

Yeast Heat Shock Factor Contains Separable Transient and Sustained Response Transcriptional Activators

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Summary

The transcriptional induction of heat shock genes in eukaryotes is mediated by the heat shock transcription factor (HSF). In yeast, this induction appears to involve the phosphorylation of DNA-bound factor. I report here that HSF contains two distinct transcriptional activation regions. In response to a temperature upshift, an N-terminal region mediates transient increases in HSF activity and a C-terminal region is essential for sustained increases. These sustained and transient activities are regulated over different temperature ranges, and increases in both are associated with rises in the level of HSF phosphorylation. I propose that the two HSF activation regions are regulated independently in response to different stimuli.

Introduction

The induction of eukaryotic heat shock genes in response to a temperature upshift is mediated by a short DNA sequence, the heat shock element (HSE; Pelham, 1982; Pelham and Bienz, 1982; Topol et al., 1985; Xiao and Lis, 1988; Amin et al., 1988), which has been highly conserved during evolution (Bienz and Pelham, 1987). HSEs are binding sites for the heat shock transcription factor (HSF), a protein that has been identified in *Drosophila* (Wu, 1985; Parker and Topol, 1984; Wu et al., 1987), HeLa (Kingston et al., 1987; Sorger et al., 1987), and yeast cells (*Saccharomyces cerevisiae*; Wiederrecht et al., 1987; Sorger and Pelham, 1987). HSF from yeast is an 833 amino acid protein encoded by a single-copy gene with no close homologs (Sorger and Pelham, 1988; Wiederrecht et al., 1988). HSF binds to DNA as a trimer. Both an oligomerization region, located between residues 327–424 (Sorger and Nelson, 1989), and a DNA binding surface, comprising residues 167–284 (Wiederrecht et al., 1988), are required for high-affinity association with DNA.

Upon heat shock, a preexisting pool of unactivated HSF is converted into a form capable of efficiently stimulating transcription (Zimarino and Wu, 1987; Kingston et al., 1987; Sorger et al., 1987). We have proposed that, in yeast, this conversion is mediated by phosphorylation (Sorger et al., 1987; Sorger and Pelham, 1988). This may also be true of human HSF (Larson et al., 1988). In human and *Drosophila* cells, HSF binds to DNA only after heat shock

(Zimarino and Wu, 1987; Kingston et al., 1987; Sorger et al., 1987). In yeast, by contrast, HSF is bound to DNA both before and after heat shock: induction appears to involve a change in the ability of DNA-bound factor to stimulate transcription (Sorger et al., 1987; Jakobsen and Pelham, 1988; Sorger and Pelham, 1988).

Heat shock proteins (hsps) are synthesized not only in response to stress but also under conditions of normal growth (Craig, 1985; Lindquist, 1986; Slater and Craig, 1987; Craig et al., 1987). In yeast, some hsps perform essential functions (e.g., Deshaies et al., 1988; Chirico et al., 1988), and others are required at greater concentration for growth at elevated temperatures (Craig and Jakobsen, 1984; Borkovich et al., 1989). The sustained (often called constitutive) activity of yeast heat shock promoters may be regulated by several factors (e.g., Werner-Washburne et al., 1987; Slater and Craig, 1987), but three lines of evidence suggest that HSF plays a major role. First, in the absence of heat shock, HSF binds to HSEs with high affinity in vitro (Sorger and Pelham, 1987) and in vivo (Jakobsen and Pelham, 1988), and the *HSF* gene is essential for viability (Sorger and Pelham, 1988; Wiederrecht et al., 1988). Second, the overexpression of HSF in wild-type yeast at non-shock temperatures (30°C) significantly increases the levels of a major hsp70 protein (Sorger and Pelham, 1988). Third, in the *SSA1* promoter, one of the few yeast heat shock promoters to have been studied in detail (Slater and Craig, 1987), HSF binding sites mediate 50%–80% of the sustained activity (Park and Craig, 1989).

Here I describe an analysis of the regions of HSF involved in transcriptional activation. Truncated HSF proteins were introduced into cells in the absence of wild-type factor and examined for their ability to enhance transcription and for their effects on cell growth. The transient and sustained activities of HSF were found to be mediated by physically separable transcriptional activators. The analysis of HSF truncations reveals a requirement for continuous high-level HSF activity during growth at elevated temperatures.

Results

Transient and Sustained Changes in HSF Activity

To characterize the activity of wild-type HSF at various temperatures, I examined a synthetic heat shock promoter that contains a single HSE inserted into a disabled *CYC1* promoter and linked to the *lacZ* gene (pHSE- β G). In cells carrying pHSE- β G, β -galactosidase levels increase dramatically in response to heat shock. In contrast, low enzyme activities are observed under all conditions with variant promoters bearing point mutations in the HSE that interfere with binding by HSF (Sorger and Pelham, 1987; Sorger et al., 1987). Thus, β -galactosidase levels in cells carrying the pHSE- β G promoter reflect the activity of HSF in enhancing transcription.

When wild-type cells carrying the pHSE- β G promoter were grown at 20°C and then shifted to and maintained

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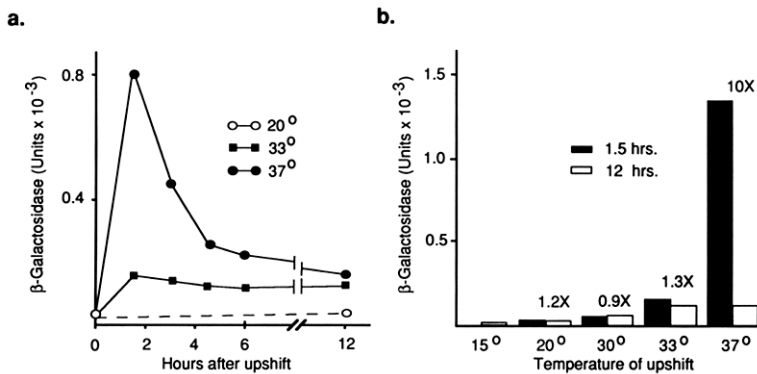


Figure 1. Activity of Wild-Type HSF

(a) Cells carrying the highly heat-inducible pHSE- β G synthetic promoter and plasmid-borne wild-type HSF were grown at 20°C and then shifted to and maintained at either 33°C (squares) or 37°C (black circles); β -galactosidase activity was measured over a 12 hr period. Open circles indicate samples maintained at 20°C. The averages of two measurements from a single experiment are shown. Similar results were obtained in two or more independent experiments but the level of induction varied by ~ 2 -fold.

(b) Cells carrying pHSE- β G and wild-type HSF were grown at 15°C and shifted to the indicated temperatures; β -galactosidase levels were determined 1.5 hr (black bars) or 12 hr (open bars) after upshift. The numbers above each set of data bars indicate the ratios of activities at 1.5 and 12 hr. Measurements from triplicate samples are shown; similar results were obtained in two experiments.

at 37°C, enzyme levels increased transiently by 30-fold within 1.5 hr and then declined to a new basal level that was 5-fold greater than the level at 20°C; the new level of activity remained constant indefinitely (Figure 1, circles). To examine the temperature dependence of this response, cells grown at 15°C were shifted to and maintained at temperatures between 20°C and 37°C, and enzyme levels were measured at the peak of the early response (1.5 hr) and after they had stabilized at a new basal level (12 hr). β -galactosidase activity 12 hr after upshift varied with temperature, being 8-fold higher at 33°C than at 15°C, but at any given temperature within this range enzyme levels at 1.5 and 12 hr were similar (Figure 1b). The absence of a transient response in this temperature range was confirmed by monitoring a time course of promoter activity following a shift from 20°C to 33°C (Figure 1a, squares). In contrast, following a shift from 20°C to 37°C, enzyme levels were 10-fold greater at 1.5 hr than at 12 hr, as expected from the induction time course in Figure 1a.

These data suggest that there are two components of the response of the pHSE- β G promoter to a temperature upshift and that they are regulated over different temperatures: sustained promoter activity increases with rising temperature over the range of normal growth temperatures between 15–33°C, but transient activity is induced only over a narrower range of 34.5–39°C (Figure 1b and data not shown). The possibility that these phenomenologically distinct responses are functionally different is investigated below. With pHSE- β G, the transient response is quantitatively more significant than the sustained response. Although it is a synthetic construct, pHSE- β G appears to be representative of a class of naturally occurring promoters known as heat-inducible promoters (e.g., the SSA3 and SSA4 gene promoters; Craig et al., 1985; Werner-Washburne et al., 1987). A member of the set of so-called constitutive heat shock promoters in which the sustained response is the more significant is discussed below.

