Mutated Yeast Heat Shock Transcription Factor Activates Transcription Independently of Hyperphosphorylation*

Naoya Hashikawa 1, Yu Mizukami, Hiromi Imazu, and Hiroshi Sakurai 2

From the Division of Health Sciences, Graduate School of Medical Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, Ishikawa 920-0942, Japan

In the yeast *Saccharomyces cerevisiae*, the HSF encoded by the *HSF1* gene regulates transcription under normal physiological conditions as well as under stress conditions, and it is essential for cell viability. The genes targeted by Hsf1 encode proteins that function in a broad range of biological processes, including protein folding and degradation, detoxification, energy generation, carbohydrate metabolism, and cell wall organization (12, 13). Mammalian cells contain three HSF isoforms, HSF1, HSF2, and HSF4. Among these, HSF1 has roles in stress-induced transcription, extra-embryonic development, and postnatal growth (14, 15). Both *S. cerevisiae* Hsf1 and mammalian HSF1 are inducibly phosphorylated concomitantly with activation (16–22). Phosphorylation of mammalian HSF1 is implicated in both positive and negative regulation of the activator function, but the mechanistic details of this regulation are incompletely understood (23, 24). *S. cerevisiae* Hsf1 is constitutively phosphorylated and is presumably controlled in a negative manner by the cAMP-dependent kinase (25). Further phosphorylation by the AMP-activated kinase Snf1 is required for glucose starvation-induced transcription mediated by Hsf1 (19). Under heat shock and oxidative stress conditions, Hsf1 is extensively phosphorylated and acquires a stronger activating ability, although the responsible kinase(s) is unknown (17, 18).

The heat-induced hyperphosphorylation of Hsf1 is regulated intramolecularly by two domains, CE2 and CTM (26). The CE2 region functions negatively to restrict the activity of Hsf1 at low temperature and/or to convert the protein from an active to an inactive form (27, 28). The CTM is required for extensive phosphorylation, but this requirement is bypassed in an *hsf1* mutant that lacks CE2, suggesting that the CTM alleviates the repressive effect of CE2 with respect to hyperphosphorylation (26). The CTM was originally identified as a domain essential for heat shock- and oxidative stress-induced transcription of the Hsf1 target gene *CUP1*. However, the CTM is dispensable when the gap-type HSE of *CUP1* is replaced by a perfect-type HSE (29). A subsequent study revealed that the CTM regulates the activating ability of Hsf1 and thereby affects recruitment of the transcription machinery (30). Therefore, the CTM and heat-induced phosphorylation are implicated in gene-specific activation and in mediating interactions between Hsf1 and the transcription machinery.

In the present study, we investigated the role of hyperphosphorylation on HSE-specific activation by Hsf1. The CTM, through its influence on phosphorylation, was found to be required for an efficient heat shock response by approximately half of Hsf1 target genes. This requirement was related to cooperative interactions among Hsf1 trimers bound to the HSE. In addition, mutated versions of Hsf1 containing amino acid substitutions in the DBD activated transcription independently of hyperphosphorylation. These results suggest that phosphorylation is involved in protein-protein interactions and in mediating conformational change of the DBD, a region that under normal conditions negatively regulates the transcriptional activity of Hsf1.

The eukaryotic heat shock transcription factor HSF regulates the transcription of various genes under numerous stressful conditions. HSF proteins share common structural motifs, including a winged helix-turn-helix DNA-binding domain (DBD), a hydrophobic repeat region essential for three-stranded coiled-coil formation, and a C-terminal transactivation domain (1–3). HSF binds to a conserved DNA sequence motif termed the heat shock element (HSE) by forming a homotrimer through the hydrophobic repeat regions, and the DBD of each monomer recognizes a 5-bp sequence, 5′-nGAA-n-3′. The organization of the three nGAA units varies among functional HSEs (4–13). The perfect-type HSE consists of three or more contiguous inverted repeats of the unit (nTTCnnGAA-nTTCn), the gap-type HSE consists of two inverted units separated from a third unit by a 5-bp gap (nTTCnnGAA5bpGAAAn), and the step-type HSE consists of direct repeats of the nGAA or nTTC motif separated by 5 bp (nGAA5bpGAAAn5bpGAAAn).

