J. and Lindquist,S. (1989) *Mol. Cell. Biol.*, **9**, 3919–3930.
Buchman,A. and Kornberg,R.D. (1990) *Mol. Cell. Biol.*, **10**, 887–897.
Chasman,D.I., Lue,N.F., Buchman,A.R., LaPointe,J.W., Lorch,Y. and Kornberg,R.D. (1990) *Genes Dev.*, **4**, 503–514.
Chen,W. and Struhl,K. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 2691–2695.
Clark-Adams,C.D., Norris,D., Osley,M.A., Fassler,J.S. and Winston,F. (1988) *Genes Dev.*, **2**, 150–159.
Costlow,N.A., Simon,J.A. and Lis,J.T. (1985) *Nature*, **313**, 147–149.
Croston,G.E., Laybourn,P.J., Paranjape,S.M. and Kadonaga,J.T. (1992) *Genes Dev.*, **6**, 2270–2281.
Devlin,C., Tice-Baldwin,K., Shore,D. and Arndt,K.T. (1991) *Mol. Cell. Biol.*, **11**, 3642–3651.
Elgin,S.C.R. (1988) *J. Biol. Chem.*, **263**, 19259–19262.
Farrelly,F.W. and Finkelstein,D.B. (1984) *J. Biol. Chem.*, **259**, 5745–5751.
Fascher,K.D., Schmitz,J. and Horz,W. (1990) *EMBO J.*, **9**, 2523–2528.
Felsenfeld,G. (1992) *Nature*, **355**, 219–224.
Gilmour,D.S. and Lis,J.T. (1986) *Mol. Cell. Biol.*, **6**, 3984–3989.
Gilmour,D.S., Thomas,G.H. and Elgin,S.C.R. (1989) *Science*, **245**, 1487–1490.
Gross,D.S. and Garrard,W.T. (1988) *Annu. Rev. Biochem.*, **57**, 159–197.

Gross,D.S., Collins,K.W., Hernandez,E.M. and Garrard,W.T. (1988) *Gene*, **74**, 347–356.
Gross,D.S., English,K.E., Collins,K.W. and Lee,S. (1990) *J. Mol. Biol.*, **216**, 611–631.
Grunstein,M. (1990a) *Annu. Rev. Cell Biol.*, **6**, 643–678.
Grunstein,M. (1990b) *Trends Genet.*, **6**, 395–400.
Han,M. and Grunstein,M. (1988) *Cell*, **55**, 1137–1145.
Harlow,E. and Lane,D. (1988) *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Hayes,J.J., Tullius,T.D. and Wolffe,A.P. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 7405–7409.
Hoffman,C.S. and Winston,F. (1987) *Gene*, **57**, 267–272.
Jakobsen,B.K. and Pelham,H.R.B. (1988) *Mol. Cell. Biol.*, **8**, 5040–5042.
Jongstra,J., Reudelhuber,T.L., Oudet,P., Benoist,C., Chae,C.-B., Jeltsch,J.-M., Mathis,D.J. and Chambon,P. (1984) *Nature*, **307**, 708–714.
Kornberg,R.D. and Lorch,Y. (1991) *Cell*, **67**, 833–836.
Kunkel,G.R. and Martinson,H.G. (1981) *Nucleic Acids Res.*, **9**, 6869–6888.
Kunkel,T.A., Roberts,J.D. and Zakour,R.A. (1987) *Methods Enzymol.*, **154**, 367–382.
Larson,J.S., Schuetz,T.J. and Kingston,R.E. (1988) *Nature*, **335**, 372–375.
Laybourn,P.J. and Kadonaga,J.T. (1991) *Science*, **254**, 238–245.
Lee,M.-S. and Garrard,W.T. (1991) *EMBO J.*, **10**, 607–615.
Lee,M.-S. and Garrard,W.T. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 9166–9170.
Lee,S. and Gross,D.S. (1993) *Mol. Cell. Biol.*, **13**, 727–738.
Lee,Y., Hayes,J.J., Pruss,D. and Wolffe,A.P. (1993) *Cell*, **72**, 73–84.
Lorch,Y., LaPointe,J.W. and Kornberg,R.D. (1987) *Cell*, **49**, 203–210.
Lorch,Y., LaPointe,J.W. and Kornberg,R.D. (1992) *Genes Dev.*, **6**, 2282–2287.
Lu,Q., Wallrath,L.L., Allan,B.D., Glaser,R.L., Lis,J.T. and Elgin,S.C.R. (1992) *J. Mol. Biol.*, **225**, 985–998.
Lu,Q., Wallrath,L.L., Granok,H. and Elgin,S.C.R. (1993) *Mol. Cell. Biol.*, **13**, 2802–2814.
