Different Mechanisms Are Involved in the Transcriptional Activation by Yeast Heat Shock Transcription Factor through Two Different Types of Heat Shock Elements*5

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THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 14, pp. 10333–10340, April 6, 2007
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The hydrophobic repeat is a conserved structural motif of eukaryotic heat shock transcription factor (HSF) that enables HSF to form a homotrimer. Homotrimeric HSF binds to heat shock elements (HSEs) consisting of three inverted repeats of the sequence nGAAn. Sequences consisting of four or more nGAAn units are bound cooperatively by two HSF trimers. We show that in Saccharomyces cerevisiae cells oligomerization-defective Hsf1 is not able to bind HSEs with three units and is not extensively phosphorylated in response to stress; it is therefore unable to activate genes containing this type of HSE. Several lines of evidence indicate that oligomerization is a prerequisite for stress-induced hyperphosphorylation of Hsf1. In contrast, oligomerization and hyperphosphorylation are not necessary for gene activation via HSEs with four units. Intragenic suppressor screening of oligomerization-defective hsf1 showed that an interface between adjacent DNA-binding domains is important for the binding of Hsf1 to the HSE. We suggest that Saccharomyces cerevisiae HSEs with different structures are regulated differently; HSEs with three units require Hsf1 to be both oligomerized and hyperphosphorylated, whereas HSEs with four or more units do not require either.

The eukaryotic heat shock transcription factor (HSF) governs constitutive and stress-inducible mRNA synthesis of various genes, including heat shock protein (HSP) genes. A helix-turn-helix DBD and two hydrophobic repeat regions, HR-A and HR-B, are HSF motifs that are conserved from yeast to humans. The DBD recognizes a 5 bp sequence, nGAAn-3’, and HR-A/B mediates interactions between three monomers to form a trimer via a triple-stranded coiled-coil. Homotrimeric HSF binds to three inverted repeats of a unit, termed the heat shock element (HSE), located in the regulatory regions of target genes (4–6).

The Saccharomyces cerevisiae HSE encoded by the HSF1 gene regulates transcription of 1.5–3.0% of the total genes of yeast (7, 8). The target genes of Hsf1 contain the “perfect type” HSE, which consists of three contiguous inverted repeats of the nGAAn unit (3P type, nTTCnnGAAnTTCC), as well as an HSE containing one (gap type, nTTCnnGAAn(5 bp)nGAAn) or two (step type, nGAAn(5 bp)nGAAn(5 bp)nGAAn) gaps between the repeating units (8–11). The three nGAAn units of these HSEs are bound by a single Hsf1 trimer, whereas HSEs consisting of four or five contiguous units (4P or 5P type) are bound by two Hsf1 trimers in a cooperative manner (12,13).

Under normal physiological conditions, trimerized yeast Hsf1 binds the HSE and maintains low level constitutive transcription for cell viability (14–17). Thermal stress increases the abilities of Hsf1 to bind stably to the HSE and to acquire high level transcriptional activity (7). In this process, hypophosphorylated Hsf1 is hyperphosphorylated by an unknown protein kinase (14, 18). This modification is regulated intramolecularly by two domains, CE2 and CTM; the former represses hyperphosphorylation and the latter alleviates the repressive effect (19). Temperature-sensitive hsf1 mutations cause defects in cell wall integrity, spindle pole body duplication, and cell cycle progression at non-permissive temperatures (20–22). In metazoans, homotrimer formation of HSFs is generally inhibited by an additional hydrophobic repeat, HR-C, that intramolecularly binds the conserved HR-A/B (23–27). The monomeric HSF interacts with chaperone complexes and localizes in cytoplasm, where various stresses stimulate trimerization, nuclear translocation, and binding to the HSE (28–34). The transcriptionally active form of mammalian HSF1 becomes inducibly phosphorylated (35, 36).

In the present study, we examined the functional domains of yeast Hsf1 by analyzing the ability of Hsf1 derivatives lacking various regions to activate transcription. The oligomerization
function of the HR-A/B region was found to be required for heat- or ethanol-induced transcription of genes containing HSEs with three, but not four, nGAAAn units. Oligomerization-defective Hsf1 inhibited activation of the genes at two steps, HSE binding and stress-induced hyperphosphorylation. Analysis of extragenic multiplicity suppressor genes showed a connection between oligomerization and hyperphosphorylation, and analysis of intragenic suppressor mutations showed that DBD-DBD interactions were important for HSE binding. These observations suggest that the number of nGAAAn units is a critical determinant of the regulatory pathway used by Hsf1.

