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

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The heat shock transcription factor Hsf1 of the yeast *Saccharomyces cerevisiae* regulates expression of genes encoding heat shock proteins and a variety of other proteins as well. To better understand the cellular roles of Hsf1, we screened multicopy suppressor genes of a temperature-sensitive *hsf1* mutation. The *RIM15* gene, encoding a protein kinase that is negatively regulated by the cyclic AMP-dependent protein kinase, was identified as a suppressor, but Rim15-regulated stress-responsive transcription factors, such as Msn2, Msn4, and Gis1, were unable to rescue the temperature-sensitive growth phenotype of the *hsf1* mutant. Another class of suppressors encoded cell wall stress sensors, Wsc1, Wsc2, and Mid2, and the GDP/GTP exchange factor Rom2 that interacts with these cell wall sensors. Activation of a protein kinase, Pkc1, which is induced by these cell wall sensor proteins upon heat shock, but not activation of the Pkc1-regulated mitogen-activated protein kinase cascade, was necessary for the *hsf1* suppression. Like Wsc-Pkc1 pathway mutants, *hsf1* cells exhibited an osmotic remedial cell lysis phenotype at elevated temperatures. Several of the other suppressors were found to encode proteins functioning in cell wall organization. These results suggest that Hsf1 in concert with Pkc1 regulates cell wall remodeling in response to heat shock.

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 HSF encoded by the *HSF1* locus is essential for the growth of *S. cerevisiae* at 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 (nTTCnnGAAnnTTCn) and discontinuous [e.g., nTTCnnGAAn(5 bp)nGAAn] arrays of repeats can function as HSEs (1, 28, 37). A genome-wide Hsf1-binding analysis revealed that Hsf1 binds to the 5′ 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.

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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

TABLE 1. Yeast strains used in this study

Strain	Genotype (plasmid)
HS126	*MATα ade2 ura3 leu2 his3 trp1 can1 hsf1*::*HIS3* YCp-*URA3-HSF1* (pSK906)
HS131	YCp-*TRP1-hsf1-AR1*Δ-*ba1* (pK137) of HS134
HS133	YCp-*URA3-hsf1-AR1*Δ-*ba1* (pK136) of HS134
HS134	*LEU2*::*SSA4-lacZ* of HS126
HS144	YCp-*LEU2-hsf1-AR1*Δ-*N583-HSF1*(316-529) (pK144 shown as Hsf1-Hs) of HS126
HS154	YCp-*LEU2-hsf1-AR1*Δ-*N583-hsf*$^{+}$(269-594) (pK154 shown as Hsf1-Sp-CTMΔ) of HS126
HS170T	YCp-*TRP1-HSF1* (pK157) of HS126
HS171T	YCp-*TRP1-hsf1-ba1* (pK159) of HS126
HS174	*gis1*::*LEU2* YCp-*TRP1-HSF1* (pK157) of HS126
HS175	*gis1*::*LEU2* YCp-*TRP1-hsf1-ba1* (pK159) of HS126
HS176	*msn2*::*LEU2 msn4*::*ADE2* YCp-*TRP1-HSF1* (pK157) of HS126
HS177	*msn2*::*LEU2 msn4*::*ADE2* YCp-*TRP1-hsf1-ba1* (pK159) of HS126
HS194	*wsc1*::*ADE2* YCp-*TRP1-HSF1* (pK157) of HS134
HS195	*wsc1*::*ADE2* YCp-*TRP1-hsf1-ba1* (pK159) of HS134
YAY9	YCp-*TRP1-hsf1-F256S* (pAY9) of HS134
YN49	YCp-*TRP1-hsf1-N583* (pN49) of HS134

hsf1 derivatives were created by plasmid shuffling, and null mutations were introduced by using a one-step gene disruption method (16, 44, 53). Rich medium containing glucose (YPD) and enriched synthetic glucose medium (ESD) were prepared as described previously (44).