The homotrimeric heat shock transcription factor (HSF) binds to the heat shock element of target genes and regulates transcription in response to various stresses. The Hsf1 protein of *Saccharomyces cerevisiae* is extensively phosphorylated upon heat shock; a modification that is under positive regulation by its C-terminal regulatory domain (CTM). Hyperphosphorylation has been implicated in gene-specific transcriptional activation. Here, we surveyed genes whose heat shock response is reduced by a CTM mutation. The CTM is indispensable for transcription via heat shock elements bound by a single Hsf1 trimer but is dispensable for transcription via heat shock elements bound by Hsf1 trimers in a cooperative manner. Intragenic mutations located within or near the wing region of the winged helix-turn-helix DNA-binding domain suppress the temperature-sensitive growth phenotype associated with the CTM mutation and enable Hsf1 to activate transcription independently of hyperphosphorylation. Deletion of the wing partially restores the transcriptional defects of the unphosphorylated Hsf1. These results demonstrate a functional link between hyperphosphorylation and the wing region and suggest that this modification is involved in a conformational change of a single Hsf1 trimer to an active form.

The abbreviations used are: DBD, DNA-binding domain; HSF, heat shock factor; HSE, heat shock element; RT-PCR, reverse transcription-PCR.

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1 Recipient of a Japan Society of the Promotion of Science fellowship (DC2).
2 To whom correspondence should be addressed. Tel.: 81-76-265-2588; Fax: 81-76-234-4369; E-mail: sakurai@kenrouku.kanazawa-u.ac.jp.
EXPERIMENTAL PROCEDURES

Plasmids and Yeast Strains—Wild type HSF1, hsf1-ba1, and hsf1-AR1Δ-ba1 genes were cloned into yeast centromeric plasmids marked with URA3 or TRP1 (YCp-URA3 or YCp-TRP1) (26, 29). Nucleotide substitution mutations were introduced by the megaprimer PCR method (31). The plasmid YCp-TRP1-hsf1-wingΔ was created by deleting the wing region (amino acids 239–249) and substituting serine 250 with aspartate and aspartate 251 with proline (32). The hsf1-ba1 mutation was introduced into this plasmid to create YCp-TRP1-hsf1-wingΔ-ba1. The reporter genes were constructed by inserting HSE-containing oligonucleotides (see Fig. 2A) into the Xhol site lying upstream of the CYC1 promoter of pLG670Z (YPE-URA3-CYC1-lacz) (33).

All strains were derived from HS126 (MATα ade2 his3 leu2 trp1 ura3 can1 hsf1::HS3 YCp-URA3-HSF1) (26, 34). For construction of hsf1 mutants, YCp-TRP1-hsf1 derivatives were introduced into HS126, and the resident YCp-URA3-HSF1 plasmid was evicted by streaking transformed cells on 5-fluoroorotic acid-containing medium (29). Cells containing intragenic mutations that suppressed the temperature-sensitive growth defect associated with the hsf1-AR1Δ-ba1 mutation were isolated as previously described (26). Cells were grown in rich glucose (YPD) and enriched synthetic glucose (ESD) media (29).

RNA Analysis—Cells were grown at 28 °C to an optical density of 1.0 at 600 nm and were heat shocked at 39 °C. Total RNA was prepared and quantified by absorbance at 260 nm (26).

Miniarray filter hybridization analysis was carried out using yeast GeneFilters (Invitrogen). 33P-labeled cDNA was prepared from total RNA of HSF1 and hsf1-ba1 cells grown in YPD medium at 39 °C for 15 min. Hybridization, quantification, and data analysis were carried out as previously described (13). Data were deposited at the NCBI gene expression omnibus (GEO; ncbi.nlm.nih.gov/geo/) with GEO accession number GSE3361. The hybridization of each sample was performed in duplicate. In each set of experiments, -fold changes were calculated from normalized intensities. Open reading frames that displayed at least a 1.5-fold lower signal intensity in hsf1-ba1 compared with HSF1 were selected, and the average -fold changes of duplicate experiments were calculated. Open reading frames that displayed at least a 2.0-fold decrease in hsf1-ba1 relative to HSF1 are listed in Table 1. The presence of the HSE in each gene was determined as described previously (13). The nucleotide sequences of putative HSEs are shown in supplemental data.

