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Constitutive Binding of Yeast Heat Shock Factor to DNA In Vivo

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We measured the binding of yeast heat shock factor (HSF) to DNA in vivo by using an interference assay in which HSF excludes GAL4 from a synthetic promoter element containing overlapping binding sites for each protein. The results show that HSF binds to DNA in unstressed cells and that binding is not sufficient for transcriptional activation.

The promoters of heat shock genes are activated when cells are thermally stressed. Activation requires a specific DNA sequence, the heat shock element (HSE), which is the binding site for a protein, the heat shock factor (HSF) (reviewed in reference 1). The basic mechanism of heat shock gene activation has been conserved in essentially all eucaryotes; organisms as diverse as *Saccharomyces cerevisiae*, *Drosophila melanogaster* and humans contain HSEs with similar sequences (1).

Activation of heat shock transcription in *D. melanogaster* is accompanied by a change in the DNA-binding properties of HSF: HSF in control cells cannot be detected by a DNA-binding assay, and the HSEs are not associated with protein in the nuclei of such cells; however, following heat shock, binding activity is readily detectable (9, 10). Similar results have been obtained with human cells (4, 6). These studies suggest that the principal regulatory event in the heat shock response is the conversion of HSF from an inactive (non-DNA-binding) state to an active, DNA-binding configuration.

Recently, we found that *S. cerevisiae* differs from *D. melanogaster* and human cells in that HSF capable of binding to DNA can be extracted from *S. cerevisiae* whether or not is has been heat shocked (6). HSF extracted from heat-shocked cells, however, appears to be more extensively phosphorylated than HSF extracted from control cells. We suggested that in *S. cerevisiae* HSF is bound to DNA all the time but that its ability to promote transcription is modulated by a heat-induced change in its phosphorylation state (6; P. K. Sorger and H. R. B. Pelham, Cell, in press). However, we were unable to exclude the possibility that HSF is normally prevented from binding to DNA by some interaction that is disrupted when the cells are lysed. We now provide evidence that yeast HSF is indeed bound to HSEs in vivo in the absence of stress and hence that its activity must be regulated at a step subsequent to DNA binding.

To test the binding of HSEs in vivo, we devised a binding interference assay. An oligonucleotide that contains overlapping binding sites for HSF (1, 7) and for the transcriptional activator protein GAL4 (2, 5, 8) was synthesized. If inactive HSF is bound tightly to a site that overlaps the GAL4 site, it should prevent GAL4 binding and thus repress transcription. The binding sites were inserted at position −178 upstream of a fusion gene consisting of the CYC1 TATA box and the *Escherichia coli lacZ* structural gene on a multicopy yeast plasmid (pLGΔ−178; 3). The level of transcription can easily be monitored by assaying β-galactosidase activity, and it has previously been shown that synthetic sequences containing either a GAL4-binding site (2) or an HSE (7) can activate β-galactosidase production from this plasmid. GAL4 is active only in cells grown on galactose; in cells grown on glucose it does not bind to DNA, and little β-galactosidase is produced (2, 5).

Figure 1 shows the sequences of the various oligonucleotides that we tested. The sequence shown (which is that of construct 4) contains the overlapping binding sites; the asterisks indicate the bases which match the extended consensus HSE sequence C--GAA--TTC--GAA--TTC--G, which can be thought of as two overlapping copies (HSE A and HSE B) of the minimal HSE (C--GAA--TTC--G). We have shown that the extended sequence binds much more tightly to yeast HSF in vitro than does a single minimal HSE and supports a higher level of transcription in vivo (6, 7). Also shown is the 17-base-pair GAL4-binding-site consensus sequence (2, 5, 8). Sequence 4 differs from this sequence at three positions, as do most of the natural binding sites. The remaining sequences contain base changes designed to inactivate one or more of the HSE A, HSE B, or GAL4 sites. The HSEs were altered by changing the innermost A and T residues of the consensus sequence into G and C, respectively; we have shown that HSE sequences with these alterations are unable to bind yeast HSF in vitro and do not support transcription in vivo (7). The GAL4 site was disrupted by changing 7 bases, including the highly conserved residues at each end of the consensus sequence. We presume that these changes prevent GAL4 binding, but we have been unable to test this directly because we could not detect GAL4 bound to DNA in crude yeast extracts. Also shown in Fig. 1 are the sequences of two oligonucleotides with either a single (HSE1) or a double (HSE2) HSE which we have previously tested in this system (7); these were included as controls.

