Saccharomyces cerevisiae Heat Shock Transcription Factor Regulates Cell Wall Remodeling in Response to Heat Shock

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All organisms respond to thermal stress by activating a gene expression program governed by stress-responsive transcription factors. The heat shock transcription factor (HSF), a protein evolutionarily conserved from yeasts to humans, regulates expression of a set of proteins called heat shock proteins (HSPs), many of which function as molecular chaperones (28, 37). In the yeast Saccharomyces cerevisiae, other transcriptional networks are also induced by heat shock. A pair of partially redundant transcription factors, Msn2 and Msn4, whose activity is controlled by the cyclic AMP (cAMP)-dependent protein kinase (PKA), activates expression of genes encoding several HSPs, enzymes for carbohydrate metabolism, and proteins involved in protection against oxidative stress (48). The transcription factors Rlm1 and Swi4, which are targets of a stress-inducible mitogen-activated protein kinase (MAPK), stimulate transcription of cell wall protein genes and cell cycle-regulated genes, respectively (14, 17).

Among these transcription factors, only HSF1 encoded by the HSF1 locus is essential for the growth of S. cerevisiae as normal, as well as elevated temperatures. Like HSFs of other eukaryotes, yeast Hsf1 forms a homotrimer and binds to a regulatory sequence, the heat shock element (HSE), of target genes. The HSE consists of multiple inverted repeats of the 5-bp sequence nGAAn (where n is any nucleotide). Both continuous (nTTCnnGAAntTTCn) and discontinuous [e.g., nT-TCnnGAAAn(5 bp)nGAAAn] arrays of repeats can function as HSEs (1, 28, 37). A genome-wide Hsf1-binding analysis revealed that Hsf1 binds to the S’ upstream region of approximately 165’ of 6,200 loci in the yeast genome (15). An expression analysis with an hsf1 mutant showed that Hsf1 activates transcription of at least 59 genes upon heat shock (53). The products of these genes are implicated in a broad range of biological functions, including protein folding and maturation, energy generation, carbohydrate metabolism, maintenance of cell integrity, cell signaling, and transcription (15, 53).

The Hsf1 protein consists of discrete domains necessary for DNA binding, for trimer formation, for activation of transcription (named AR1 and AR2), for repression of the activation ability (CE2 [for conserved element 2]), and for regulation of the CE2 function (CTM [for C-terminal modulator]) (19, 32, 44, 47). Notably, the CTM domain is required for the growth of yeast at elevated temperatures for heat-induced hyperphosphorylation of Hsf1 and for transcriptional activation of genes containing the discontinuous HSE but not the continuous HSE. All of the defects associated with loss of CTM function are bypassed when CE2 has simultaneously been deleted, suggesting that CE2 inhibits hyperphosphorylation and HSE architecture-specific transcriptional activation and that in response to heat shock CTM restrains the inhibitory functions of CE2 (16).

Whereas the DNA-binding domain of Hsf1 is essential for viability of yeast, the other domains exhibit differential requirements for growth (16, 19, 32, 47). Here, we isolated multicopy suppressor genes that rescue the temperature sensitivity of an hsf1 mutant lacking the CTM function. Analyses of these isolates revealed the involvement of Hsf1 in cell wall remodeling and, additionally, showed functional interactions between Hsf1 and two protein kinases, Pkc1, an upstream regulator of the MAPK cascade, and Rim15, a downstream target of PKA.

MATERIALS AND METHODS

Yeast strains and media. The yeast strains used in the present study are listed in Table 1. All strains were derived from HS126 (16). Cells containing various
**RESULTS**

**Isolation of multicopy suppressor genes of the temperature-sensitive growth defect associated with CTM mutations.** CTM function is inactivated by the “ba1” mutation in which two arginine residues in the CTM are replaced by glutamic acid (Fig. 1A, hsf1-AR1\-ba1 mutation), show a severe growth defect at 38°C (16). Unlike other hsf1 mutants, whose temperature sensitivity is suppressed by elevated expression of Hsp90 (27, 55), introduction of a multicopy plasmid bearing the Hsp90 gene HSP82 into hsf1-AR1\-ba1 cells failed to recover normal growth at 38°C (Fig. 1B).