Reverse transcription-PCR (RT-PCR) analysis was done as described previously (26). The amounts of PCR products were compared after normalizing RNA samples to the levels of control ACT1 mRNA (encoding actin) by using GelPro Analyzer software (Media Cybernetics). The nucleotide sequences of gene-specific primers are available upon request.

For Northern blot hybridization analysis, total RNA (8 μg) was treated with glyoxal, electrophoresed on an agarose gel, and blotted on a Hybond-N+ membrane (Amersham Biosciences). Each open reading frame of interest was amplified by PCR from yeast genomic DNA and labeled with the AlkPhos Direct Labeling Kit (Amersham Biosciences). Hybridization, washing, and detection were as recommended by the manufacturer. The chemiluminescence of CDP-Star was captured by a FAS-1000 lumino image analyzer (Toyobo, Tokyo, Japan) and analyzed by using GelPro Analyzer software as above.

The transcripts of HSE-CYC1-lacz reporter genes were analyzed as follows. The reporters were introduced into HSF1 and hsf1-ba1 cells, and transformed cells were grown in ESD medium lacking uracil and tryptophan at 28 or 39 °C for 20 min. Total RNA was subjected to RT-PCR and analyzed as above, except that the reverse transcription reaction was carried out in the presence of a lacZ-specific primer to improve the synthesis of the cDNA.

Gel Retardation Analysis—Cells were grown in YPD medium at 28 °C to an optical density of 1.2 at 600 nm. Cells were disrupted by vortexing with glass beads in a buffer containing 100 mM Heps-KOH (pH 7.6), 0.4 M NaCl, 1 mM EDTA, 0.2% Nonidet P-40, 6% glycerol, and 2 mM dithiothreitol. Four microliters of cleared extract was added to 12 μl of reaction mixture (1 mM EDTA, 6% glycerol, 0.3 μg of poly(dl-dC), and 0.1 ng of 32P-labeled probe DNA). After incubating at room temperature for 20 min, the samples were electrophoresed on an agarose gel and subjected to phosphorimaging as described previously (13). For Fig. 2B, cell extracts were prepared from a derivative of strain HS126 containing YEp-TRP1-HSF1, a multicopy plasmid YEplac112 (35) bearing HSF1, instead of YCp-URA3-HSF1, and were incubated with 0.2 ng of probe DNA.

Immunoblot Analysis—Cells were grown in YPD medium at 28 or 39 °C for 15 min, and cell extracts were subjected to immunoblotting with anti-Hsf1 antiserum (26).

RESULTS

Transcriptional Changes in Cells Bearing a CTM Mutation—Upon heat shock, the Hsf1 protein is extensively phosphorylated, but this modification is inhibited by the hsf1-bal mutation, which contains amino acid substitutions in the CTM domain (26). To study the effect of hyperphosphorylation on Hsf1-regulated transcription at the whole genome level, we compared the heat-induced gene expression profiles of HSF1 and hsf1-bal cells using miniarray filters. The signal intensity of 29 genes displayed at least a 2.0-fold decrease in hsf1-bal relative to HSF1 cells after a temperature shift from 28 to 39 °C for 15 min (Table 1). To confirm these transcriptional changes, the expression levels of these genes were analyzed by Northern blot hybridization and RT-PCR (Fig. 1A, left panel). In HSF1 cells, a temperature shift from 28 to 39 °C led to a rapid increase in the levels of transcripts from all genes analyzed. However, transcript accumulation was severely inhibited by the hsf1-bal mutation. We previously identified 59 Hsf1 target genes by the same method but with an hsf1 allele that confers a severe transcriptional defect (13), and 22 of the 29 genes identified here were also previously recognized as Hsf1 targets (Table 1). The remaining seven genes are also likely to be targets, because they are bound by Hsf1 (Ref. 12 and data not shown). These results show that CTM-regulated hyperphosphorylation is required for the efficient heat shock response of approximately half of Hsf1 target genes, and we refer to these as CTM-dependent genes. Notably, several of them encode proteins involved in cell wall organization (Table 1), and it was recently discovered that Hsf1 has a role in cell wall remodeling at elevated temperatures (34).