Multicopy suppressor screening. Strain HS131 (YCp-*TRP1-hsf1-AR1*Δ-*ba1*) was transformed with a genomic library cloned into the YEp24 multicopy vector (YEp-*URA3*). Transformants were selected for uracil prototrophy on ESD medium lacking uracil and were incubated at 38°C to identify plasmids, allowing growth of *hsf1-AR1*Δ-*ba1* cells at the restrictive temperature. Plasmids were recovered from the cells, and the nucleotide sequences of the regions flanking the inserts were determined. To identify genes responsible for suppression, candidate genes were subcloned into YEp24 or YEplac195 (YEp-*URA3*) and were retested for the ability to suppress the temperature sensitivity. The original isolates bearing *ROM2* did not contain the coding sequence of the N-terminal 326 amino acids. The full-length *ROM2* gene amplified by PCR was used for further analysis. The *HSP82*, *MSN2*, *MSN4*, *GIS1*, *PKC1*, *MPK1*, *RLM1*, and *SWI4* genes were amplified by PCR from yeast genomic DNA and were cloned into YEp24 or YEplac195. Plasmids bearing constitutively active alleles *PKC1*^{R398P}, *BCK1-20*, and *MKK1*^{S386P} were kindly provided by Kunio Matsumoto.

RNA analysis. Cells were grown to an optical density of 1.0 at 600 nm (OD_{600}) under the conditions described in the figure legends. Total RNA was prepared from the cells, quantified by determining the absorbance at 260 nm, and subjected to reverse transcription-PCR (RT-PCR) analysis as described previously (16). The amounts of PCR products were compared after normalizing RNA samples to the levels of control *ACT1* mRNA (encoding actin) (16, 44).

Immunoblot analysis. Wild-type *HSF1* and *hsf1-ba1* cells were grown in YPD medium at 28 or 39°C to an OD_{600} of 2.0. Cells were harvested from 2 ml of culture, and protein extracts were prepared as described previously (22). Identical protein samples were separated on two sodium dodecyl sulfate-polyacrylamide gels; one gel was stained with Coomassie brilliant blue to confirm equivalent loading of protein samples, and the other was subjected to immunoblotting with an antibody recognizing phosphorylated Mpk1 (Phospho-p44/42 MAPK antibody; Cell Signaling Technology, Inc.).

Cell lysis assay. Cell suspensions were spotted on YPD medium or YPD containing 1 M sorbitol, incubated at 28°C for 1 day, and then incubated at 38°C overnight. Subsequently, the plates were overlaid with an alkaline phosphatase assay solution as described previously (34).

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 residues (Fig. 1A) (44). Cells expressing the Hsf1-ba1 protein exhibit slow-growth at elevated temperatures and, when combined with deletion of the nonessential activation domain AR1 (Fig. 1A, *hsf1-AR1*Δ-*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*Δ-*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*Δ-*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 Pkc1, 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*Δ-*ba1* phenotype (9). The *ZDS1* gene, the most frequently isolated gene in this screen, and its paralog *ZDS2* have 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-ba1* cells at 38°C (data not shown), and we used *hsf1-ba1* cells in the analyses presented below.

Rts1 affects transcription activation by Hsf1. We first examined whether Hsf1 regulates transcription of the suppressors