Table 1 summarizes the results obtained. We consider first the results obtained at 30°C. Construct 1, which lacks functional binding sites, produced only very low levels of β-galactosidase on both glucose and galactose whether or not the cells were heat shocked. Construct 2, with only a GAL4 site, produced high levels of β-galactosidase on galactose but very low levels on glucose, as expected. Construct 3, with HSEs but no GAL4 site, produced low but detectable levels of β-galactosidase on both galactose and glucose, and in each case the activity was increased by heat shock; these results were very similar to those obtained with the HSE2 plasmid.

The crucial result was obtained with construct 4, with the overlapping HSE and GAL4 sites. In cells grown on galactose, this plasmid produced about 40-fold less activity than

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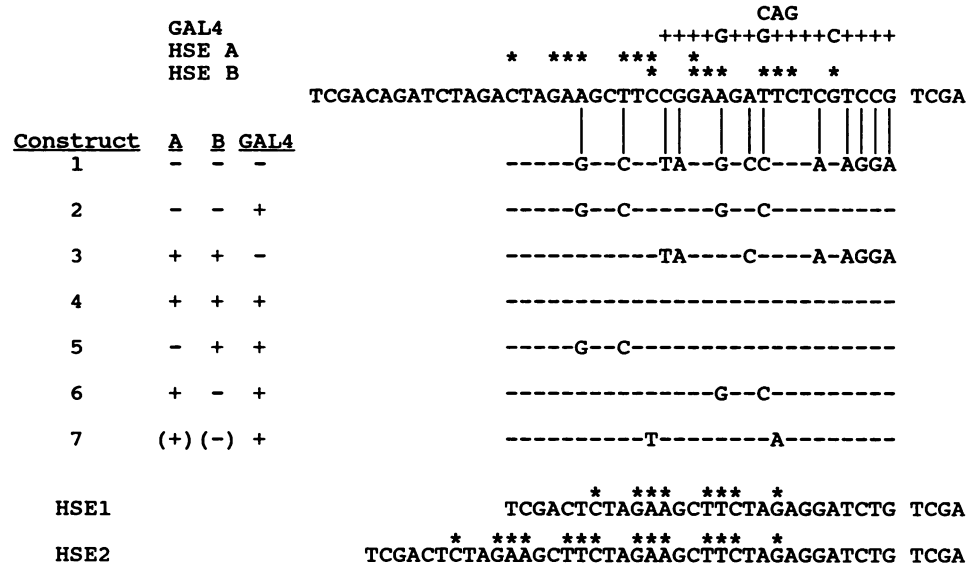

FIG. 1. Structure of synthetic promoter elements. The sequence shown at the top is the synthetic fragment inserted at the *Xho*I site at position −178 of pLGΔ−178 (3) to make construct 4. Matches to two overlapping minimal HSE consensus sequences are indicated by asterisks, and matches to the GAL4 binding-site-consensus sequence are indicated by plus signs. Where the GAL4 consensus sequence differs, the preferred base is indicated; in the central three positions two bases are equally preferred. The individual constructs differ from the sequence shown as indicated (they all have identical flanking sequences). The presence or absence of the HSE A, HSE B, or GAL4 site in each construct is indicated at the left by plus and minus signs. Also shown are the inserts present in the HSE1 and HSE2 constructs described previously (7).

did construct 2, indicating that HSF is capable of excluding GAL4 from the DNA or at least interfering with its function, even in unshocked cells. The activity increased upon heat shock, as expected for a functional HSE. Construct 5 also produced a low level of activity on galactose; this plasmid lacks HSE A but retains HSE B that overlaps the GAL4 site. Such single HSEs generally produced a low basal level of expression and were relatively weakly heat inducible (e.g., HSE1). Nevertheless, HSE B was evidently still capable of binding HSF and blocking the action of GAL4. However, when a single HSE did not overlap the GAL4 site (HSE A in construct 6), induction by galactose was not prevented. The activity of HSE A on its own could still be detected when the cells were grown on glucose to repress GAL4 activity (construct 6). Surprisingly, HSE B in construct 5 produced very little heat-inducible activity on glucose, as discussed below.