HSF Residues 1–424 Are Sufficient for Heat-Inducible Transcriptional Activation

To localize the regions of HSF involved in transcriptional activation, yeast centromeric plasmids encoding truncated forms of the factor (Figure 2) were transformed into a haploid *S. cerevisiae* strain that harbors a disruption of the chromosomal *HSF* gene and a wild-type *HSF* gene on a *URA3*-containing plasmid. The wild-type *HSF* gene was eliminated from transformants by counterselection on 5-fluoroorotic acid (Boeke et al., 1984). This procedure generates strains in which the only *HSF* gene is the plasmid-encoded truncation.

The set of N- and C-terminal truncations detailed in Figure 2 was introduced into cells and HSF levels were estimated from immunoblots; truncated HSF proteins were found to accumulate to levels similar to that of wild-type factor (e.g., Figure 3c). Of the truncations shown in Figure 2, all but HF/1–393 and HF/1–327 were able to substitute for wild-type HSF in cells grown at 20°C. Thus, large parts of the HSF polypeptide are dispensable for viability at 20°C. No viable cells lacking wild-type HSF, but carrying HF/1–327 or HF/1–393, were isolated at any temperature. This is consistent with the observation that C-terminal truncation to residue 393 or 327 interferes with oligomerization and with binding to DNA *in vitro* (Sorgor and Nelson, 1989). Surprisingly, the HF/327 Δ 584 protein, although lacking sequences essential for trimerization, could substitute for HSF at 20°C to yield slow growing cells; when synthesized *in vitro* this protein bound to DNA poorly and appeared to be monomeric (H. Nelson and P. K. S., unpublished data).

The transcriptional activities of truncated HSF proteins were first determined using the synthetic transient response promoter (pHSE- β G). Cells were grown at 20°C, exposed to a 39°C heat shock, and allowed to recover for 1.5 hr at room temperature. This procedure results in heat shocks essentially identical to the 1.5 hr time points of Figure 1 but is more reproducible. In cells containing pHSE-

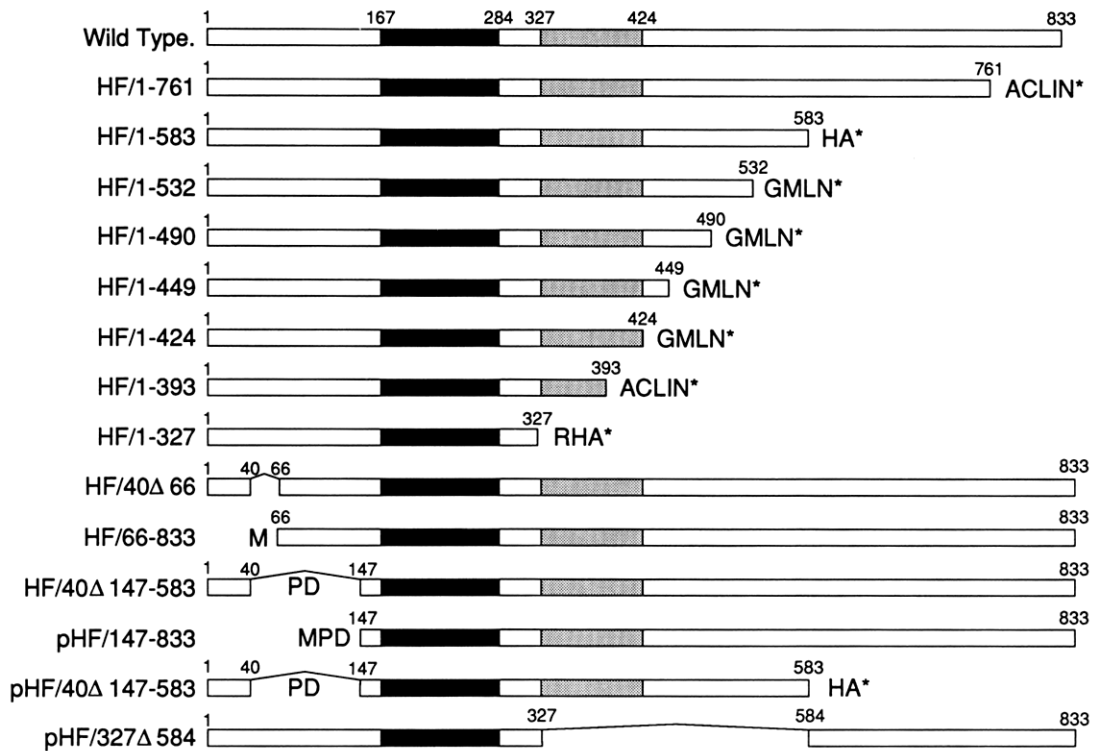


Figure 2. Structures of Truncated HSF Proteins

Linear representations of truncated HSF proteins with the DNA binding region (amino acids 167–284; Wiederrecht et al., 1988) indicated by a black bar and the trimerization region (amino acids 327–424; Sorger and Nelson, 1989) by a stippled bar. Numbers indicate the final HSF residue in each region, and sloping lines indicate internal deletions. Letters denote non-HSF sequences (in one-letter code) and asterisks denote stop codons.

β G and wild-type HSF encoded by a plasmid-borne gene, β -galactosidase levels increased 20-fold in response to heat shock (Figure 3a). HSF truncated C-terminally at residue 424 (HF/1–424) supported a similar level of heat

induction (25-fold), although absolute enzyme levels were 3-fold lower. Significant heat-inducible activity was observed with the other C-terminal deletions shown in Figure 2, and in no case was there evidence for a qualitatively

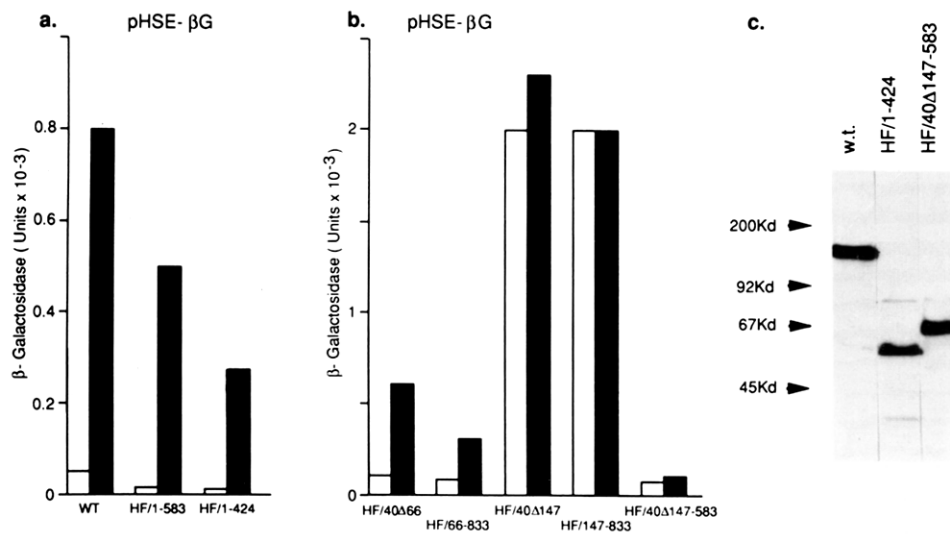


Figure 3. Activities and Expression of Truncated HSF Proteins

(a) and (b) β -galactosidase enzyme levels in cells carrying either wild-type (WT) or various truncated HSF proteins and the transient response pHSE- β G heat shock promoter. Enzyme levels were measured at 20°C (open bars) and after heat shock to 39°C for 30 min and recovery at 20°C for 1.5 hr (black bars). An average value derived from three to eight independent determinations is shown. Note the change of vertical scale. (c) Immunoblots with anti-HSF antisera of cells grown at 20°C, synthesizing the indicated HSF proteins.

different response (data not shown). Larger proteins were generally more active, HF/1–583, for example, being 70% as active as wild-type factor (Figure 3a).

These results demonstrate that residues 1–424 contain an inducible transcriptional activation region sufficient for the transient response to heat shock. The 3-fold lower activity the HF/1–424 protein relative to wild-type HSF may be a consequence of its somewhat lower DNA binding affinity and of a reduced tendency to self-associate (Sorger and Nelson, 1989). Alternatively, sequences C-terminal to residue 424 may contribute to the activity of the factor, particularly in the absence of heat shock (Figure 3). These C-terminal sequences are clearly dispensable for heat inducibility, however, and HF/1–424 was therefore chosen for further study as being representative of the C-terminally truncated HSF proteins (see below).