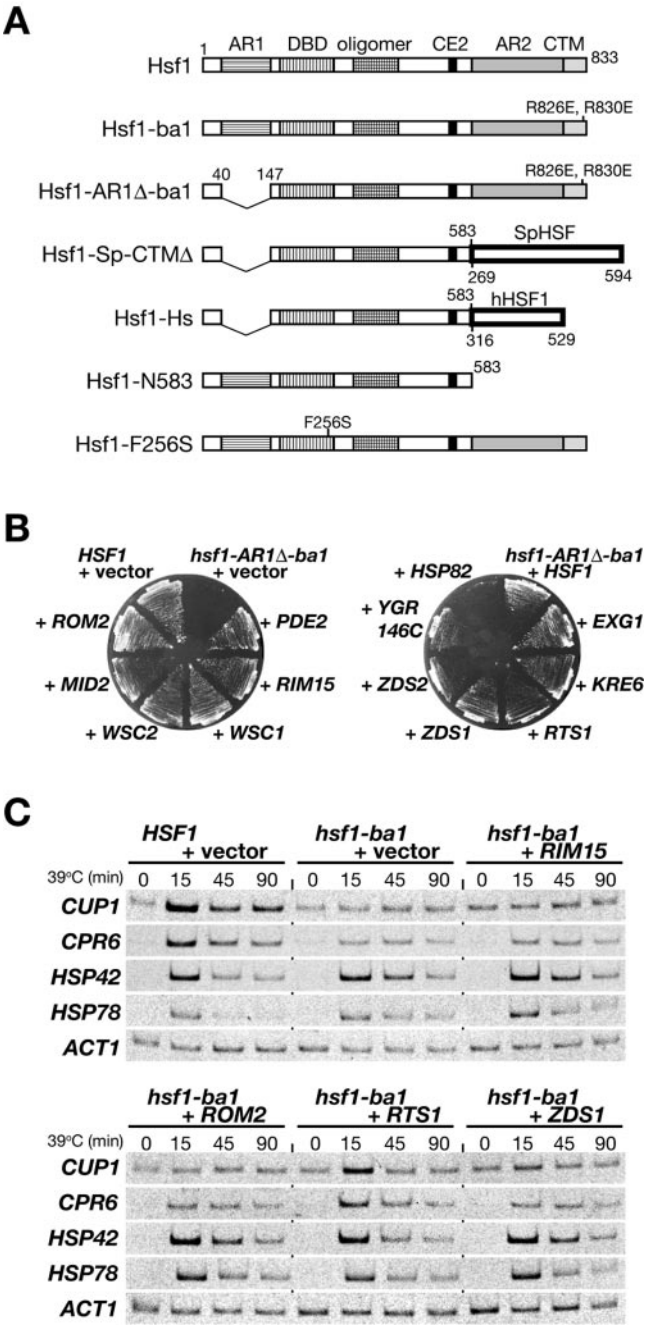

FIG. 1. Characterization of multicopy suppressor genes. (A) Schematic diagram of structural motifs of Hsf1 and Hsf1 mutant constructs. The motifs indicated above Hsf1 are as follows: AR1 and AR2, activation domains; DBD, DNA-binding domain; oligomer, oligomerization domain; CE2, conserved element 2; CTM, C-terminal modulator. Numbers represent amino acid positions. Hsf1-Sp-CTMΔ 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-ba1* cells. Wild-type *HSF1* cells harboring empty vector (+ vector) and *hsf1-AR1Δ-ba1* 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-ba1* 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, YEp-*RIM15*, YEp-*ROM2*, 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*.

TABLE 2. List of multicopy suppressor genes

Gene	Description and product[a]	No. of isolates
HSF1	Heat shock transcription factor	2
PDE2	High-affinity cAMP phosphodiesterase	3
RIM15	Trehalose-associated protein kinase related to *S. pombe cek1+*	1
WSC1	Cell wall integrity and stress response component 1	16
WSC2	Cell wall integrity and stress response component 2	13
MID2	Protein required for mating	1
ROM2	GDP/GTP exchange protein for Rho1 and Rho2	3
EXG1	Exo-1,3-β-glucanase	1
KRE6	Protein required for β-1,6-glucan biosynthesis	6
RTS1	B′-type regulatory subunit of protein phosphatase 2A	2
ZDS1	Zillion different screens 1	33
ZDS2	Zillion different screens 2	2
YGR146C	Unknown	6

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

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′ upstream 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-

fected by the *hsf1-ba1* mutation, so as to enable cells to grow at elevated temperatures.