Requirement for the CTM Is Dependent on HSE Architecture—Previous analyses implied that a requirement for CTM function can be ascribed to HSE architecture (26, 29). We classified Hsf1 target genes with respect to their HSEs (Fig. 1B). The CTM-dependent genes contained gap-type or step-type HSEs or HSEs of indeterminable composition. The CTM was also necessary for the efficient heat shock response of several genes containing three perfect inverted repeats of the nGAA n unit (3P type). Notably, none of the genes containing four or more nGAA repeats was recognized as CTM-dependent in the genome-wide survey. Consistent with this, heat-induced transcription of these genes was not significantly affected by the hsf1-bal mutation as judged by Northern blot hybridization and RT-PCR analysis (Fig. 1A, right panel).

We evaluated the HSE-specific requirement for the CTM by using reporter genes containing HSEs with different arrangements of the
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TABLE 1
Genes whose heat shock-induced expression is reduced in hsf1-ba1 cells

| Open reading frame | Gene  | Description and product* |
|--------------------|-------|--------------------------|
| YBR067C            | Tip1  | Cell wall mannospeptidase |
| YER150W            | Sp11  | Stationary phase-induced cell wall protein |
| YIL042W*           | Mhp1  | Microtubule-associated protein |
| YOL109W            | Zeo1  | Zeocin resistance |

| Protein folding and degradation | | |
| YBR072W | Hsp26 | Heat shock protein 26 |
| YCR021C | Hsp30 | Heat shock protein 30 |
| YBL075C | Ssa3  | Hsp70 family |
| YPL106C | Sse1  | Hsp70 family |
| YBR169C | Sse2  | Hsp70 family |
| YBR101C | Fes1  | Hsp70 nucleotide exchange factor |
| YML130C | Ero1  | Required for oxidative protein folding in the ER |
| YLR216C | Cpr6  | Cyclophilin 40, peptidyl-prolyl cis-trans isomerase |
| YHR053C | Clp1-1 | Copper-binding metallothionein |
| YHR055C | Clp1-2 | Copper-binding metallothionein |
| YBR082C | Ubc4  | Ubiquitin-conjugating enzyme E2 |
| YDL020C* | Rpn4  | Transcription factor that stimulates expression of proteasome genes |
| YOR007C | Sgt2  | Small glutamine-rich TPR-containing protein |

| Cell wall organization | | |
| YBR067C            | TIP1  | Cell wall mannospeptidase |
| YER150W            | SP11  | Stationary phase-induced cell wall protein |
| YIL042W*           | MHP1  | Microtubule-associated protein |
| YOL109W            | ZEO1  | Zeocin resistance |

| Unclassified | | |
| YCL050C | APA1 | Diadenosine tetrathosphate phosphatase |
| YHR049W | FSH1 | Family of serine hydrolases |
| YHR104W | GRE3 | Aldose reductase |
| YHR082C* | KSP4 | Putative serine/threonine kinase |
| YDL193W* | NLS1 | Prenyltransferase |
| YLR327C | Rbf9 | Protein putative involved in cytoplasmic ribosome function |
| YIL089W* | Sip4 | Involved in Sfn1-regulated transcriptional activation |
| YBR025C | Unknown | |
| YDR110W* | Unknown | |
| YIL144W | Unknown | |
| YLR064W | Unknown | |
| YOL032W | Unknown | |

| nGaAn unit inserted upstream of the CYC1 promoter fused to the lacZ gene (HSE-CYC1-lacZ). The mRNA levels of lacZ in HSF1 and hsf1-ba1 cells were compared after normalizing them to ACT1 mRNA as a control (Fig. 2A). In HSF1 cells, the heat-induced mRNA levels of CYC1-lacZ reporters increased in parallel with increases in the number of nGaAn units, ranging from a 3-fold induction for a reporter containing a step-type HSE (HSEstep) to a 10-fold induction for a reporter containing six perfect inverted nGaAn repeats (HSE6P). The hsf1-ba1 mutation did not alter the levels of basal reporter transcription. In heat-shocked hsf1-ba1 cells, the level of HSE6P-CYC1-lacZ mRNA was comparable with that in HSF1 cells. However, transcription of reporters containing three nGaAn units, including HSE3P, HSEGap, and HSEstep, was not notably activated above the uninduced levels. The observed HSF1-specific requirement for the CTM is consistent with the results of expression analysis for individual genes. |