N-Terminal Sequences Appear to Mask a C-Terminal Activity

I next analyzed cells carrying HSF proteins N-terminally truncated at residue 66 (HF/66–833) or deleted for residues 40–66 (HF/40 Δ 66). Like C-terminal truncations, these derivatives were capable of heat-inducible transactivation (with an induction ratio of 4- to 6-fold; Figure 3b). However, more extensive deletion produced proteins (HF/40 Δ 147 and HF/147–833) that were highly active at both 20°C and following heat shock. This suggests that the N-terminal region of HSF is involved in inhibiting the transcriptional activity of the factor in the absence of stress.

By analogy with those mutations in the glucocorticoid receptor that make it a constitutive transcriptional activator in the absence of a hormone agonist (Godowski et al., 1987; Hollenberg et al., 1987), it seemed possible that the HF/40 Δ 147 mutation in HSF unmasks a normally cryptic activation sequence. To identify this activator, a protein truncated at both its N- and C-termini (HF/40 Δ 147–583; see Figure 2) was constructed. This protein had low activity before and after heat shock (Figure 3b). Low activity was not a consequence of reduced accumulation of the truncated proteins, since HF/40 Δ 147–583 was as abundant as HF/1–424 (Figure 3c). Nor did it appear to reflect poor binding to DNA: HF/40 Δ 147–583 bound well to a synthetic HSE in vitro and formed DNA–protein complexes qualitatively similar to those seen with HF/1–424 (H. Nelson and P. K. S., unpublished data). I infer from this that the HF/40 Δ 147–583 protein is stable and able to bind to HSEs but is unable to enhance transcription efficiently.

The primary conclusion from these data is that the HF/40 Δ 147 mutation appears to unmask a cryptic C-terminal activation region. This is demonstrated by the finding that the high activity of HF/40 Δ 147 in the absence of heat shock is reduced some 20-fold by the removal of sequences C-terminal to residue 583. I show below that this C-terminal activator can enhance transcription when fused to the DNA binding domain of the bacterial repressor LexA and that it is involved in the sustained response of HSF to a temperature upshift.

In addition, a region of HSF between residues 40–147 seems to be essential for the transcriptional activity of the factor after heat shock. This is suggested by the observa-

Table 1. Transcriptional Activity of LexA–HSF Fusion Proteins in Wild-Type Cells

Construct	Junction		Amount of Protein	Transcriptional Activity	
	N	C		20°C	H.S.
1. LEX/1-424 ^a	1	6	++	120	600
2. LEX/327-424 ^a	2	6	+	600	900
3. LEX/211-833	3	0	++	2000	~
4. LEX/373-833	4	0	++	2400	~
5. LEX/417-833	4	0	ND	2400	~
6. LEX/584-833	0	0	++++	2300	2300
7. LEX/584-783	0	0	++	400	400
8. LEX/783-833	0	0	++	5	10
9. LEX/584-657	0	7	+	5	30
10. LEX/681-783	5	0	++	10	25
11. gcn4 Δ 19 ^b	~	~	—	2800	2600
12. CNTRL ^c	~	~	ND	<4	<4

Junction amino acids not included in wild-type HSF were introduced N- or C-terminally with DNA linkers; a key to this single-digit numerical code is found in the Experimental Procedures. 0 indicates that no additional amino acids were introduced. The approximate relative amounts of protein were judged by immunoblotting of whole-cell extracts with anti-LexA antisera. — indicates that no protein was detected; ND, level not determined. To determine transcriptional activity, β -galactosidase enzyme levels in cells carrying LexA–HSF fusion proteins and a *lexA* operator–*CYC1-lacZ* promoter fusion construct were measured. Cells were grown at 20°C and heat shocked (H.S.) to 39°C for 30 min. Average values as determined in at least three independent experiments are shown. Where necessary, independent measurements were scaled to the activity of the LEX/584–833 construct. ^a Activity of these fusion proteins is affected by interaction with endogenous HSF (see text).

^b Positive control. Construct YCp88-gcn4 Δ 19 is from Hope et al. (1988).

^c Negative control. Only the *lexA*-operator promoter fusion construct was present in these samples.

tion that HF/40 Δ 147–583 is 6-fold less active following heat shock than HF/1–583, a protein capable of mediating heat-inducible transcriptional activation (Figure 3a).

The C-Terminal but Not N-Terminal Activator Functions when Fused to LexA

To localize the transcriptional activators further, HSF sequences were fused to the DNA binding domain of the bacterial repressor LexA (amino acids 1–87; Brent and Ptashne, 1985; Hope and Struhl, 1986; Godowski et al., 1988). When expressed in yeast cells, these LexA–HSF fusion proteins accumulated to levels roughly similar to that of wild-type HSF as assessed by immunoblotting (data not shown). Their ability to enhance transcription was determined with a *lexA* operator–*CYC1* promoter linked to *lacZ* (Hope and Struhl, 1986).

To determine whether heat-inducible activity could be conferred on a fusion protein, the N-terminal activator was linked to LexA (LEX/1–424). In wild-type cells carrying LEX/1–424, enzyme levels increased 5-fold after heat shock (Table 1, line 1). However, the analysis of 20 C-terminal and N-terminal truncations (listed in Experimental Procedures) demonstrated that inducible activity required the presence of the HSF trimerization region between residues 327–424 (line 2 and data not shown), and we

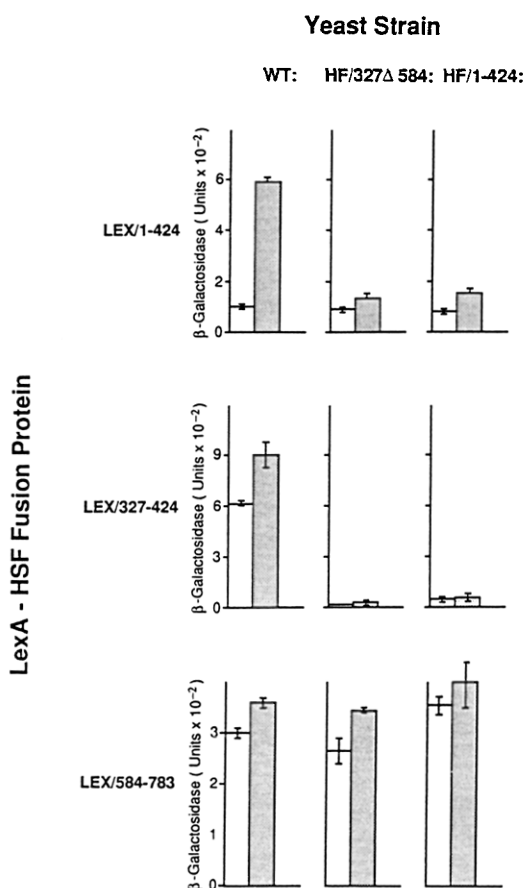


Figure 4. Activities of LEX-HSF Fusion Proteins in Strains Carrying Truncated HSF Molecules

Plasmids encoding various LexA-HSF fusion proteins (arranged in rows) were introduced into either a wild-type (WT) strain or strains carrying truncated HSF proteins (arranged in columns). β -galactosidase levels were determined at 20°C and after heat shock to 39°C for 30 min. Mean values and ranges from a typical experiment performed on duplicate samples are shown. All samples were analyzed in parallel to minimize variability; qualitatively similar results with each combination of fusion protein and truncated HSF protein were obtained in two or more experiments.

have shown that LexA-HSF chimeras containing the trimerization region interact with wild-type HSF *in vitro* to form mixed complexes (Sorger and Nelson, 1989).

To determine the contribution of this interaction to the activity of LEX/1-424 and LEX/327-424 *in vivo*, they were analyzed either in cells carrying HF/327Δ584 (Figure 2), which lacks the oligomerization region and which should not form mixed complexes (this was confirmed *in vitro*; data not shown), or in cells carrying HF/1-424, in which the transcriptional activity of these complexes would be reduced. It was found that in cells carrying HF/1-424 and HF/327Δ584, the heat inducibility of LEX/1-424 and high activity of HF/327-424 were absent (Figure 4). Low activity was not a consequence of low-level expression, since fusion proteins were equally abundant in mutant and wild-type cells (data not shown); nor was it due to a general defect in transcription: a fusion containing the C-terminal

activation region (LEX/584-783; see below) was active in all strains (Figure 4, bottom row).