To explore cellular functions of Hsf1 further, we screened multicopy suppressor genes that rescue the temperature-sensitive growth of hsf1-AR1\-ba1 cells (Fig. 1B). In addition to the expected wild-type HSF1 gene, various genes were identified as suppressors (Table 2). The PDE2 gene encodes a Camp phosphodiesterase that downregulates Pka-dependent responses (48). The RIM15 gene product is a protein kinase containing a PAS domain that is known to act as a sensor for a variety of stimuli, and its kinase activity is negatively regulated by Pka (5, 39, 52). The plasma membrane proteins encoded by WSC1, WSC2, and MID2 play the role of stress sensors and bind to and activate Rom2, a Gdp/Gtp exchange protein for the small GTP-binding protein Rho1 (12, 18, 22, 33, 36, 38, 51). Rho1 is an upstream regulator of the protein kinase Pkci, which affects actin filament organization and cell wall biogenesis (14, 17). EXG1 and KRE6 encode the major exo-1,3-β-glucanase and a protein required for β-1,6-glucan synthesis, respectively (24, 41). The B’ regulatory subunit of protein phosphatase 2A encoded by RTS1 was also involved in suppression of the hsf1-AR1\-ba1 phenotype (9). The ZDS1 gene, the most frequently isolated gene in this screen, and its paralog ZDS2 have both been identified in numerous other screens designed to isolate genes that act as negative regulators of CDC42 (3), positive effectors of replication origin function (54), or stabilizers of linear centromeric plasmids (43). Other studies show that Zds1 has properties reminiscent of the Pka anchoring proteins (13). However, the exact functions of Zds1 and Zds2 have not been established. The YGR146C gene has been recognized as an Hsf1-bound gene, but the molecular function of its product is not known (15). All of the suppressors were also able to improve the slow-growth phenotype of hsf1-AR1\-ba1 cells at 38°C (data not shown), and we used hsf1-AR1\-ba1 cells in the analyses presented below.

**Rts1 affects transcription activation by Hsf1.** We first examined whether Hsf1 regulates transcription of the suppressors...
identified above. Hsf1 did not bind to or activate any of the suppressor genes, with the exception of YGR146C, as judged from previous genome-wide analyses (15, 53). Although the 5'S region of YGR146C contains an HSE and binds Hsf1 (15), the mRNA levels of YGR146C were not affected by the hsf1-ba1 mutation (data not shown). We thus concluded that none of the suppressors are the direct targets of Hsf1.

We then tested the effects of suppressors on transcription activation by Hsf1-ba1. We analyzed the mRNA levels of Hsf1 target genes by using quantitative RT-PCR (16). As shown in Fig. 1C, heat-induced accumulation of the mRNAs from CUP1 and CPR6, which contain the discontinuous HSE, was severely compromised in hsf1-ba1 cells relative to wild type. In contrast, the hsf1-ba1 mutation did not significantly affect transcriptional activation through the continuous HSEs of HSP42 and HSP78. Among the suppressor genes we tested, the heat shock response of CUP1 and CPR6 in the hsf1-ba1 cells was only restored by introduction of RTS1 (Fig. 1C and data not shown). The RTS1 gene has been previously isolated as a multicopy suppressor of hsp60-ts, a temperature-sensitive allele of HSP60 that encodes a mitochondrial GroEL homologue (46). Null mutations of RTS1 result in a low-level of heat-induced transcription of Hsf1 target genes, such as HSP60 and HSP10 (a mitochondrial GroES homologue) (46). Our finding that overexpression of RTS1 in hsf1-ba1 cells restores the heat shock response of CUP1 and CPR6 supports the hypothesis that Rts1 affects the ability of Hsf1 to activate transcription. How Rts1 regulates the Hsf1 activity will be the focus of a future study. The products of the other suppressor genes may collaborate with unknown protein(s), whose expression or function is af-