Requirement for the CTM Is Affected by Cooperative Interactions among Hsf1 Trimers—Two nGaAn units can be positioned head to head (nGaAnnTTCn) or tail to tail (nTTCnGaAn), and HSE oligonucleotides containing two head to head and two tail to tail repeats are denoted as 4Phh and 4Ptt, respectively (5, 6, 36). In heat-shocked HSF1 cells, the relative orientations of nGaAn units did not significantly affect the mRNA levels of reporters (Fig. 2A). In hsf1-ba1 cells, HSE4Ptt-CYC1-lacZ transcripts accumulated to the same extent as in HSF1 cells, but HSE4Phh-CYC1-lacZ transcripts accumulated to only 50% of HSF1 control levels. |

It has been suggested that the 4Ptt configuration readily binds two HSF trimers, whereas 4Phh binds a second trimer with difficulty (36). To address this proposal, HSF-Hsf1 interactions were then examined by gel retardation analysis using extracts prepared from HSF1 cells. As shown in Fig. 2B, lanes 2–4 and 5–7, the addition of increasing amounts of protein caused the formation of DNA–protein complexes containing one and two Hsf1 trimers bound to the HSE3P and HSE6P fragments, respectively. The amounts of the complexes formed with HSE6P were greater than those formed with HSE3P because of cooperative binding of trimers (5, 13, 37). The HSE4Ptt and HSE4Phh fragments formed complexes with mobilities comparable with those formed with HSE6P and HSE3P, respectively, but with higher affinities than seen for HSE3P (compare lanes 2–7 with lanes 8–13). Therefore, the HSE4Ptt fragment allows the cooperative binding of two Hsf1 trimers, whereas the HSE4Phh fragment does not stably support two bound trimers, presumably because of slightly weaker interactions between the trimers. |

CTM-dependent genes contain three nGaAn units and bind a single Hsf1 trimer. When two trimers bind cooperatively to the 4Ptt-type and 6P-type HSEs, however, transcription is activated independently of the CTM. The 4Phh-type HSE exhibits an intermediate property with respect to Hsf1 binding and the CTM dependence of transcription. These observations imply that CTM function is related either to the overall number of Hsf1 trimers bound or to trimer-trimer interactions. To distinguish between these two possibilities, a 5-bp spacer was inserted into the middle of HSE6P, which contains six inverted repeats of the nGaAn unit, to create the HSE6P+5 oligonucleotide (37, 38). This fragment bound two Hsf1 trimers in a non-cooperative manner, as judged by complex mobility and by the extent of complex formation (Fig. 2B, lanes 14–16). Consistent with these binding properties, the mRNA level of the CYC1-lacZ reporter containing HSE6P+5 was lower than that of the HSE6P-CYC1-lacZ reporter but higher than that of the HSE3P-CYC1-lacZ reporter in heat-shocked HSF1 cells (Fig. 2A). Importantly, the hsf1-ba1 mutation abrogated the transcriptional activation of the HSE6P+5-CYC1-lacZ reporter. We conclude that cooperative interactions among trimers allow Hsf1 to bypass the require-
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FIGURE 1. Effect of the hsf1-ba1 mutation on transcription of Hsf1 target genes. A, mRNA levels of Hsf1 target genes in hsf1-ba1 cells. HSF1 wild type and hsf1-ba1 cells were grown in YPD medium at 28 °C, and then the temperature was shifted to 39 °C. At the indicated times, aliquots of cells were removed and stored at −80 °C. Total RNA prepared from each sample was subjected to Northern blot (SSE1, CPR6, HSP104, KAR2, SIS1, and ACT1) or RT-PCR (all other genes) analysis. Left and right panels show transcripts of CTM-dependent and CTM-independent genes, respectively. The ACT1 gene encoding actin was used as a control. B, classification of CTM-dependent genes by HSE type. Sixty-six Hsf1 target genes, including 59 genes identified in the previous study (13) and seven genes (KSP1, MHP1, NUS1, RPN4, SIP4, TIP1, and YDR210W) identified in this study, are classified. Genes for which a consensus HSE could not be readily identified were classified as "unknown." Genes containing the perfect-type HSE are further divided into two groups based on the number of nGAAn units. CTM-dependent genes are shown in bold.