From these data I conclude that the ability of LexA-HSF chimeras that contain the HSF oligomerization region to enhance transcription following heat shock derives from their interaction with endogenous HSF. HSF need not, therefore, be bound to a promoter via its own DNA binding region for correct regulation. However, the ability of the N-terminal activator to promote heat-inducible transcription is lost when it is covalently coupled to LexA. This is also true of fragments of the activator: when correction is made for the interaction of fusion proteins with endogenous HSF, none of a series of 12 LexA-HSF fusion proteins containing overlapping sequences from the N-terminal region of HSF between residues 1-583 was capable of heat-inducible transcriptional activation (constructs are listed in Experimental Procedures; data not shown).

As described above, the unmasking effect observed with the HF/40Δ147 truncation suggests the presence of an activation region near the C-terminus of HSF (Figure 3b). To localize this activator, C-terminal HSF sequences were fused to LexA. LexA-HSF chimeras containing HSF sequences 211-833 or 373-833 enhanced transcription strongly at 20°C; they were as active as LexA fusions containing amino acids 85-281 of GCN4 (Table 1, lines 3, 4, and 11). Progressive deletion of the HSF sequences present in these fusion proteins located a region between residues 584-833 that retained full activity (lines 5-8). The activities of LEX/584-783 and LEX/584-833 were not affected by heat shock (lines 6 and 7). Cells carrying LEX/584-783 had about 20% of the β -galactosidase levels of cells with LEX/584-833; however, the former protein was only 40% as abundant as the latter (data not shown), indicating that the intrinsic activities of the two proteins were less than 2-fold different.

Because we have not demonstrated that the trimerization region is the only part of HSF involved in self-association, it was necessary to consider the possibility of interaction between LEX/584-783 and endogenous HSF. The fusion protein was therefore introduced into strains carrying HF/1-424 and HF/327Δ584; LEX/584-783 activity was similar in each of these strains (Figure 4), consistent with the view that LEX/584-783 does not interact appreciably with endogenous HSF. It has not been possible to define the sequences comprising this activator further: two smaller fusions (LEX/584-657 and LEX/681-783) are detectable in cells (data not shown), but they are less than 10% as active as LEX/584-783 (lines 9 and 10).

These data indicate that a 200 amino acid sequence lying between residues 584-783 contains an autonomous transcriptional enhancement region whose activity is not affected by heat shock. This region has a predicted net charge at neutral pH of -18 and contains 46 serine and threonine residues.

Cells Carrying C-Ternally Truncated HSF Molecules Are Temperature Sensitive for Growth

Loss-of-function mutations in several constitutively expressed hsp's have been shown to interfere with cell growth at elevated temperatures (Craig and Jacobsen,

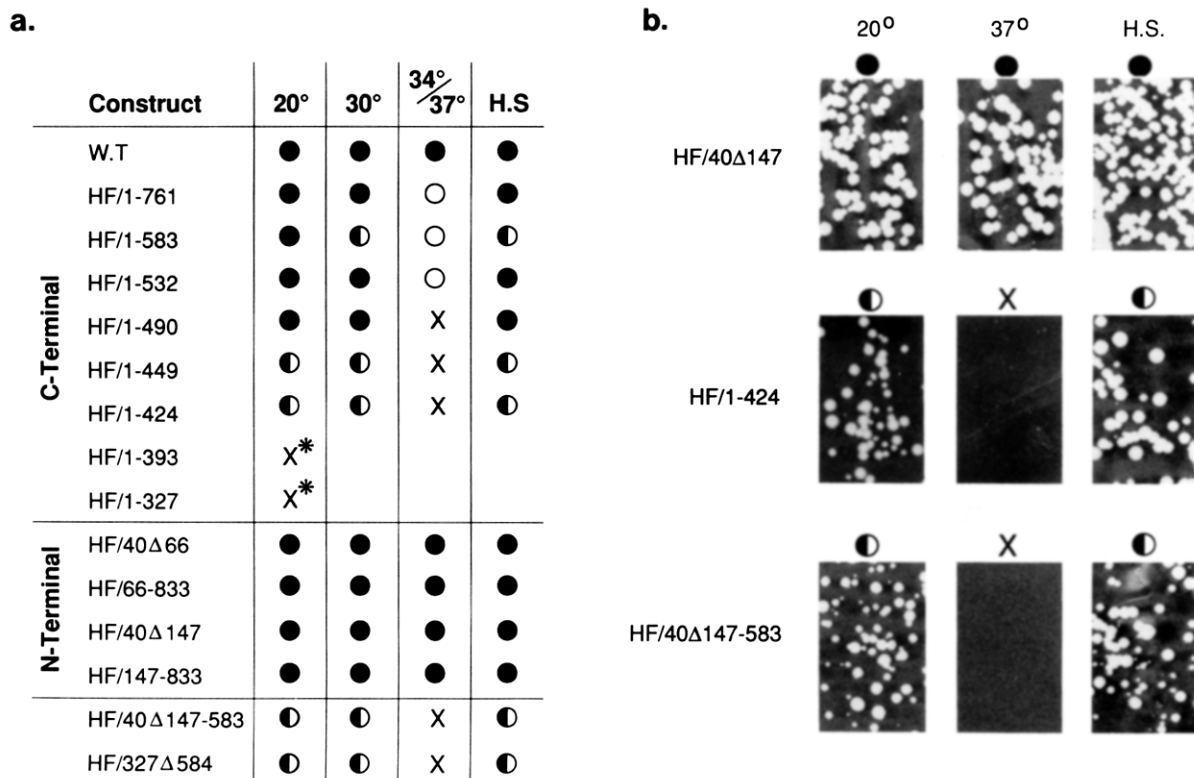


Figure 5. Growth Properties of Cells Carrying Truncated HSF Proteins

The growth on rich plates of cells (YEPD) carrying the indicated HSF proteins at 20°C, 30°C, 34°C, and 37°C and of cells grown at 20°C and heat shocked to 39°C for 45 min. The results obtained at 34°C and at 37°C were very similar.

(a) Summary of growth properties of cells harboring constructs shown in Figure 1 or wild-type HSF (W.T.). A black circle represents wild-type growth, half-open circle represents moderate growth with pronounced variability in colony size, open circles represent very poor growth, and an X indicates no growth. Constructs indicated with asterisks were tested for their ability to substitute for wild-type HSF down to 15°C. In strains marked with a half-open circle, large colonies probably differ from small colonies in containing cells with more copies of the HSF-encoding plasmid. This implies that the copy number of plasmids encoding truncated HSF proteins varies somewhat from cell to cell and that some truncations may not be sufficient for viability in single copy.

(b) Photographs of plates of cells carrying truncated HSF proteins. Cells were grown at 20°C, plated on rich plates, and incubated at the indicated temperatures for 36–48 hr. Very small colonies were often seen when strains carrying C-terminally deleted proteins were incubated at 37°C (e.g., the HF/1-424 plate). These colonies contain cells that could not divide further when transferred to a fresh plate at 37°C; thus, they appear to arise from cells that can undergo only a limited number of divisions at 37°C.

1984; Borkovich et al., 1989) and it seemed likely that deletions in HSF might confer on cells a similar phenotype. On rich medium, cells harboring C-terminally truncated HSF proteins (Figure 2) were unable to form colonies at temperatures above 33°C (Figure 5) but, when grown at 20°C, were able to survive a transient heat shock at 39°C. In liquid culture, the doubling time of wild-type and of HF/1-424 strains at 20°C was identical (3.3 hr) and at 33°C quite similar (1.8 versus 2.6 hr), but above 34.5°C, division of cells carrying HF/1-424 was arrested within 4 hr and viability slowly decreased. In contrast, cells carrying N-terminally truncated proteins were indistinguishable from wild-type cells (Figure 5). Similar results were obtained with high copy number (2 μ m) vectors (B. Jakobsen, personal communication), suggesting that the temperature-sensitive phenotype was not a consequence of low-level expression of truncated HSF proteins.