EXPERIMENTAL PROCEDURES

Plasmids and Yeast Strains—The wild type HSF1 gene was cloned into a yeast centromeric plasmid containing a TRP1 marker (YCP-TRP1) (19, 37). The detailed structures of the hsf1 derivatives are shown in Figs. 1A, 2A, and 3A. Nucleotide substitution mutations were introduced into YCP-TRP1-HSF1 by site-directed mutagenesis (13). In YCp-TRP1-hsf1-gcn4, the HR-A/B region was substituted with the dimerization domain (YEp-HR-A/B) region was substituted with the dimerization domain (YEp-LEU2). For chromatin immunoprecipitation analysis, cells containing the multicopy plasmid of HSF1 were grown to an optical density of 2.0 at 600 nm and treated with 1% formaldehyde for 10 min. Chromatin immunoprecipitation analysis was carried out as described (41), using an anti-HA antibody (Invitrogen). DNA was prepared from immunoprecipitates and input chromatin samples (1% of each chromatin sample used for immunoprecipitation) and was subjected to PCR. The PCR products were separated by polyacrylamide gel electrophoresis, visualized by ethidium bromide staining, and quantified by GelPro Analyzer software (Media Cybernetics). All experiments were performed at least three times with similar results. The following regions relative to the translation initiation site were amplified by PCR: −334 to +26 of HSP78, −386 to −93 of BTN2, −485 to −133 of HSP10, −449 to −8 of CPR6, −230 to +18 of CUP1, −417 to −165 of HSC82, −339 to +46 of SSA3, and −211 to +83 of GAL7.

RESULTS

HR-A/B of Hsf1 Affects the Heat Shock Response of Target Genes—The DBD and the N-terminal linker segment are essential for Hsf1 to function to support cell viability (42–44). To examine whether other regions affect the activator function of Hsf1, we constructed cells expressing deletion derivatives of Hsf1 (Fig. 1A). In HSF1 wild type cells, a temperature shift from 28 to 39 °C caused accumulation of transcripts from the Hsf1 target genes HSP78, HSP10, CUP1, and SSA3, as determined with an RT-PCR analysis (Fig. 1B). Deletion of various regions did not significantly affect the heat-induced mRNA levels of HSP78, which contains the 4Ptt-type HSE that consists of four continuous inverted repeats of the nGAAAn unit in a tail-to-tail orientation (nTTCnnGAAnnTTCnnGAAn) (13). The mRNA levels of other genes containing HSEs of the 3P type (three continuous units, HSP10), gap type (CUP1), and step type (SSA3) increased by almost normal amounts in cells expressing most of the Hsf1 derivatives but not in those with the Hsf1-A/Δ and Hsf1-CTMΔ constructs. This is in agreement with our previous observation that the CTM is necessary for the heat shock response via the HSEs consisting of three nGAAAn units, but not four or more units (13), and indicates that although some Hsf1 domains are not essential for the activator function, the HR-A/B and CTM are necessary for gene-specific transcription.

Trimerization of Hsf1 Is Necessary for a Subset of Target Genes to Respond to Stress—The HR-A/B region of Hsf1 consists of two α-helical coiled-coils, A and B, (Fig. 2A) and has a
role in homotrimer formation (16, 45, 46). Consistent with previous results (42, 44), hsf1-A/BΔ cells exhibited a growth defect at elevated, but not normal, temperatures (Fig. 2B). We analyzed trimer formation of Hsf1 and Hsf1-A/BΔ proteins by treating cell extracts with the chemical cross-linker EGS and immunoblotting with anti-Hsf1 serum. In the absence of EGS, wild type Hsf1 protein was detected as a band on the gel with a mass consistent with the monomeric form. However, when EGS was added to the cell extracts, two additional bands were observed, corresponding to higher molecular weights than the monomer. These bands are consistent with the formation of a dimer and a trimer, as expected for a protein with coiled-coil motifs.