Mutation in the DBD Restore Transcriptional Activity but Not Hyperphosphorylation of the Hsf1-ba1 Protein—The slow growth phenotype of hsf1-ba1 cells at elevated temperatures is exacerbated by deletion of the non-essential activation domain AR1 (hsf1-AR1/H9004-ba1) (26, 34). We isolated intragenic mutations suppressing the temperature-sensitive growth phenotype associated with the hsf1-AR1Δ-ba1 mutation (Fig. 3A, left panel). Three mutations were located in the DBD, including alterations of glycine to arginine at 234 (G234R), glutamine to arginine at position 239 (Q239R), and glycine to arginine at position 244 (G244R). The suppressor mutations also permitted hsf1-ba1 cells to grow almost normally at 38 °C (Fig. 3A, right panel).

As shown by RT-PCR analysis (Fig. 3B), Hsf1-ba1 protein containing the Q239R mutation restored robust heat shock response of all CTM-dependent genes analyzed, including APA1, CUP1, SSA3, FSH1, SGT2, and YJL144W, to levels roughly equal that of the HSF1 control. The introduction of the G234R and G244R mutations into the hsf1-ba1 gene elevated the basal level of transcription of FSH1 and permitted the efficient heat shock response of SSA3 and a moderate response of other genes. Activation of the CTM-independent gene BTN2 was unaffected by the suppressor mutations.

It may be argued that the hsf1-ba1 mutation inhibits binding of Hsf1 to the HSE and that the suppressor mutations restore binding. Although Hsf1-ba1 protein has been shown to bind the gap-type HSE of CUP1 with an affinity similar to that of the wild type protein (29), we further examined interactions between HSE oligonucleotides and Hsf1 derivatives by gel retardation analysis. When HSE3P was used as a probe, the extent of DNA-Hsf1 complexes formed with extracts of hsf1-ba1 cells was similar to that formed with wild type extracts (Fig. 3C, compare
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Heat shock induces hyperphosphorylation of Hsf1, which causes the protein to migrate more slowly on denaturing polyacrylamide gels (16–18, 26, 28). As judged by immunoblot analysis, wild type Hsf1 protein to migrate more slowly on denaturing polyacrylamide gels (16–18, 26, 28). Therefore, the observed HSE-specific transcription is not because of changes in DNA binding affinities.

Heat shock induces hyperphosphorylation of Hsf1, which causes the protein to migrate more slowly on denaturing polyacrylamide gels (16–18, 26, 28). As judged by immunoblot analysis, wild type Hsf1 protein from heat-shocked cells migrated more slowly than did Hsf1 from control cells (Fig. 3D). However, retardation was inhibited by the hsf1-ba1 mutation, because of decreased phosphorylation of the protein (26). Heat shock also did not induce slower migration of Hsf1-ba1 proteins containing the G234R, Q239R, or G244R mutations. These results show that the suppressor mutations improve the activating ability of Hsf1-ba1 without restoring hyperphosphorylation.

Transcriptional Defects of hsf1-ba1 Cells Are Partially Suppressed by Deletion of the Wing Region—The HSF DBD contains a winged helix-turn-helix motif consisting of three helices, four β-sheets, and a flexible long loop (termed the wing) with a less ordered structure (39, 40). The Gly-234 and Gln-239 residues are located at the N and C termini of the third β-sheet, respectively. This sheet is short and is rotated away from the plane formed by other sheets. The Gly-244 residue is located at the plane formed by other sheets. The Gly-244 residue is located at the plane formed by other sheets.

Thus, the identified suppressors may alter the conformation of the wing, which presumably influences DBD-DBD interactions (41).