Two simple explanations for this phenomenon were in-

vestigated and rejected. First, the possibility that the HF/1-424 protein was temperature sensitive for synthesis was excluded: when HF/1-424 was expressed in wild-type cells, the levels of protein were identical following growth at either 20°C or 37°C for 48 hr (data not shown). Second, the possibility that truncated HSF proteins were thermally inactivated at higher temperatures was tested as follows. Cells grown at 20°C and carrying HF/1-424 and the pHSE- β G reporter plasmid were exposed to continuous heat shock at 37°C for 1 or 2 hr and harvested immediately. β -galactosidase levels were observed to increase 12-fold within the first hour and 24-fold after 2 hr. Since this progressive rise in enzyme levels reflects transcriptional activation by the factor for 2 hr at 37°C, the HF/1-424 protein cannot be thermolabile.

These data indicate that cells are temperature sensitive for growth in rich medium if they harbor HSF proteins in which the activation region located C-terminally to residue

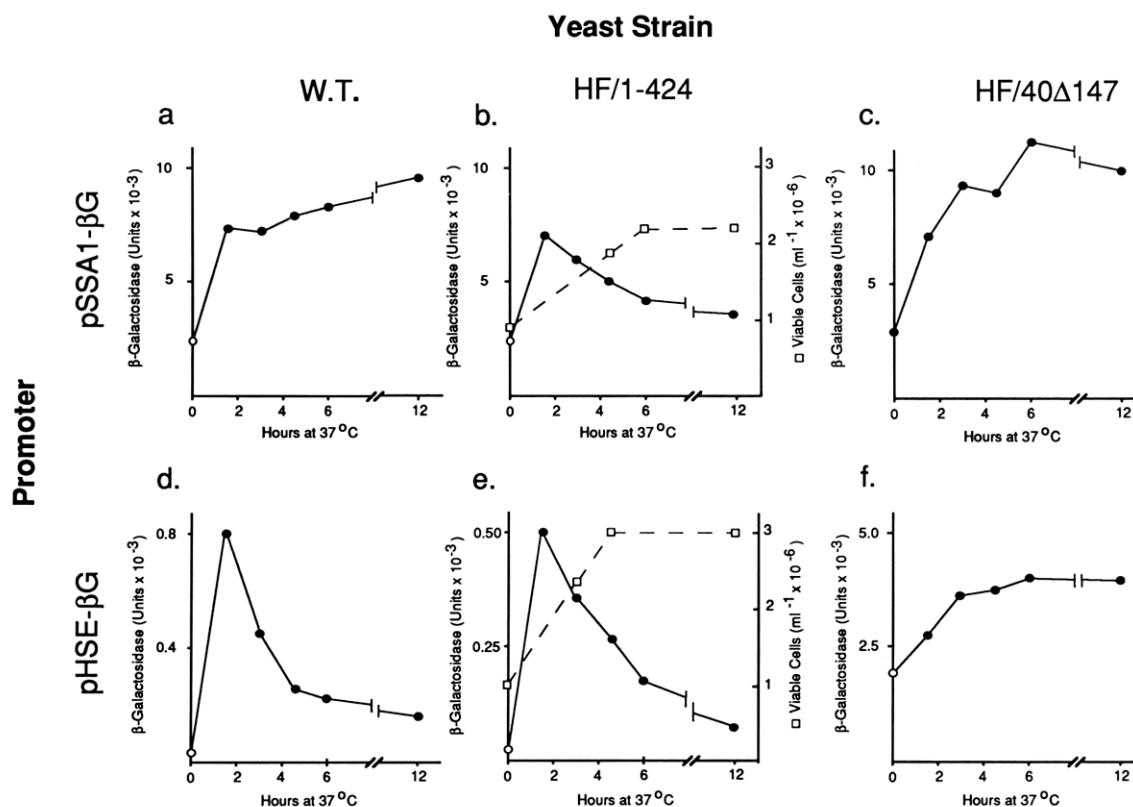


Figure 6. Time Course of the Response of Promoters to a Shift in Growth Temperature

Note different vertical scales. Cells growing exponentially at 20°C were shifted to and maintained at 37°C, and β-galactosidase levels were determined at the indicated times. An average of four independent measurements from two time courses is shown; the error is estimated to be ±20%. In (a), (c), (d), and (f), no significant changes in enzyme levels were detected between 12 and 48 hr. In (b) and (e), squares indicate the number of viable cells per ml as judged by plating samples at 20°C.

(a) Cells harboring the sustained response pSSA1-βG promoter construct and a plasmid-encoded wild-type *HSF* gene.

(b) pSSA1-βG in cells harboring HF/1-424.

(c) pSSA1-βG in cells harboring HF/40Δ147.

(d) Cells harboring the heat-inducible pHSE-βG promoter construct and a plasmid-encoded wild-type *HSF* gene.

(e) pHSE-βG in cells harboring HF/1-424.

(f) pHSE-βG in cells harboring HF/40Δ147.

583 has been truncated or deleted. This suggests that the C-terminal activator performs an essential function above 33°C.

C-Terminal HSF Sequences Are Involved in the Sustained Response of Heat Shock Promoters to Elevated Growth Temperatures

The C-terminal activator is dispensable for the transient response to a temperature upshift (Figure 3a), but it seemed possible that it was involved in sustained HSF activity. In cells carrying truncated HSF proteins the sustained activity determined with the pHSE-βG promoter was very low (Figure 3a, 20°C data bars), and to measure it accurately it was necessary to examine the promoter from the constitutively expressed *SSA1* gene (a yeast *hsp70*; Slater and Craig, 1987). At 20°C in cells bearing wild-type HSF, the activity of a construct that contains the *SSA1* promoter fused to *lacZ* (pSSA1-βG) was 100 times greater than that of pHSE-βG (Figure 6). The 1200 bp

SSA1 promoter is complex and contains binding sites for several transcription factors, but 50%–80% of its activity is dependent on binding by HSF (Park and Craig, 1989).

When cells carrying pSSA1-βG were shifted from 20°C to 37°C, β-galactosidase levels increased 5- to 6-fold and then remained at a high constant level (Figure 6a; Slater and Craig, 1987; Park and Craig, 1989). This induction profile is very different from that observed with the transient response pHSE-βG promoter (Figure 6d) and indicates that the sustained activity of pSSA1-βG is quantitatively more significant than its transient activity.

To investigate the role of C-terminal HSF sequences, β-galactosidase levels were determined 24 hr after temperature upshift in cells that carried pSSA1-βG and either plasmid-encoded wild-type HSF or HF/1-424. In cells with wild-type factor, enzyme levels were 4-fold higher at 33°C than at 20°C (Figure 7). In cells carrying HF/1-424 the increase between 20°C and 33°C was only 1.7-fold; this 70% increase may not be mediated by HSF because unrelated

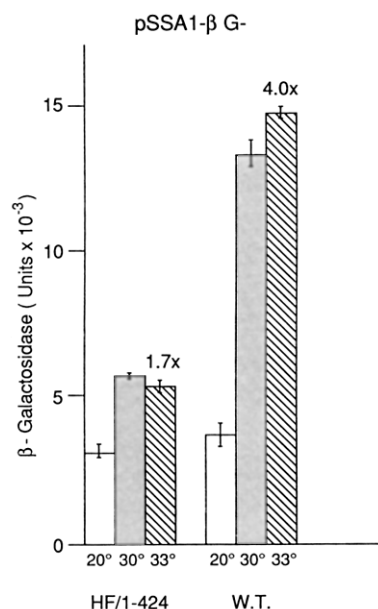


Figure 7. Sustained Transcriptional Activities of HF/1-424 and Wild-Type Proteins

Cells carrying the indicated plasmid-encoded proteins and the sustained response pSSA1- β G promoter were grown at 20°C and then shifted to the indicated temperatures for 24 hr. Mean values and ranges from a typical experiment performed in triplicate are shown. In similar experiments, virtually identical induction ratios were observed.

proteins, such as LexA-GCN4 chimeras, are typically 1.5-fold more active at the higher temperature, possibly reflecting better cell growth (unpublished data). Thus, C-terminal HSF sequences appear to be required for increases in sustained SSA1 activity at elevated temperatures.

However, there are two weaknesses in this experiment. First, because the induction of the SSA1 promoter between 20°C and 33°C is modest in wild-type cells (4-fold), only a limited reduction (2.5-fold) could be observed in cells with truncated HSF. Second, the experiment measures a loss rather than a gain of function. To establish meaningful qualitative differences in HSF activity between cells harboring wild-type HSF and truncations in which the C-terminal activator had been deleted (HF/1-424) or in which it had been unmasked (HF/40 Δ 147), the induction profiles of the transient (pHSE- β G) and sustained response (pSSA1- β G) promoters were examined over a 12 hr period.