Lue,N.F., Buchman,A.R. and Kornberg,R.D. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 486–490.
Lutter,L.C. (1978) *J. Mol. Biol.*, **124**, 391–420.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499–560.
McDaniel,D., Caplan,A.J., Lee,M.S., Adams,C.C., Fishel,B.R., Gross,D.S. and Garrard,W.T. (1989) *Mol. Cell. Biol.*, **9**, 4789–4798.
Meisterernst,M., Horikoshi,M. and Roeder,R.G. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 9153–9157.
Nelson,D.A. (1982) *J. Biol. Chem.*, **257**, 1565–1568.
Nieto-Sotelo,J., Wiederrecht,G., Okuda,A. and Parker,C.S. (1990) *Cell*, **62**, 807–817.
Park,H.O. and Craig,E.A. (1989) *Mol. Cell. Biol.*, **9**, 2025–2033.
Perlmann,T. and Wrange,O. (1988) *EMBO J.*, **7**, 3073–3079.
Pham,T.A., Hwung,Y.-P., McDonnell,D.P. and O'Malley,B.W. (1991) *J. Biol. Chem.*, **266**, 18179–18187.
Piña,B., Bruggemeier, and Beato,M. (1990) *Cell*, **60**, 719–731.
Rabindran,S.K., Giorgi,G., Clos,J. and Wu,C. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 6906–6910.
Reed,K.C. and Mann,D.A. (1985) *Nucleic Acids Res.*, **13**, 7207–7221.
Richard-Foy,H. and Hager,G.L. (1987) *EMBO J.*, **6**, 2321–2328.
Roth,S.Y., Shimizu,M., Johnson,L., Grunstein,M. and Simpson,R.T. (1992) *Genes Dev.*, **6**, 411–425.
Rougvie,A.E. and Lis,J.T. (1990) *Mol. Cell. Biol.*, **10**, 6041–6045.
Scharf,K.-D., Rose,S., Zott,W., Schoff,F. and Nover,L. (1990) *EMBO J.*, **9**, 4495–4501.
Schuetz,T.J., Gallo,G.J., Sheldon,L., Tempst,P. and Kingston,R.E. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 6911–6915.
Schmid,A., Fascher,K.-D. and Horz,W. (1992) *Cell*, **71**, 853–864.
Shimizu,M., Roth,S.Y., Szent-Gyorgyi,C. and Simpson,R.T. (1991) *EMBO J.*, **10**, 3033–3041.
Shrader,T.E. and Crothers,D.M. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 7418–7422.
Simpson,R.T. and Whitlock,J.P. (1976) *Cell*, **9**, 347–353.
Sorger,P.K. (1990) *Cell*, **62**, 793–805.
Sorger,P.K. and Pelham,H.R.B. (1988) *Cell*, **54**, 855–864.
Sorger,P.K., Lewis,M.J. and Pelham,H.R.B. (1987) *Nature*, **329**, 81–84.
Straka,C. and Horz,W. (1991) *EMBO J.*, **10**, 361–368.
Struhl,K. (1985a) *Nature*, **317**, 822–824.
Struhl,K. (1985b) *Proc. Natl Acad. Sci. USA*, **82**, 8419–8423.
Szent-Gyorgyi,C., Finkelstein,D.B. and Garrard,W.T. (1987) *J. Mol. Biol.*, **193**, 71–80.
Taylor,I.C.A., Workman,J.L., Schuetz,T.J. and Kingston,R.E. (1991) *Genes Dev.*, **5**, 1285–1298.
Thomas,G.H. and Elgin,S.C.R. (1988) *EMBO J.*, **7**, 2191–2201.
Towbin,H., Staehelin,T. and Gordon,J. (1979) *Proc. Natl Acad. Sci. USA*, **76**, 4350–4354.
van Holde,K.E. (1989) *Chromatin*. Springer-Verlag, New York.
Winston,F. and Carlson,M. (1992) *Trends Genet.*, **8**, 387–391.
Winter,E. and Varshavsky,A. (1989) *EMBO J.*, **8**, 1867–1877.
Workman,J.L. and Buchman,A.R. (1993) *Trends Biochem. Sci.*, **18**, 90–95.
Workman,J.L. and Roeder,R.G. (1987) *Cell*, **51**, 613–622.
Workman,J.L., Roeder,R.G. and Kingston,R.E. (1990) *EMBO J.*, **9**, 1299–1308.
Workman,J.L., Taylor,I.C.A. and Kingston,R.E. (1991) *Cell*, **64**, 533–544.
Wu,C. (1980) *Nature*, **286**, 854–860.
Wu,C. (1984) *Nature*, **309**, 229–234.
Xiao,H. and Lis,J.T. (1988) *Science*, **239**, 1139–1142.

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