### Table 2. List of multicopy suppressor genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description and product</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSF1</td>
<td>Heat shock transcription factor</td>
<td>2</td>
</tr>
<tr>
<td>PDE2</td>
<td>High-affinity cAMP phosphodiesterase</td>
<td>3</td>
</tr>
<tr>
<td>RIM15</td>
<td>Trehalose-associated protein kinase related to S. pombe cell+</td>
<td>1</td>
</tr>
<tr>
<td>WSC1</td>
<td>Cell wall integrity and stress response component 1</td>
<td>16</td>
</tr>
<tr>
<td>WSC2</td>
<td>Cell wall integrity and stress response component 2</td>
<td>13</td>
</tr>
<tr>
<td>MID2</td>
<td>Protein required for mating</td>
<td>1</td>
</tr>
<tr>
<td>ROM2</td>
<td>GDP/GTP exchange protein for Rho1 and Rho2</td>
<td>3</td>
</tr>
<tr>
<td>EXG1</td>
<td>Exo-1,3-β-glucanase</td>
<td>1</td>
</tr>
<tr>
<td>KRE6</td>
<td>Protein required for β-1,6-glucan biosynthesis</td>
<td>6</td>
</tr>
<tr>
<td>RTS1</td>
<td>B-type regulatory subunit of protein</td>
<td>2</td>
</tr>
<tr>
<td>ZDS1</td>
<td>Zillion different screens 1</td>
<td>33</td>
</tr>
<tr>
<td>ZDS2</td>
<td>Zillion different screens 2</td>
<td>2</td>
</tr>
<tr>
<td>YGR146C</td>
<td>Unknown</td>
<td>6</td>
</tr>
</tbody>
</table>

*Derived from the Saccharomyces Genome Database and/or the MIPS Comprehensive Yeast Genome Database.*

FIG. 1. Characterization of multicopy suppressor genes. (A) Schematic diagram of structural motifs of Hsf1 and Hsf1 mutant constructs. The motifs indicated above Hsf1 are as follows: AR1 and AR2, activation domains; DBD, DNA-binding domain; oligomer, oligomerization domain; CE2, conserved element 2; CTM, C-terminal modulator. Numbers represent amino acid positions. Hsf1-Sp-CTM contains amino acids 269 to 594 of S. pombe HSF but lacks the C-terminal 15 amino acids that function as a CTM domain. Hsf1-Hs contains amino acids 316 to 529 of human HSF1. (B) Multicopy suppression of growth defects at elevated temperature of hsf1-AR1Δ-bal cells. Wild-type HSF1 cells harboring empty vector (+ vector) and hsf1-AR1Δ-bal cells harboring empty vector or the vector bearing the suppressor genes (indicated by “+” and the gene name) were streaked on YPD medium and were incubated at 38°C for 2 days. The growth of hsf1-AR1Δ-bal cells harboring YEp-HSP82 is also shown. (C) RT-PCR analysis of multicopy suppressor gene effects on heat shock response target gene transcription. Wild-type HSF1 cells harboring empty vector (+ vector) and hsf1-ba1 cells harboring empty vector, YEPRIM15, YEPROM2, YEp-RTS1, or YEp-ZDS1 were grown in ESD medium lacking uracil 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 RT-PCR analysis with primers for several heat-inducible genes (CUP1, CPR6, HSP42, and HSP78) and a control gene, ACT1.
ected by the hsfl-ba1 mutation, so as to enable cells to grow at elevated temperatures.

**Suppression of the temperature sensitivity of hsfl-ba1 cells by RIM15.** Cells with deficient PKA activity exhibit increased resistance toward heat stress (48). Accordingly, downregulation of PKA by Pde2 phosphodiesterase and overexpression of Rim15, a kinase acting immediately downstream of and negatively regulated by PKA, were responsible for suppression of the hsfl-ba1 mutation. The Rim15 kinase is required for proper establishment of the Go program and for extension of life span (10, 35, 39). In response to nutrient limitation, the transcription factors Msn2, Msn4, and Gis1 cooperatively mediate the entire Rim15-dependent transcription response and induce expression of various genes, including HSP12, HSP26, and SSA3 (5, 35, 39).

We analyzed effect of RIM15 overexpression in the hsfl-ba1 cells on the heat shock response of HSP12, HSP26, and SSA3 (Fig. 2A). In logarithmically growing hsfl-ba1 cells, heat-induced accumulation of the HSP12, HSP26, and SSA3 mRNAs was reduced by ca. 60, 40, and 15% compared to HSFI wild-type controls, respectively, and the levels were not affected by multiple copies of RIM15. We then examined the growth of hsfl-ba1 cells harboring multiple copies of MSN2, MSN4, and GIS1 and found that they are unable to rescue the growth defect (Fig. 2B). Wild-type HSFI cells containing either msn2Δ msn4Δ double null mutations or a gis1Δ null mutation were able to grow at 38°C (Fig. 2C). When the hsfl-ba1 mutation was combined with the null mutations of these genes, the combinations did not exacerbate the heat sensitivity of hsfl-ba1 cells. Furthermore, RIM15 rescued the temperature sensitivity of hsfl-ba1 msn2Δ msn4Δ and hsfl-ba1 gis1Δ cells. Taken together, we conclude that Msn2, Msn4, and Gis1 are dispensable for suppression by Rim15 and suggest that Rim15 regulates the functions of different sets of proteins in response to distinct stressors, heat and nutrient limitation.