Transcription of several Hsf1 target genes is also regulated by other stress-responsive transcription factors, including Msn2 and Msn4 (50). To verify the role of trimerization on HSE-specific activation by Hsf1, we constructed reporter genes containing various HSEs placed upstream of the CYC1 promoter-lacZ fusion (HSE-CYC1-lacZ) (13). In HSF1 cells, insertion of the 4Ptt-, 3P-, gap-, and step-type HSEs increased the mRNA levels from the reporter genes under heat or ethanol stress conditions (Fig. 2E). In hsf1-A/BΔ cells, transcription of HSE4Ptt-CYC1-lacZ was activated in response to stress; however, reporter genes containing the 3P-, gap-, and step-type HSEs were completely inactive. These results show that trimerization is required for stress-induced transcription via HSEs containing three nGAAn units. Note that under normal conditions, the HSE4Ptt-CYC1-lacZ mRNA was elevated in hsf1-A/BΔ cells compared with HSF1 cells. The constitutive transcription of HSP42, HSP78, and BTN2 was slightly increased in trimerization-defective mutants (see supplemental data). It has been shown by Nieto-Sotelo et al. (43) that the HRA/B region is involved in repression of Hsf1 at non-shock temperatures.

**Dimerization Domain of Gcn4 Substitutes for the Trimerization Domain of Hsf1**—To analyze the relationship between the trimerization defect and phenotypic changes, the HRA/B region of Hsf1 was replaced with the dimerization domain of the transcriptional activator Gcn4 (Fig. 3A) (38, 39). Cells expressing Hsf1-gcn4 recovered almost normal growth at 38 °C although the protein predominantly formed dimers in the cross-linking assay (Fig. 3, B and C). In hsf1-gcn4 cells, all of the genes analyzed were normally activated by heat or ethanol treatment (Fig. 3D, lanes 4–6). The dimerization domain of Gcn4 was functionally indistinguishable from the authentic trimerization domain of Hsf1, suggesting that oligomerization rather than some unknown HRA/B function is required for gene activation. However, the possibility remains that in yeast cells Hsf1-gcn4 forms a homotrimer when it binds to the HSE, because a recombinant polypeptide of the Hsf1 DBD-Gcn4 dimerization domain fusion forms a dimer in solution but forms a trimer in the presence of the HSE (39).
Oligomerization of Hsf1 in Gene Activation

Oligomerization of Hsf1 is necessary for stress-induced hyperphosphorylation of Hsf1—Similar to the hsf1 mutations that are defective in oligomerization, the hsf1-CTMΔ and hsf1-ba1 mutations of the CTM inhibited transcriptional activation via HSEs containing three nGAAn units (see Fig. 1B and Fig. 3D, lanes 7–9) (13). The CTM is also required for heat-induced hyperphosphorylation of Hsf1 (19). Hyperphosphorylated Hsf1 migrates more slowly than the hypophosphorylated form on denaturing polyacrylamide gels (14, 18, 19). Immunoblot analysis showed that, in contrast to wild type Hsf1, the mobilities of Hsf1-A/Δ, Hsf1-Am, and Hsf1-Bm were not retarded when cells were heat-shocked (Fig. 4, lanes 2, 5, 8, and 11). Ethanol treatment also induced slower migration of Hsf1, but not Hsf1-A/Δ, Hsf1-Am, and Hsf1-Bm (lanes 3, 6, 9, and 12). Therefore, the oligomerization defect abrogates the stress-induced phosphorylation of Hsf1. On the other hand, the substitution of the Gcn4 dimerization domain for the HR-A/B caused heat- or ethanol-induced phosphorylation of Hsf1-gcn4 (lanes 13–15). When the hsf1-ba1 mutation was combined with the hsf1-gcn4 mutation, the resulting hsf1-gcn4-ba1 cells exhibited the same phenotypes as hsf1-ba1 cells with defects in stress-induced phosphorylation (Fig. 4, lanes 16–18), transcriptional activation via HSEs with three nGAAn units (Fig. 3D, lanes 10–12), and growth at elevated temperatures (Fig. 3B). These results strengthen the conclusion that the Gcn4 dimerization domain functionally substitutes for the HR-A/B. Taken together with the observation that oligomerization was affected neither by the heat shock (Fig. 2C, lanes 1–8) nor by the hsf1-ba1 mutation (see supplemental data), we conclude that oligomerization is required but not sufficient for hyperphosphorylation of Hsf1.