When cells bearing HF/1-424 and pHSE- β G were grown at 20°C and shifted to 37°C, the profile of promoter activity was very similar to that observed in wild-type cells (Figures 6d and 6e): enzyme levels increased 25-fold within 1.5 hr and then declined to a low level (in cells with HF/1-424, in contrast to those with wild-type HSF or HF/40 Δ 147, enzyme levels had not stabilized after 12 hr and continued to drop). Because 37°C is a nonpermissive temperature for the growth of strains carrying HF/1-424, cell viability was examined. Division was observed to cease within 4.5 hr and the number of viable cells then re-

mained constant for 12 hr (Figure 6e, squares); thus, the decline in pHSE- β G transcription after 1.5 hr is not a consequence of cell death. These data support the earlier conclusion that the N-terminal activator mediates an inherently transient response to temperature upshift.

When pSSA1- β G and HF/1-424 were combined, enzyme levels increased transiently by 3-fold following an upshift and then declined (Figure 6b). The number of viable cells increased 2-fold in the 12 hr period. This profile reveals a transient responsiveness of the pSSA1- β G promoter which is obscured in cells with wild-type HSF. Qualitative changes in the induction profile relative to that in cells with wild-type HSF confirm the conclusion that HF/1-424 truncation selectively interferes with the sustained activity of the SSA1 promoter.

When pHSE- β G and HF/40 Δ 147 were combined, enzyme levels at 20°C were 50- to 100-fold higher than in wild-type cells, and there was a sustained 2-fold increase following a shift to 37°C (Figure 6f). This profile is quite different from that observed with pHSE- β G in the presence of wild-type HSF and is typical of a sustained response promoter. This result suggests that the C-terminal activator can mediate high-level increases in transcription in response to a temperature upshift. Finally, when pSSA1- β G and HF/40 Δ 147 were combined, promoter activity was nearly identical to that observed in wild-type cells, perhaps because the C-terminal activator is fully unmasked when HSF is bound to the SSA1 promoter (see Discussion).

These data can be summarized as follows. When the N-terminal activator and a transient response promoter (pHSE- β G) are examined in combination, a wild-type response is observed. This is also true when the C-terminal activator (unmasked by HF/40 Δ 147) and the sustained response promoter (pSSA1- β G) are combined. However, when either the N-terminal activator and the sustained response promoter or the C-terminal activator and the transient response promoter are combined, the phenotype of the HSF truncation is dominant. That is, regardless of the promoter, a transient response is observed with HF/1-424 and a sustained response with HF/40 Δ 147. This strongly argues that the two HSF activators perform different functions and that, following a temperature upshift, the N-terminal activator is sufficient for transient increases in HSF activity and the C-terminal activator is necessary for sustained increases.

Increased Phosphorylation of HSF Is Correlated with Changes in Transient and Sustained Activity

We previously showed that HSF transcriptional activity and phosphorylation, as judged by mobility shifts on gels, are closely correlated (Sorgor et al., 1987; Sorgor and Pelham, 1988). To establish that transient activation of HSF is associated with increased total phosphorylation rather than with the modification of a small subset of sites that determine electrophoretic mobility, cells were labeled with 32 P and incubated at 20°C or exposed to a 20 min heat shock at 39°C. A 5- to 6-fold increase in the incorporation of 32 P into the protein was observed after heat shock (Fig-

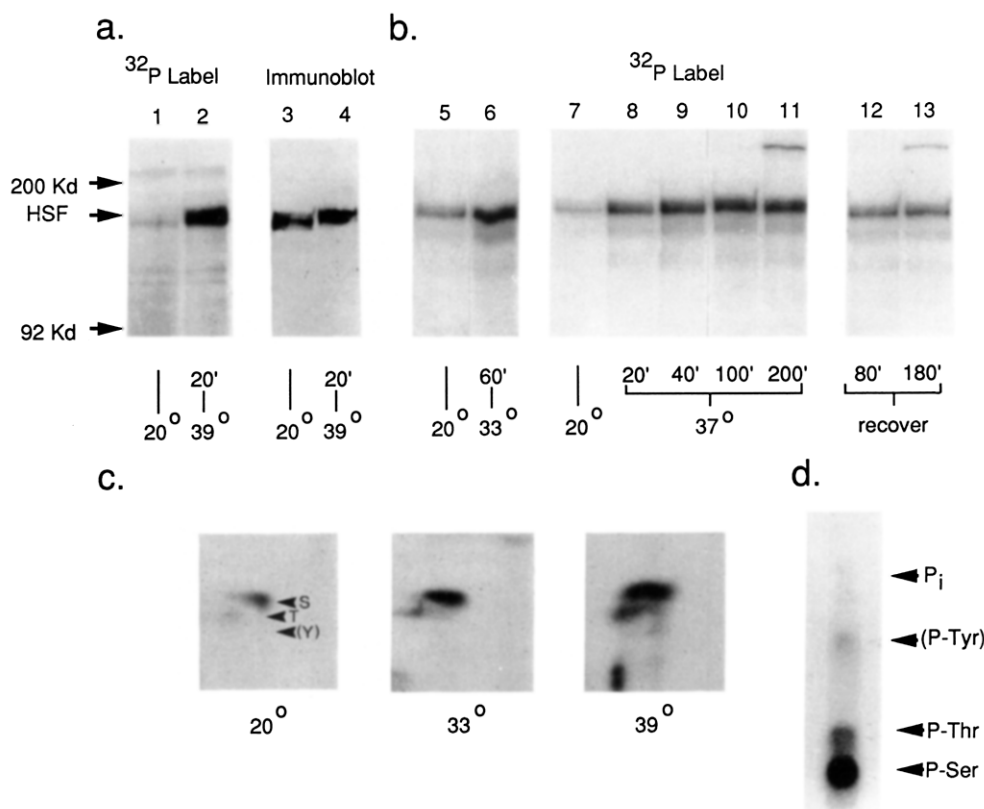


Figure 8. Analysis of HSF Phosphorylation

HSF was overproduced in wild-type cells by induction of an *HSF* gene under the control of the *GAL1* promoter (Sorger and Pelham, 1988).

(a) Effect of transient heat shock. SDS-containing gel of HSF isolated by immunoprecipitation from cells labeled with [^{32}P]orthophosphate and incubated at 20°C (lane 1) or heat shocked at 39°C for 20 min (lane 2). As a control for recovery of HSF, samples isolated in parallel but from unlabeled cells were analyzed by immunoblotting before (lane 3) and after (lane 4) heat shock. Each lane contains an equal amount of total protein.

(b) Phosphorylation associated with sustained activation. Cells labeled with [^{32}P]orthophosphate were incubated at 20°C for 20 min or at either 33°C or 37°C for the times indicated. The samples in lanes 12 and 13 were heat shocked at 37°C for 20 min and then allowed to recover at 20°C for the indicated times. The identity of the high molecular weight band visible in lanes 11 and 13 is not known. A composite of lanes from a single exposure of a single gel is shown.

(c) Two-dimensional thin-layer chromatographic analysis of hydrolyzed ^{32}P -labeled HSF recovered from SDS-containing gels following incubation of cells at 20°C, 33°C, and 39°C for 20 min. The positions of unlabeled phosphoserine, phosphothreonine, and phosphotyrosine included as markers are shown.

(d) One-dimensional TLC analysis of a labeled HSF isolated from cells heat shocked at 39°C for 20 min to visualize more clearly the ratio of phosphoserine and phosphothreonine. P_i indicates the position of free inorganic phosphate. The presence of phosphotyrosine in HSF could not be detected under any conditions by immunoblotting with anti-phosphotyrosine antibodies (data not shown), and the spot migrating near P-Tyr may arise from hydrolysis of adenylated proteins (Schieven et al., 1986).

ure 8a, lanes 1–4). Cell viability was not affected by these labeling conditions.

To determine whether increased phosphorylation is associated with changes in sustained HSF activity, two experiments were performed. First, cells were labeled at 20°C and then shifted to 33°C for 60 min, conditions that induce sustained but not transient HSF activity (Figure 1). An increase of 5-fold in phosphorylation was observed (lanes 5 and 6). Next, cells were shifted from 20°C to 37°C and maintained at the elevated temperature, and samples were withdrawn at various times within a 3 hr period. Phosphorylation increased 3-fold within 20 min and 5-fold within 40 min and then remained constant (lanes 7–11), consistent with the hypothesis that the level of phosphory-

lation remains high after the transient response is shut off. To exclude the possibility that this elevated phosphorylation was a trivial consequence of the extended period of labeling, cells were heat shocked at 37°C for 20 min and then returned to 20°C to permit recovery (Sorger et al., 1987). With this treatment, the amount of incorporated ^{32}P decreased during recovery 2-fold from its maximum level (lanes 12 and 13); this indicates that high-level labeling requires continued exposure to elevated temperatures. These data demonstrate that increases in both the sustained and transient activities of HSF are associated with elevated levels of HSF phosphorylation.