**Suppression of the temperature sensitivity of hsfl-ba1 cells by activation of the Wsc-Pkc1 pathway.** Upon heat shock, plasma membrane sensor proteins encoded by WSC1, WSC2, and MID2 activate Rom2 to promote GTP loading of Rho1, which in turn activates Pkc1 (36). Pkc1 then activates the downstream MAPK cascade consisting of Bck1, a pair of redundant MAPK kinases Mkk1 and Mkk2, and a MAPK Mpk1/Slit2 (14, 17). Mpk1 activates the transcription factors Rim1 and Swi4, which regulate expression of cell wall genes and cell cycle-regulated genes, respectively (2, 20).

We examined whether the temperature sensitivity of hsfl-ba1 cells could be suppressed by overexpressing components of the Pkc1-MAPK pathway. As shown in Fig. 3A, a multicopy plasmid bearing PKC1 enabled hsfl-ba1 cells to grow at 38°C. A constitutively active allele of PKC1 (PKC1<sup>RES</sup>) also rescued the temperature sensitivity, indicating that activation of Pkc1 is correlated with suppression of the hsfl-ba1 phenotype. However, introduction of the downstream MAPK cascade components as constitutively active alleles (BCK1<sup>20</sup> and M KK1<sup>RES</sup>) or a multicopy gene (MPK1) was not sufficient to support the growth of hsfl-ba1 cells at 38°C. Overexpression of RLM1 or SWI4 did not rescue the growth defect. These data suggest that an alternative Pkc1 pathway mediates suppression of the temperature sensitivity of hsfl-ba1 cells (see Discussion).

Because the components of the Wsc-Pkc1 pathway are necessary for the growth of cells at normal or elevated temperatures (14, 17), it is possible that the temperature sensitivity of hsfl-ba1 cells is due to inefficient activation of this pathway. Heat-responsive activation of the Wsc-Pkc1 pathway causes phosphorylation of Mpk1 and increases its catalytic activity (21). We analyzed the activated form of Mpk1 by using an antibody that recognizes only phosphorylated Mpk1 (51). When the temperature of control HSFI cells was shifted from 28 to 39°C, the amount of phosphorylated Mpk1 increased significantly, as shown by immunoblot analysis (Fig. 3B). Sim-
FIG. 3. Effect of components of the Wsc-Pkc1-Mpk1 pathway on the growth of \textit{hsf1-ba1} cells. (A) Suppression of temperature-sensitive growth defects of \textit{hsf1-ba1} cells by activation of the Wsc-Pkc1 pathway. Wild-type \textit{HSF1} cells harboring empty vector (+ vector) and \textit{hsf1-ba1} cells harboring empty vector, \textit{YEp-ROM2}, \textit{YEp-PKCI}, \textit{YEp-PKCI}\textsubscript{R398P}, \textit{YCP-BCK1-20}, \textit{YCP-MKK1S385P}, \textit{YEp-MPK1}, \textit{YEp-RLM1}, or \textit{YEp-SWI4} were streaked on YPD medium and were incubated at 38°C for 2 days. (B) Heat-induced phosphorylation of Mpk1 in \textit{hsf1-ba1} cells. Wild-type \textit{HSF1} and \textit{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 protein extracts were prepared. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subjected to immuno- blot analysis with an antibody recognizing phosphorylated Mpk1.