Suppression of the temperature sensitivity of *hsf1-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 *hsf1-ba1* mutation. The Rim15 kinase is required for proper establishment of the G_0 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 *hsf1-ba1* cells on the heat shock response of *HSP12*, *HSP26*, and *SSA3* (Fig. 2A). In logarithmically growing *hsf1-ba1* cells, heat-induced accumulation of the *HSP12*, *HSP26*, and *SSA3* mRNAs was reduced by ca. 60, 40, and 15% compared to *HSF1* wild-type controls, respectively, and the levels were not affected by multiple copies of *RIM15*. We then examined the growth of *hsf1-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 *HSF1* cells containing either *msn2*Δ *msn4*Δ double null mutations or a *gis1*Δ null mutation were able to grow at 38°C (Fig. 2C). When the *hsf1-ba1* mutation was combined with the null mutations of these genes, the combinations did not exacerbate the heat sensitivity of *hsf1-ba1* cells. Furthermore, *RIM15* rescued the temperature sensitivity of *hsf1-ba1 msn2*Δ *msn4*Δ and *hsf1-ba1 gis1*Δ 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 *hsf1-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/Slt2 (14, 17). Mpk1 activates the transcription factors Rlm1 and Swi4, which regulate expression of cell wall genes and cell cycle-regulated genes, respectively (2, 20).

We examined whether the temperature sensitivity of *hsf1-ba1* cells could be suppressed by overexpressing components of the Pkc1-MAPK pathway. As shown in Fig. 3A, a multicopy plasmid bearing *PKC1* enabled *hsf1-ba1* cells to grow at 38°C. A constitutively active allele of *PKC1* (*PKC1*^{R398P}) also rescued the temperature sensitivity, indicating that activation of Pkc1 is correlated with suppression of the *hsf1-ba1* phenotype. However, introduction of the downstream MAPK cascade components as constitutively active alleles (*BCK1-20* and *MKK1*^{S386P}) or a multicopy gene (*MPK1*) was not sufficient to support the growth of *hsf1-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 *hsf1-ba1* cells (see Discussion).