To determine the amino acids that are phosphorylated, labeled HSF from cells incubated at 20°C, 33°C, and 39°C

were subjected to acid hydrolysis and analyzed by thin-layer chromatography (Figure 8c). At 20°C the principal phosphoamino acid was serine; at 39°C phosphoserine and phosphothreonine were present in an approximate ratio of 3:1, respectively (Figure 8d). HSF isolated from cells incubated at either 20°C, 33°C, or 39°C lacked phosphotyrosine as determined by immunoblotting with anti-phosphotyrosine antibodies using PDGF receptor from stimulated cells as a positive control (data not shown; Morrison et al., 1988). These data indicate that HSF is phosphorylated by a serine-threonine protein kinase and demonstrate that the levels of both phosphoserine and phosphothreonine are elevated in activated HSF.

Discussion

The exposure of yeast cells to elevated temperatures results in both transient and sustained changes in HSF activity. The transient changes are characterized by a rapid 30-fold increase that persists for less than 1 hr and constitutes the classical heat shock response (Miller et al., 1979; McAlister et al., 1979; Craig, 1985; Lindquist, 1986). This response is induced over a narrow range of temperatures above 34.5°C. In contrast, changes in sustained activity are regulated over the range of temperatures at which yeast cells can grow. The analysis of HSF described in this paper suggests that the two responses are mediated by physically separable transcriptional activators.

The transient activity of HSF was assayed with a synthetic construct (pHSE-βG) that behaves like a classical heat shock promoter (Craig, 1985; Sorger and Pelham, 1987). Sustained HSF activity was low when assayed with this promoter and was therefore measured using the yeast SSA1 promoter (pSSA1-βG). This so-called constitutive heat shock promoter responds to a temperature upshift with a permanent increase in transcription which, although distinct from the classical response (Slater and Craig, 1987), is dependent on binding by HSF (Park and Craig, 1989). The nature of the difference between these promoters is not yet clear; it is not simply related to the number of heat shock elements (Sorger and Pelham, 1987).

HSF Contains Two Distinct Transcriptional Activation Regions

A fragment of HSF containing residues 1–424 is able to stimulate high-level transcription from the pHSE-βG pro-

motor following a temperature upshift. This indicates that the N-terminus of HSF contains an activation region sufficient for the transient response to heat shock (AR1; Figure 9). C-terminal sequences contribute somewhat to the activity of AR1 but are not necessary for heat inducibility; in contrast, residues 40–147 do appear to be essential. A trimerization region between residues 327–424 (Sorger and Nelson, 1989) and a DNA binding region comprising amino acids 167–284 (Wiederrecht et al., 1988) are necessary *in vitro* for the high-affinity binding of HSF to DNA; *in vivo*, truncation of these sequences produces proteins incapable of maintaining cell viability. There is no evidence from the analysis of LexA-HSF fusion proteins that a high-level transcriptional activator exists in a short contiguous sequence located between residues 1–424. Rather, I infer that the integrity of the entire 424 amino acid N-terminal region of HSF is essential for correct regulation.

The deletion of N-terminal residues (HF/147–833 or HF/40Δ147) results in a 40-fold increase in HSF activity in the absence of heat shock. This appears to be a consequence of unmasking a second activation region (AR2), which is located near the C-terminus of HSF (residues 584–783) and which retains its activity when coupled to the DNA binding domain of LexA. The mechanistic basis of unmasking is unclear, but a similar phenomenon has been observed following deletion of the hormone binding region of the glucocorticoid receptor (Godowski et al., 1987; Hollenberg et al., 1987). In HSF, N-terminal sequences involved in masking AR2 are also essential for the heat inducibility of AR1. This suggests that the activators may interact physically, but detailed analysis will be required to determine whether the sequences required for AR1 are identical to those that mask AR2.

Two experiments implicate AR2 in the sustained activity of HSF. First, in cells carrying HSF proteins that lack AR2, the sustained but not the transient responsiveness of the SSA1 promoter to a temperature upshift is lost. Second, the HF/40Δ147 mutation (which appears to unmask AR2) transforms the transiently inducible (pHSE-βG) promoter into a strong sustained response promoter. This indicates, by demonstrating a gain of function, that AR2 is able to stimulate transcription at a high level in conditions that do not induce the classical heat shock response.

Based on these observations, I propose that the N-terminal activator comprising residues 1–424 (AR1) mediates the transient response of HSF to elevated temperatures and that the C-terminal activator between residues 584–783 (AR2) mediates the sustained response. Following a

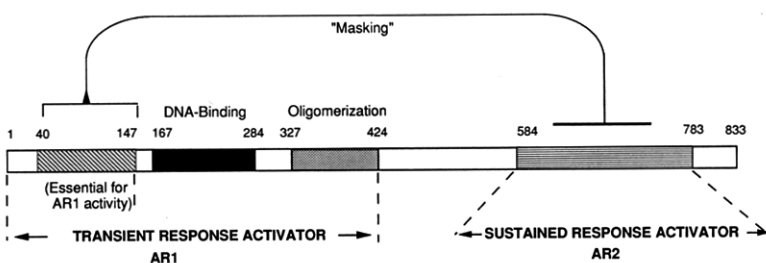


Figure 9. Schematic Representation of the Organization of Proposed HSF

Representation of HSF sequences involved in DNA binding, oligomerization, and transcriptional activation as indicated. The numbers refer to the final amino acid in each region. A bracket and curved line indicate that the deletion of residues 40–147 leads to the unmasking of AR2.

shift of cells to temperatures above 33°C, HSF enters a transient activity state that drives high-level transcription of heat-inducible genes via AR1. At 37°C this state persists for less than 1.5 hr and the factor then enters a second activated state in which AR2 directs increased transcription of the subset of heat shock genes that is expressed constitutively.

We previously demonstrated a close correlation between the extent of HSF phosphorylation and the ability to stimulate transcription. Here I show that both the transient and sustained activities of HSF appear to be regulated by serine-threonine protein kinases and phosphatases. A prediction of our model for HSF action is that there should exist at least two qualitatively different activated forms of HSF. Consistent with this we have shown that the electrophoretic mobilities of HSF-DNA complexes, which are affected by their phosphorylation state, are significantly altered when cells are shifted to temperatures above 33°C, but that little change is seen between 15°C and 33°C (Sorger et al., 1987; Sorger and Pelham, 1988). Thus, the conformation of the protein appears to differ under conditions that induce sustained (below 33°C) or transient (above 33°C) activity. The details of HSF phosphorylation appear to be complex, and preliminary tryptic analysis suggests that the protein is modified at up to 10 sites at 20°C and at an additional 5 or more sites at 39°C (unpublished data). Identification of these sites will be necessary to permit a direct test of the role of phosphorylation in HSF activation.

The data presented here also suggest that differences between heat-inducible and constitutive heat shock promoters may be a result of differential utilization of HSF activators. In particular, AR2, which is normally weakly active when HSF is bound to a transient response promoter, appears to be highly active when HSF binds to a sustained response promoter. This phenomenon may be a consequence of the interaction of HSF with a second as yet unidentified protein. Selectivity in the utilization of transcriptional activation regions has been reported to occur in the promoters of genes regulated by steroid hormone receptors (e.g., Tora et al., 1988; Lees et al., 1989).

In rich media, cells harboring HSF derivatives that lack AR2 are not viable above 33°C, indicating that the C-terminal activator performs an essential function at elevated temperatures. It seems likely that temperature sensitivity is a consequence of insufficient synthesis in nonpermissive conditions of one or more heat shock proteins. Yeast hsp82 and hsc82 (heat shock cognate protein) are examples of hsps required in greater amounts at higher temperatures (Borkovich et al., 1989). Similarly, the hsp70s encoded by the *SSA1* and *SSA2* genes are dispensable at 20°C but essential for growth at 37°C (Craig and Jacobsen, 1984). However, a direct demonstration that it is the lack of AR2 activity that is responsible for temperature sensitivity will require the identification and analysis of those genes whose products are limiting for cell growth above 33°C. The data presented here do not demonstrate that it is the *SSA1* gene product that is lacking. In addition, the role of HSF in the sustained transcription of heat shock genes cannot be mediated solely by AR2, because

the *HSF* gene is essential for viability at temperatures at which AR2 is dispensable (i.e., below 33°C; Sorger and Pelham, 1988; Wiederrecht et al., 1988).