FIG. 4. Suppression of the lysis phenotype of \textit{hsf1-ba1} cells by osmotic stabilization. (A) Growth of cells containing \textit{hsf1-ba1} and \textit{wsc1\textDelta} mutations under various conditions. Wild-type \textit{HSF1} cells (WT) and their derivatives containing \textit{hsf1-ba1} and \textit{wsc1\textDelta} mutations were streaked onto YPD medium or YPD medium containing 1 M sorbitol and were incubated at 28, 35, or 38°C for 2 days. (B) Cell lysis assay of \textit{hsf1-ba1} and \textit{wsc1\textDelta} cells grown at elevated temperature. Suspensions of the indicated cells were spotted on YPD medium or YPD medium containing 1 M sorbitol. Plates were incubated at 28°C for 1 day and then switched to 38°C and incubated overnight. The plate was overlaid with an alkaline phosphatase assay solution and incubated at 38°C for 1 h.

FIG. 5. Growth phenotype of \textit{hsf1-ba1} cells grown either at 28 or at 39°C. Therefore, the \textit{hsf1-ba1} mutation does not affect the heat-regulated activation of the \textit{wsc1-Pkc1-Mpk1} pathway.

Cell wall defect of \textit{hsf1-ba1} cells. Inactivation of the Wsc-Pkc1 pathway leads to cell wall defects (12, 18, 22, 25, 33, 34, 38, 51). As reported previously (12, 18, 51), \textit{wsc1\textDelta} cells exhibit a weak heat sensitivity, and the wild-type phenotype was restored by addition of an osmotic stabilizer, such as sorbitol, to the medium (Fig. 4A). We found that \textit{hsf1-ba1} cells are able to grow on medium containing sorbitol at 38°C. To test the cell lysis phenotype of the \textit{hsf1-ba1} mutant, we conducted a cell lysis assay, in which leakage of alkaline phosphatase from lysed cells is detected by a nonpermeable alkaline phosphatase substrate added to the culture plates (34). Cells expressing wild-type Hsf1 were negative controls, since the wild-type cells do not lyse even at elevated temperatures. The \textit{hsf1-ba1} cells, as well as \textit{wsc1\textDelta} cells, underwent cell lysis on the standard plate but not on the osmotically stabilized plate (Fig. 4B). We then combined the \textit{hsf1-ba1} mutation with \textit{wsc1\textDelta}. The growth of \textit{hsf1-ba1 wsc1\textDelta} cells was inhibited at 35°C, but the addition of sorbitol to the medium allowed this mutant to grow even at 38°C (Fig. 4A). The osmotic remedial cell lysis phenotype of \textit{hsf1-ba1} cells was significantly exacerbated by combination with the \textit{wsc1\textDelta} mutation, but sorbitol protected the cells from lysis, as judged by the alkaline phosphatase leakage assay (Fig. 4B). These results show that a cell wall defect is responsible for the temperature-sensitive growth inhibition of \textit{hsf1-ba1} cells and that Hsf1 is necessary for proper cell wall remodeling upon heat shock.

Cell wall integrity of various \textit{hsf} mutants. To confirm the involvement of Hsf1 in cell wall remodeling, we analyzed the growth phenotype of cells containing various temperature-sensitive mutations in \textit{HSF1} (see Fig. 1A). The Hsf1-Sp-CTM\textDelta construct is the fusion protein of the central region (DNA-binding, oligomerization, and CE2 domains) of \textit{S. cerevisiae} Hsf1 and the C-terminal region (without CTM domain) of \textit{Schizosaccharomyces pombe} HSF. The Hsf1-Hs fusion contains the central region of \textit{S. cerevisiae} Hsf1 and the C-terminal activation domain of human HSF1 (16). Hsf1-N583 is the C-terminally truncated form of Hsf1 lacking the AR2 and CTM domains (27, 44, 47). Hsf1-F256S contains a substitution of phenylalanine to serine at the 256 position in the DNA-binding domain (53). Among these \textit{hsf} mutants, the temperature sensitivity of cells expressing \textit{hsf1-Sp-CTM\textDelta} or \textit{hsf1-Hs} was rescued when \textit{ROM2} was overexpressed or when the medium contained sorbitol (Fig. 5). Thus, several \textit{hsf} mutations cause defects in cell wall organization at elevated temperatures.

DISCUSSION

The temperature-sensitive growth phenotype of \textit{hsf1-ba1} cells was suppressed by activation of the Wsc-Pkc1 pathway, which mediates maintenance of cell wall integrity. The \textit{hsf1-ba1} mutation consistently led to an osmotic remedial cell lysis phenotype at elevated temperatures. The activation of the


Evangelista, C. C., Jr., A. M. Rodriguez Torres, M. P. Limbach, and R. S.