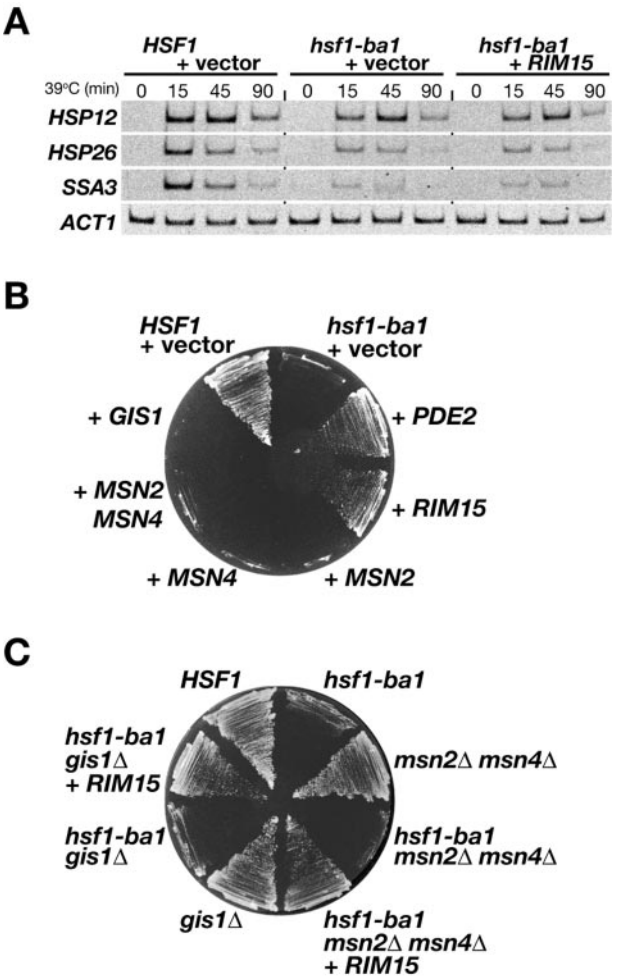

FIG. 2. Effect of components of the PKA pathway on the growth of *hsf1-ba1* cells. (A) RT-PCR analysis of *RIM15* overexpression effects on *HSP12*, *HSP26*, and *SSA3* transcription. Wild-type *HSF1* cells harboring empty vector (+ vector) and *hsf1-ba1* cells harboring empty vector or YEp-*RIM15* were grown in ESD medium lacking uracil, and total RNA prepared from each sample was subjected to RT-PCR analysis, as described for Fig. 1C. (B) Growth of *hsf1-ba1* cells harboring multiple copies of the PKA pathway genes at elevated temperature. Wild-type *HSF1* cells harboring empty vector (+ vector) and *hsf1-ba1* cells harboring empty vector or the vector bearing various genes (indicated by "+" and the gene name) were streaked onto YPD medium and were incubated at 38°C for 2 days. (C) Growth of *hsf1-ba1* cells containing mutations in the PKA pathway genes at elevated temperature. Wild-type *HSF1* cells, their derivatives containing the indicated mutations, and mutant cells harboring YEp-*RIM15* were streaked on YPD medium and were incubated at 38°C for 2 days.

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 *hsf1-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 *HSF1* cells was shifted from 28 to 39°C, the amount of phosphorylated Mpk1 increased significantly, as shown by immunoblot analysis (Fig. 3B). Sim-

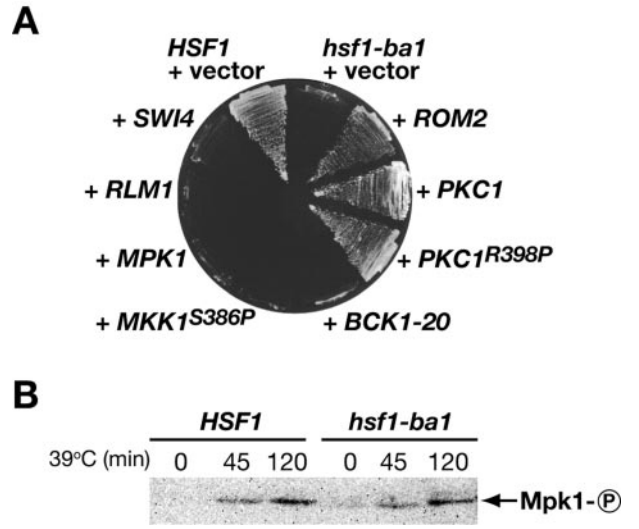

FIG. 3. Effect of components of the Wsc-Pkc1-Mpk1 pathway on the growth of *hsf1-ba1* cells. (A) Suppression of temperature-sensitive growth defects of *hsf1-ba1* cells by activation of the Wsc-Pkc1 pathway. Wild-type *HSF1* cells harboring empty vector (+ vector) and *hsf1-ba1* cells harboring empty vector, YEp-*ROM2*, YEp-*PKC1*, YCp-*PKC1*^{R398P}, YCp-*BCK1-20*, YCp-*MKK1*^{S386P}, YEp-*MPK1*, YEp-*RLM1*, or YEp-*SWI4* were streaked on YPD medium and were incubated at 38°C for 2 days. (B) Heat-induced phosphorylation of Mpk1 in *hsf1-ba1* cells. Wild-type *HSF1* 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 protein extracts were prepared. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subjected to immunoblot analysis with an antibody recognizing phosphorylated Mpk1.