The Oligomerization Defect Causes a Cell Wall Organization Defect at Elevated Temperatures—Cells containing mutations in the CTM exhibit an osmotic remedial cell lysis phenotype at 28 °C and are suppressed by the addition of a hyperosmotic stabilizer, such as sorbitol, to the medium or by activation of cell wall integrity signaling (20, 51). The signal is required but not sufficient for hyperphosphorylation of Hsf1. The Oligomerization Defect Causes a Cell Wall Organization Defect at Elevated Temperatures—Cells containing mutations in the CTM exhibit an osmotic remedial cell lysis phenotype at elevated temperatures (20). This phenotype is caused by a defect in cell wall organization and is suppressed by the addition of an osmotic stabilizer, such as sorbitol, to the medium or by activation of cell wall integrity signaling (20, 51). The signal pathway consists of cell wall stress sensors, Wsc1–3, Mid2, and Mtl1; a GDP/GTP exchange protein, Rom2, that is coupled to the sensors; a small G-protein, Rho1, that is regulated by...
Rom2; and a protein kinase, Pkc1, activated by Rho1 (51). Cells expressing Hsf1-A/Bm, which contains all six amino acid changes of Hsf1-Am and Hsf1-Bm, recovered normal growth at 37 °C when 1 M sorbitol was added to the medium or when WSC1 was expressed as a multicopy gene (Fig. 5). These results confirm a functional connection between the CTM-regulated and oligomerization-regulated phosphorylation/transcription pathways. It is known that Pkc1 activates the downstream mitogen-activated protein kinase cascade (51). Similar to hsf1-ba1 cells (20), a constitutively active allele of the mitogen-activated protein kinase cascade component (MKK1S386P) failed to suppress the temperature sensitivity of hsf1-A/Bm cells (Fig. 5), suggesting that alternative Pkc1 pathway branches are involved in cell wall remodeling in these hsf1 mutant cells.

An Amino Acid Substitution in the DBD Suppresses Several Phenotypes Associated with the Oligomerization Defect—To further explore the role of oligomerization in Hsf1 function, we attempted to isolate intragenic mutations that suppressed the temperature sensitivity of hsf1-A/Bm. An amino acid substitution, V203A, located in the DBD suppressed the growth defect of hsf1-A/Bm cells at 37 °C (Fig. 6A). Interestingly, the same substitution has been identified and characterized as a muta-
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![Figure 7. Binding of Hsf1 to target promoters in yeast. A, binding of Hsf1-ba1. Cells containing Yip-LEU2-HA-HSF1 or Yip-LEU2-HA-hsf1-ba1 were grown at 28 °C in the absence (C) or presence of 8% ethanol for 30 min (E) or were grown at 39 °C for 15 min (HS). Soluble chromatin was prepared from formaldehyde-treated cells and was subjected to immunoprecipitation with an anti-HA antibody. DNA was prepared from immunoprecipitates (Imm ppt) and input chromatin samples (Input) and analyzed by PCR. PCR analysis of the input indicates that the chromatin samples had equal amounts of chromatin fragments containing the control GAL7 promoter. However, the fragments containing the Hsf1 target promoters were slightly decreased in the input of heat- or ethanol-treated cells compared with control cells (for example, HSP78; compare lanes 2 and 3 with lane 1, and lanes 8 and 9 with lane 7). It was shown that histones are displaced from the Hsf1 target promoters upon heat shock (60). This might cause excessive fragmentation of these promoter DNA's by sonication, thereby inhibiting amplification by PCR. Therefore, relative binding of Hsf1 to the active promoters might be underestimated in our analysis. B, binding of Hsf1-A/Bm and Hsf1-A/Bm-V203A. Cells containing Yip-LEU2-HA-HSF1, Yip-LEU2-HA-hsf1-A/Bm, or Yip-LEU2-HA-hsf1-A/Bm-V203A were grown at 28 °C (C) or 39 °C for 15 min (HS). The chromatin immunoprecipitation analysis was carried out as described above. C, relative levels of Hsf1 binding and transcription. The mRNA levels of the indicated genes (Fig. 6B) were normalized to control ACT1 mRNA as 100% and expressed as the mean ± S.D. of three independent experiments. The immunoprecipitation (IP)/input ratios of the indicated genes (B) were normalized to those of GAL7 and expressed as the mean ± S.D. of three independent experiments. The dashed line is the relative immunoprecipitation/input ratio of GAL7 (0.085 ± 0.021%), and the ratio above the line shows enrichment of the promoter in the precipitate relative to input. Asterisks indicate significant differences (p < 0.01) when hsf1-A/Bm cells were compared with hsf1-A/Bm-V203A cells as determined by the Student’s t test.

The constitutive and heat- or ethanol-induced mRNA levels of HSP1 were moderately lower than the binding of HSF1 to HSP10, CPR6, and SSA3 to a similar extent as heat shock, suggesting that the low level of activation of these genes by ethanol treatment can be ascribed to reasons other than the DNA binding. Under normal and stress conditions, HA-Hsf1-ba1 protein bound to various target genes as well as wild type protein (Fig. 7A, lanes 10–12). These results show that the CTM-regulated hyperphosphorylation of Hsf1 does not affect binding to the HSE.