The two base changes introduced to prevent HSF binding to HSE B in constructs 2 and 6 were within the GAL4-binding site, and they improved the match to the GAL4 consensus sequence. The formal possibility thus exists that the low galactose-inducible activity in construct 4 (relative to that in constructs 2 and 6) was due to a low affinity for GAL4 rather than the presence of an overlapping HSF-binding site. We therefore designed a further construct (construct 7) in which the HSF-binding site of construct 4 was altered by two different base changes which reduced HSE A to a seven of eight match to the consensus sequence and HSE B to a six of eight match. Only one of these changes was within the GAL4 site, and it did not alter the match to the GAL4 consensus sequence because it was a T-to-A change at the central position of the dyad symmetric element. These two changes increased the galactose-inducible activity 70-fold, relative to that in construct 4, to a level even higher than that in

TABLE 1. Activity of constructs under various conditions

Construct or insert	Presence (+) or absence (−) of the following site:			β-Galactosidase activity[a]					
				30°C, galactose		30°C, glucose		15°C, galactose, control	15°C, glucose, control
	HSE A	HSE B	GAL4	Control	Heat shocked[b]	Control	Heat shocked[b]		
1	−	−	−	3	3	1	2	3	1
2	−	−	+	4,800	4,800	9	11	1,700	1
3	+	+	−	70	540	150	1,900	16	7
4	+	+	+	130	470	16	1,000	7	1
5	−	+	+	170	200	5	22	17	2
6	+	−	+	3,900	4,500	8	260	1,500	2
7	(+)	(−)	+	9,600	10,000	13	100		
HSE1	+			4	100	9	710	2	3
HSE2	+	+		42	520	130	1,800	5	5

[a] β-Galactosidase activity was determined as described previously (7). Data are the averages of two determinations; variations of up to 20% were observed.
[b] Cells were heat shocked for 30 min at 40°C and then allowed to recover for 1 h at room temperature before being harvested.

TABLE 2. Orientation-dependent activity of construct 5

Construct	β-Galactosidase activity[a]	
	Control	Heat shocked
5	9	26
5R[b]	23	160
HSE1	12	490

[a] Cells were grown at 30°C in glucose and heat shocked as described in Table 1, footnote *b*. Data are the averages of three determinations.

[b] Construct 5R has the same insert in the *Xho*I site as construct 5, but in the reverse orientation.

constructs 2 and 6 (Table 1). We conclude that the activity in constructs 2, 4, 5, 6, and 7 is determined principally by the presence or absence of HSE B and not by minor variations in the sequence of the GAL4 site.

At 30°C, basal activity was detectable from promoters containing a double HSE (e. g., HSE2 and construct 3), and it could be argued that HSF was slightly activated at this temperature. We therefore repeated the assays after growing the cells at 15°C for 2 weeks. At this temperature the basal activity caused by HSF was extremely low, whereas GAL4-driven expression of β-galactosidase was only reduced about threefold (Table 1). Constructs 4 and 5 still had very low activity, demonstrating that even at this temperature, HSF was able to block the action of GAL4.

Not every construct had the activity expected at 30°C. The basal activity of construct 4 on glucose was low, and construct 5 showed very little heat inducibility, as compared with HSE1, even though it had a similar match to the HSE consensus sequence. Both constructs were able to bind HSF, because in each case the galactose-inducible activity of the overlapping GAL4-binding site was efficiently blocked. Surprisingly, inversion of the construct 5 oligonucleotide increased the heat-inducible activity sixfold (Table 2). This effect is curious because the consensus bases in the HSF-binding site are arrayed symmetrically, and one would not expect such a site to be sensitive to inversion. The effect was seen in glucose-containing medium and thus was not due to abortive binding of GAL4. The results could, however, be explained by the binding of a putative repressor to the GAL4 consensus sequence (8a). Comparison of the various constructs indicated that a minor change in the distance from HSE to TATA is unlikely to account for the difference in activity.

Taken together, our results allow firm conclusions to be drawn about the behaviour of HSF in vivo. First, they show that HSF can bind to HSEs even at 15°C, when very little transcriptional activity can be detected. This conclusion implies that the regulation of HSF activity occurs after the DNA-binding step in *S. cerevisiae*. Second, although single HSEs bind HSF weakly in vitro, their low activity in vivo (as compared with that of double HSEs) is not due to a failure of HSF to bind. This low activity must reflect a less efficient interaction of HSF with other components of the transcriptional machinery when only a single HSE is available. It remains to be seen whether these properties are common features of regulatory transcription factors.

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