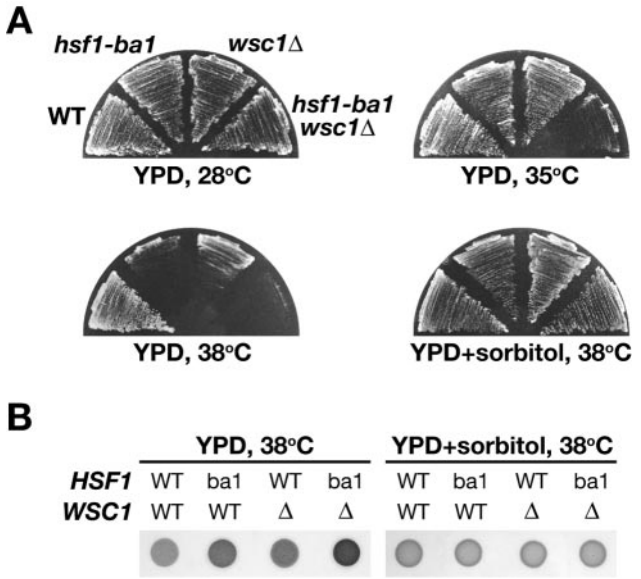

FIG. 4. Suppression of the lysis phenotype of *hsf1-ba1* cells by osmotic stabilization. (A) Growth of cells containing *hsf1-ba1* and *wsc1*Δ mutations under various conditions. Wild-type *HSF1* cells (WT) and their derivatives containing *hsf1-ba1* and *wsc1*Δ mutations were streaked onto YPD medium or YPD medium containing 1 M sorbitol and were incubated at 28, 35, or 38°C for 2 days. (B) Cell lysis assay of *hsf1-ba1* and *wsc1*Δ 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.

ilar levels of the activated Mpk1 were detected in the extracts of *hsf1-ba1* cells grown either at 28 or at 39°C. Therefore, the *hsf1-ba1* mutation does not affect the heat-regulated activation of the Wsc-Pkc1-Mpk1 pathway.

Cell wall defect of *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), *wsc1*Δ 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 *hsf1-ba1* cells are able to grow on medium containing sorbitol at 38°C. To test the cell lysis phenotype of the *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 *hsf1-ba1* cells, as well as *wsc1*Δ cells, underwent cell lysis on the standard plate but not on the osmotically stabilized plate (Fig. 4B). We then combined the *hsf1-ba1* mutation with *wsc1*Δ. The growth of *hsf1-ba1 wsc1*Δ 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 *hsf1-ba1* cells was significantly exacerbated by combination with the *wsc1*Δ 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 *hsf1-ba1* cells and that Hsf1 is necessary for proper cell wall remodeling upon heat shock.

Cell wall integrity of various *hsf1* mutants. To confirm the involvement of Hsf1 in cell wall remodeling, we analyzed the growth phenotype of cells containing various temperature-sensitive mutations in *HSF1* (see Fig. 1A). The Hsf1-Sp-CTMΔ construct is the fusion protein of the central region (DNA-binding, oligomerization, and CE2 domains) of *S. cerevisiae* Hsf1 and the C-terminal region (without CTM domain) of *Schizosaccharomyces pombe* HSF. The Hsf1-Hs fusion contains the central region of *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 *hsf1* mutants, the temperature sensitivity of cells expressing *hsf1-Sp-CTM*Δ or *hsf1-Hs* was rescued when *ROM2* was overexpressed or when the medium contained sorbitol (Fig. 5). Thus, several *hsf1* mutations cause defects in cell wall organization at elevated temperatures.

DISCUSSION

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

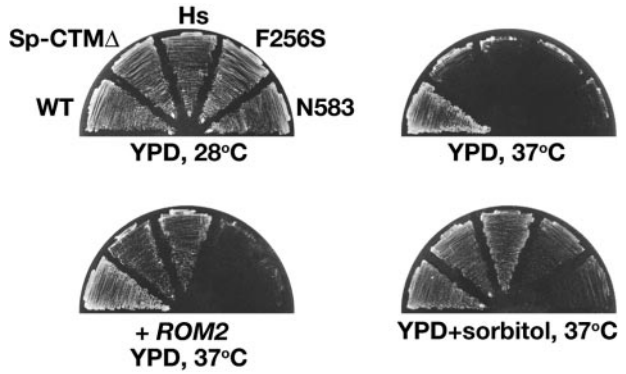

FIG. 5. Growth of various *hsf1* mutants under cell wall-stabilizing conditions. Cells expressing Hsf1 (WT), Hsf1-Sp-CTMΔ (Sp-CTMΔ), Hsf1-Hs (Hs), Hsf1-F256S (F256S), or Hsf1-N583 (N583) were streaked onto YPD medium or YPD containing 1 M sorbitol. Plates were incubated at 28 or at 37°C for 2 days. The lower left panel shows growth of the indicated *hsf1* mutant cells harboring YEp-*ROM2* on YPD medium at 37°C.