In heat-shocked cells, the binding of HA-Hsf1-A/Bm to BTN2 and HSP78 was moderately lower than the binding of HA-Hsf1, and HA-Hsf1-A/Bm containing the V203A substitution retained normal binding affinity (Fig. 7B, lanes 4, 8, and 12). However, the change of binding affinity did not significantly affect the heat shock response of these genes (Fig. 7C). The binding of HA-Hsf1-A/Bm to the other genes we examined, whose transcription was significantly reduced by the mutation, was almost completely absent, irrespective of growth temperatures (Fig. 7B, lanes 7 and 8). Among these, binding to CUP1 and HSC82 was slightly but reproducibly stimulated by the V203A substitution in cells grown under normal conditions (lane 11). The elevated binding was correlated with transcription that elevates Hsf1 binding to and transcription of CUP1 (which encodes copper metallothionein) (52–54). In hsf1-V203A cells, the constitutive and heat- or ethanol-induced mRNA levels of CUP1 were robustly increased, whereas the mRNA levels of FSH1 and SSA3 were slightly decreased (Fig. 6B, lanes 7–9). The addition of the V203A substitution to the hsf1-A/Bm background background stimulated constitutive, but not stress-induced, transcription of HSC82 in addition to CUP1 (lanes 10–12). Transcriptional activation of several genes, including HSP42, HSP78, and APA1, was slightly elevated in hsf1-A/Bm-V203A cells relative to hsf1-A/Bm cells. However, the V203A suppressor failed to restore oligomerization (see supplemental data) and stress-inducible phosphorylation (Fig. 4, lanes 22–24) to Hsf1-A/Bm.

We analyzed the binding of Hsf1 protein to target genes with the chromatin immunoprecipitation technique, using cells expressing HA-tagged Hsf1. The addition of the HA tag did not significantly alter the activity of wild type and mutant Hsf1 (see supplemental data). An anti-HA antibody precipitated the promoter fragments of HSP78, HSP10, and CPR6 from extracts of HA-HSF1 wild type cells grown at 28 °C (Fig. 7A, lane 4). Heat or ethanol treatment resulted in efficient precipitation of these fragments as well as the SSA3 promoter fragment (lanes 5 and 6). The failure of the anti-HA antibody to precipitate the non-target promoter GAL7, whose transcription is regulated by galactose availability, confirmed the specific binding of HA-Hsf1 to these genes. Therefore, Hsf1 binds with lower affinity to many, if not all, of the targets under normal growth conditions and with higher affinity to most of the targets in response to stress. Ethanol treatment induced the binding of HA-Hsf1 to HSP10, CPR6, and SSA3 to a similar extent as heat shock, suggesting that the low level of activation of these genes by ethanol treatment can be ascribed to reasons other than the DNA binding. Under normal and stress conditions, HA-Hsf1-ba1 protein bound to various target genes as well as wild type protein (Fig. 7A, lanes 10–12). These results show that the CTM-regulated hyperphosphorylation of Hsf1 does not affect binding to the HSE.

Binding of Mutant Hsf1 Proteins to Target Genes in Yeast—
tional stimulation of these genes (Fig. 7C). Therefore, we ascribe the HSE type-specific transcriptional defect of Hsf1-A/Bm to defects in both HSE binding and hyperphosphorylation, whereas the partial suppression of the defect by the V203A substitution is because of elevated binding to several genes, including CUP1 and HSC82.

DISCUSSION

Although some regions of Hsf1 were not essential for the activation of HSE-containing genes, HR-A/B and CTM were indispensable for heat- or ethanol-induced transcription of genes containing 3P-, gap-, and step-type HSEs. The oligomerization mediated by HR-A/B was required for the binding of a single Hsf1 trimer to HSEs with three nGAAn units but not for the binding of two trimers to 4Ptt-type HSEs, and stress-induced hyperphosphorylation of Hsf1 was abrogated by the oligomerization defect. The CTM mutations also impeded hyperphosphorylation as well as the transcriptional activation via three-unit but not four-unit HSEs (13, 19) without affecting binding to various HSEs (see Fig. 7A). Therefore, activation by a single trimer is dependent on oligomerization-mediated HSE binding that is regulated by HR-A/B and on hyperphosphorylation that is regulated by HR-A/B and the CTM. In contrast, activation by two trimers circumvents both regulatory steps. In S. cerevisiae cells, therefore, the presence or absence of trimer-trimer interactions on the promoter is an important determinant for how Hsf1 activates transcription of a gene.