Independent Regulation of HSF Activators

Evidence from several organisms indicates that hsp levels are regulated by a homeostatic mechanism (DiDomenico et al., 1982; Werner-Washburne et al., 1987; Stone and Craig, 1990). It might be expected, therefore, that in cells carrying HSF proteins that lack AR2 (e.g., HF1-424), the induction of AR1 activity would regulate hsp synthesis to maintain a level commensurate with growth. However, when cells carrying HF1-424 are shifted to a nonpermissive temperature (37°C), the rate of turn-off of the transient transcriptional response is similar to the rate observed in wild-type cells (Figure 6). Thus, AR1 is not permanently induced, although cells eventually die as a consequence of insufficient HSF activity. This indicates that the N-terminal activator mediates an intrinsically temporary increase in transcription in response to a temperature upshift and strongly suggests that continued exposure to elevated temperatures involves stresses that are distinct from those encountered during brief heat shock.

A further distinction between the sustained and transient activities of HSF is their temperature dependence: the former is regulated between 15°C and 33°C but the latter only above 34.5°C. To account for these differences I propose that there exist in yeast two independent networks responsible for regulating HSF activity. One network would be involved in the transient response to heat shock and would direct, via AR1, the synthesis of those hsps involved in the repair of thermolabile structures (Pelham, 1986). The other would regulate the response to changes in growth temperature and, via AR2, would act to maintain the correct balance of those hsps essential for normal cellular functions. The identification of the enzymes that modify HSF and regulate its activity should permit a test of this hypothesis.

Experimental Procedures

Plasmids

Fragments of the *HSF* gene generated with *Bal31* were fused via linkers containing stop codons in three phases to a transcriptional termination sequence comprising nucleotides 3148-3719 of the *HSF* gene (Sorger and Pelham, 1988). These truncations were then inserted into the *CEN3*-containing vector pNC160, which also carries the *TRP1*-*ARS1* gene (gift of B. Errede). Coding sequences not present in the original *HSF* gene are shown in Figure 1. Truncated HSF proteins were also constructed in vectors that carried the *HIS3* gene and *CEN4* sequences. Identical results were obtained with *TRP1* and *HIS3* vectors.

LexA-HSF fusions were constructed by inserting fragments of the *HSF* gene between the *Sall* and *EcoRI* sites of the *CEN3* vector YCp88-LexA-gcn4- Δ 19 (gift of K. Struhl; see Hope and Struhl, 1986) using linkers. At the 5' end, the *Sall* site is in-frame to encode CGA = Arg, and additional sequences (in one-letter code) were inserted as indicated in Table 1 according to the following code: 1 = VDCIVGA, 2 = P, 3 = DL, 4 = RYRDKLD, 5 = VDRDHRMLQV. At the 3' end, non-HSF sequences were inserted as follows (where * indicates a stop codon): 6 = GMLN*, 7 = WAL*. Transcription termination sequences were provided by bases 3148-3719 of the *HSF* gene.

In addition to the constructs listed in Table 1, the following LexA-HSF fusion proteins were also constructed and analyzed: LEX/1-583, LEX/1-259, LEX/1-151, LEX/66-259, LEX/211-583, LEX/211-

424, LEX/241-583, LEX/269-583, LEX/313-583, LEX/373-583, and LEX/417-583.

Yeast Strains and Enzyme Assays

Plasmids encoding truncated HSF proteins were transformed into a haploid strain derived from W303 (*ade2-1 trp1 can1-100 leu2,3-112 his3-11,15 ura3*; Shore and Nasmyth, 1987), which carried the *HSFΔ2::LEU2* chromosomal disruption of HSF and a wild-type gene on the *URA3*-containing plasmid YCp50 (Sorger and Pelham, 1988). The wild-type gene was eliminated by counterselection on 5-fluoroorotic acid plates (1 mg/ml 5-fluoroorotic acid, 7 mg/ml nitrogen base, 0.05 mg/ml uracil, and appropriate amino acid supplements) for 48 hr followed by colony purification on rich plates (Boeke et al., 1984). In cases where no *ura3*⁻ colonies were recovered at 30°C, the counterselection was repeated at 15°C. The structures of *HSF* genes were confirmed by Southern blotting, and protein levels were checked by immunoblotting.

Plasmids encoding LexA-HSF fusions were transformed into the protease-deficient strain JBB11 (*leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 GAL2*; gift of J. Boeke) or into strains containing truncated HSF molecules as described above. Strains were grown in appropriate selective media with 2% glucose.

HSF activity *in vivo* was monitored by β-galactosidase enzyme levels as described previously (Sorger and Pelham, 1987) using either of two 2μm-based multicopy plasmids: pHSE-βG, which contains a synthetic HSE sequence (HSE2) at position -178 of a *CYC1-lacZ* fusion gene lacking the *CYC1* upstream activating sequence as previously described (Sorger and Pelham, 1987), or pSSA1-βG, which contains the yeast *SSA1* promoter fused to *lacZ* (identical to plasmid pZD02 of Slater and Craig, 1987). The activities of LexA-HSF fusion proteins were determined with the plasmid YEp21-SC3423 (Hope and Struhl, 1986).

Antibodies

Anti-HSF antisera were generated in rabbits against purified protein as described previously for rats (Sorger and Pelham, 1988) and used at a dilution of 1:500. Anti-LexA antisera (kind gifts from M. Schnarr, J. Little, and R. Brent) were used at a dilution of 1:400 as follows: whole-cell extracts (prepared according to Sorger and Pelham, 1987) were incubated with antiserum for 3 hr on ice and immunoprecipitated by the addition of protein A-Sepharose. The Sepharose beads were washed with PBS and with 2 M urea, 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 0.1% Triton, 0.5% NP40, and 0.5% deoxycholate. Proteins were eluted with SDS-loading buffer that contained 1 M 2-mercaptoethanol. Samples were then electrophoresed on SDS-containing gels and immunoblotted for 12 hr essentially as described previously (Sorger and Pelham, 1988).

In Vivo Labeling with [³²P]Orthophosphate

Protease-deficient yeast strains overexpressing HSF from the pGAL1:HSF plasmid (Sorger and Pelham, 1988) were grown to OD₆₀₀ = 2 in -Ura medium containing 2% galactose and transferred to phosphate-free medium for 4 hr (Rubin, 1975), and 50 ml samples of cells were incubated with 5 mCi of [³²P]orthophosphate for 10 min at room temperature. Cells were then incubated at temperatures between 20°C and 39°C for various times, and extracts were prepared as described previously except that RNAase A was added to 0.1 mg/ml (Sorger and Pelham, 1988). Labeled extracts were reacted with anti-HSF rabbit antiserum at 1:100 dilution, immunoprecipitated with protein A-Sepharose beads that had been preincubated with unlabeled extracts, and washed three times with PBS, twice with buffer A (2 M urea, 0.1% NP40, 0.1% Triton, 0.1% deoxycholate, 50 mM Tris, 50 mM NaCl [pH 7.5]), twice with buffer B (0.1% SDS, 50 mM Tris, 150 mM NaCl [pH 7.5]), and finally with SDS sample buffer.

For phosphoamino acid analysis HSF was electrophoresed on SDS-containing gels and either eluted in 0.05 M NH₄HCO₃, 0.1% SDS or transferred to immobilion membranes by electroblotting. Labeled HSF was hydrolyzed at 100°C for 2 hr in 6 M HCl and analyzed on silica TLC plates (Merck D5589) as described by Chalberg et al. (1980) for one-dimensional analysis or by Kamps and Sefton (1989) for two-dimensional analysis. The ratio of various amino acids was determined by optical scanning of autoradiographs. The phosphotyrosine content of HSF was investigated by immunoprecipitating HSF from wild-type cells or from cells overproducing the factor followed by electrophoresis

on SDS-containing gels and finally by immunoblotting with anti-phosphotyrosine antibodies as described (Morrison et al., 1988). HSF in roughly 10-fold molar excess was compared with PDGF receptor from stimulated cells as a positive control.

Miscellaneous Techniques

Phosphatase treatment of LexA-HSF fusions were performed as described (Sorger and Pelham, 1988).

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