Wsc-Pkc1 pathway or inclusion of an osmotic stabilizer in the medium rescued the growth defects of several *hsf1* mutants at the restrictive temperature. Thus, the present study disclosed a novel cellular role of Hsf1: regulation of cell wall remodeling for adaptation to a high-temperature environment.

Although activation of the Wsc-Pkc1 pathway, which is known to activate a MAPK cascade, causes suppression of the temperature sensitivity of *hsf1-ba1* cells, expression of individual components of the MAPK cascade or Mpk1-regulated transcription factors was unable to do so. Deletion of *PKC1*, but not the downstream components, results in the osmotic remedial cell lysis phenotype even at normal growth temperatures, implying that Pkc1 affects cell wall organization through a pathway other than the MAPK cascade (25, 34, 42). In addition, Pkc1, but not the MAPK cascade, regulates cellular functions such as heat-induced depolarization of the actin cytoskeleton (7), attenuation of ribosome biogenesis upon interruption of the secretory pathway (26, 31), and nuclear perturbation caused by high osmolarity (30). We suggest that Pkc1 regulates cell wall organization through an alternative signaling pathway in collaboration with Hsf1 to prevent cell lysis at elevated temperatures.

In addition to the components of the Wsc-Pkc1 pathway, the following observations implicate additional suppressor genes in cell wall maintenance. The *S. cerevisiae* cell wall consists of β-1,3-glucan, β-1,6-glucan, chitin, and mannoproteins (23). The *EXG1* gene encoding the major exo-1,3-β-glucanase has been identified as a multicopy suppressor of the osmotic remedial cell lysis phenotype of *ypk1-1*^{ts} *ypk2*Δ mutant cells. The Ypk1 and Ypk2 protein kinases function in parallel with the Pkc1-dependent pathway for maintenance of cell wall integrity (40). We also found that multiple copies of *EXG1* improved the slow-growth phenotype of *wsc1*Δ cells at 38°C (data not shown). The *KRE6* product, which may function as a glycoside hydrolase or transglycosidase in the Golgi complex, is required for the synthesis of β-1,6-glucan (29, 41). Mild overexpression of *KRE6* rescues the cell lysis phenotype of *pkc1*Δ, indicating a functional interaction between Kre6 and Pkc1 (42). The *ZDS1* gene, in addition to *WSC1* and *ROM2*, has been identified as a multicopy suppressor of *fks1-1154 fks2*Δ, a temperature-sensitive mutant of cell wall 1,3-β-glucan synthase (45). A large-scale two-hybrid experiment showed Zds1 and Zds2 interactions with diverse gene products, including Rho1 and Pkc1 (8). Although the function of the *YGR146C* product is unknown, its transcription, as well as transcription of *EXG1* and *KRE6*, is induced upon transient cell wall damage (11). In addition, null mutations of *PDE2* cause a loss of cell wall strength and overexpression of *PDE2* suppresses the sorbitol dependence of a mutant strain with fragile cell walls, implicating the PKA pathway in the maintenance of cell wall integrity (49).

Although Msn2 and Msn4 share various target genes with Hsf1 (4, 50), and Rlm1 activates transcription of cell wall genes in response to heat shock (20), multiple copies of *MSN2*, *MSN4*, and *RLM1* failed to rescue the cell wall defect associated with the *hsf1-ba1* mutation. Rather, the kinases controlling these transcription activators are functioning with Hsf1. Hsf1 regulates heat-induced transcription of several cell wall genes, including *CWP1*, *SPI1*, *HOR7*, *YGP1*, and *ZEO1* (53). Our preliminary observations showed that the mRNA levels of *CWP1*, *SPI1*, and *ZEO1* were slightly reduced in *hsf1-ba1* cells relative to wild-type but that multiple copies of these genes were not sufficient to rescue the temperature sensitivity of *hsf1-ba1* cells (data not shown). It has been estimated that more than 1,200 *S. cerevisiae* genes are in some way related to cell wall biosynthesis (6). Our data suggest that Hsf1 regulates expression of not only HSPs but also an additional set of unknown proteins that are involved in cell wall formation and remodeling.

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