Metazoan HSFs are generally in a monomeric form, and stress-induced oligomerization is the first step for their subsequent activation of transcription (28–34). In addition to the HR-A/B, oligomerization of mammalian HSF1 is regulated by the structures of the DBD and linker regions (34, 55, 56). Although the HR-A/B of yeast Hsf1 participates in the activation by a single Hsf1 trimer as described above, Hsf1 function was retained when HR-A/B was replaced by the dimerization domain of Gcn4, suggesting the essential role of the HR-A/B region to maintain the structural integrity of the Hsf1 oligomer. A recombinant Hsf1 polypeptide containing the DBD alone will bind to HSEs cooperatively, but its binding affinity is significantly lower than that containing both the DBD and HR-A/B (44, 57). Our suppressor screening showed that the DBD has an important role for HSE binding in oligomerization-defective mutants. The suppressor mutation V203A is located near the interface of the DBD-DBD interaction (57). The DBD dimer interface is likely important for increasing the DNA binding affinity of the HSF trimer as well as for increasing cooperativity between adjacent trimers (57). The V203A substitution elevated the constitutive transcription of CUP1 and HSC82 by stimulating the binding of Hsf1-A/Bm to their HSEs. However, it failed to recover the stress response of these genes, because hyperphosphorylation was still inhibited. In our in vivo binding analysis, oligomerization-defective Hsf1 was able to bind promoters containing the 4Ptt-type HSE. This could be explained by cooperative interactions among DBD monomers. There are two orientations for two nGAAn units, nGAAnTTTcn and nTTCnGAAn, which are referred to as head-to-head and tail-to-tail repeats, respectively. In vitro binding analysis has shown that HSF binds more strongly to the tail-to-tail repeat than to the head-to-head repeat (12, 58). The 4Ptt-type HSE contains one more tail-to-tail repeat than the 3P-type HSE, which induces cooperative interactions and greatly increases binding affinity (12, 13). These findings, together with our results, indicate that the number of nGAAn units determines the strength of the DBD-DBD and DBD-DNA interactions.

The oligomerization defect impedes the heat- or ethanol-induced hyperphosphorylation of Hsf1 that is positively regulated by the CTM and is essential for activation by a single trimer. By contrast, hyperphosphorylation did not affect oligomerization. Although the phosphorylation defect caused by CTM mutations is suppressed by deletion of the CE2 region (19), the defects of Hsf1-A/Bm in hyperphosphorylation, transcriptional activation, and growth at elevated temperatures were not suppressed by the CE2 deletion (data not shown). These results show that oligomerization is a prerequisite for the inducible phosphorylation regulated by the CTM-CE2 interaction. In mammalian cells, stress-induced HSF1 is hyperphosphorylated after oligomerization of monomers (35, 36). The reason phosphorylation is involved in activation by a single trimer, but not by two trimers, remains to be elucidated. It is possible that this modification induces a conformational change in a single trimer that converts it to an active form, a change that can also be mediated by trimer-trimer interactions on the HSEs with four or more nGAAn units (13).

Analysis of additional Hsf1 target genes showed that Hsf1-A/Bm failed to mediate the heat shock response of 9 genes containing HSEs with three nGAAn units (FES1, HSP60, HSP82, MDJ1, SGT2, SSCP1, YDH1, YLR064W, and ZEO1) and 3 genes lacking an apparent HSE (AHPI, TIP1, and YIL144W) but did mediate the heat shock response of 4 genes containing HSE with at least four units (SIS1, SSA1, SSA4, and ZPR1) (data not shown). In the S. cerevisiae genome, 57 of 66 Hsf1 targets contain three-unit HSEs or lack an apparent HSE (13), and many, if not all, of these genes would be transcriptionally inhibited by the hsfs1–A/Bm mutation. The oligomerization- and phosphorylation-defective Hsf1 is able to support cell growth in medium containing an osmotic stabilizer, suggesting that the heat shock response of many target genes is not needed under this condition. Although Hsf1 directly regulates genes whose products are involved in cell wall organization, there was no significant relationship between the expression levels of these genes and the cell lysis phenotype of hsfs1–ba1 cells (20). Several hsfs1 mutations indirectly affect the mRNA levels of non-target genes (8, 59), suggesting that these transcriptional changes could cause the cell lysis phenotype. Regardless, direct and indirect functions of Hsf1 are essential for the adaptation of cells to thermal stress conditions.

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