Cicereto et al. (32) have shown that deletion of the wing region from the DBD does not cause growth defects at normal temperatures but does lead to a decrease in the HSE-binding affinity of a single Hsf1 trimer, without affecting cooperative binding of two or three trimers, as demonstrated by the gel retardation assay. As shown in Fig. 4, A and B, hsf1-wingΔ cells grew at elevated temperatures, but the heat-induced mRNA accumulation of APA1, CUP1, SSA3, and YLR144W was reduced to 20–60% of the HSF1 control. Although the temperature sensitivity associated with the hsf1-ba1 mutation was not suppressed by simultaneous deletion of the wing (hsf1-wingΔ-ba1), the transcriptional defect was partially suppressed. In hsf1-wingΔ-ba1 cells, the mRNA levels of SSA3, FSH1, and SGT2, which contain the step-type HSE, were higher than those in hsf1-ba1 cells and similar to those in hsf1-wingΔ cells (Fig. 4B). Note that transcription of SSA3 and FSH1 was significantly influenced by the G234R, Q239R, and G244R mutations (see Fig. 3B). Immunoblot analysis showed that the wing deletion failed to restore the hsf1-wingΔ phosphorylation defect, as judged by the faster migration of Hsf1-wingΔ-ba1 protein from heat-shocked cells (Fig. 4C). The wing deletion can partially mimic suppressor mutations, suggesting a functional connection between hyperphosphorylation and the role of the wing region.

DISCUSSION

The C-terminal regulatory domain CTM is required for the heat-induced hyperphosphorylation of Hsf1 (26). Here we conducted a genome-wide expression analysis and showed that the CTM is necessary for an efficient heat shock response by approximately half of Hsf1 target genes. The classification of CTM-dependent genes and further analysis of artificial reporter genes have demonstrated that the CTM is required for transcriptional activation via HSEs consisting of three nGAAAn units (3P-, gap-, and step-type HSEs) recognized by a single trimer. Mutated Hsf1 circumvented the requirement for hyperphosphorylation for activation of CTM-dependent genes. We suggest that phosphorylation is involved in mediating a conformational change of the single trimer to an active form. When two Hsf1 trimers bind to the HSE in a cooperative manner, however, trimer-trimer interactions may induce this conformational change without extensive phosphorylation of the proteins.

The suppressor mutations in the DBD allow Hsf1 to activate transcription independently of hyperphosphorylation. The DBD determines HSE binding specificity, and mutations of amino acids in this region cause gene-specific transcriptional changes (42–45). The DBD negatively regulates the transcriptional activity of Hsf1 under normal growth conditions (46–50), and it can directly sense heat shock and localization.
undergo conformational changes (48). These observations have highlighted the essential regulatory function of the DBD for gene-specific, heat-inducible transcription.
The DBD suppressor mutations are located within or near the wing. The crystallographic study of the DBD-HSE complex revealed that unlike other winged helix-turn-helix DNA-binding domains, the Hsf1 wing does not contact DNA. It was proposed that the wing of one monomer interacts with the turn of the other monomer, and that the turn-turn interface is important for DBD-DBD interactions in a single trimer and/or two trimers (41). We have shown that the wing is necessary for the efficient transcription of several genes that contain three nGAAn repeats (APA1, CUP1, and SSA3, Fig. 4B). When mammalian HSF isoforms are expressed in yeast cells, their wing regions regulate homotrimer formation and dictate gene-specific transcription (51). These observations suggest the involvement of the wing in protein-protein interactions within a single trimer and consequently in HSE type-specific binding and transcription.

Deletion of the wing partially suppresses transcriptional defects associated with the CTM mutation without restoring the hyperphosphorylation; unphosphorylated Hsf1-wingΔ-ba1 protein activated the transcription of several DBM-dependent genes, including FSH1, SSA3, and SGT2. The partial suppression conferred by the Hsf1-wingΔ-ba1 protein is likely caused by a decrease in DNA binding affinity because of the deletion of the wing (32). In addition to its positive role in DNA binding, the wing may also be partly responsible for the negative regulatory roles of the DBD. It is possible that protein-protein interactions involving the wings inhibit the activator function of a single trimer. Because the CTM has no significant effect on binding to the HSE, hyperphosphorylation may facilitate a conformational change of the DBD and especially of the wing that restrains the inhibitory function and allows acquisition of the activating ability.

Cooperative protein-DNA and protein-protein interactions are important for fine tuning transcriptional levels (52). The cooperative binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well characterized (6, 37, 38). In these studies, cooperativity has been connected with the binding of Hsf1 molecules to the HSE has been well charact