MOLECULAR AND CELLULAR BIOLOGY, Feb. 2007, p. 1505–1515 Vol. 27, No. 4
0270-7306/07/$08.00+0 doi:10.1128/MCB.01158-06

The Natural Osmolyte Trehalose Is a Positive Regulator of the Heat-Induced Activity of Yeast Heat Shock Transcription Factor▽

Laura K. Conlin and Hillary C. M. Nelson*

Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, 813A Stellar-Chance, 422 Curie Blvd., Philadelphia, Pennsylvania 19104-6059

Received 27 June 2006/Returned for modification 12 September 2006/Accepted 17 November 2006

In *Saccharomyces cerevisiae*, the intracellular concentration of trehalose increases rapidly in response to many environmental stresses, including heat shock. These high trehalose levels have been correlated with tolerance to adverse conditions and led to the model that trehalose functions as a chemical cochaperone. Here, we show that the transcriptional activity of Hsf1 during the heat shock response depends on trehalose. Strains with low levels of trehalose have a diminished transcriptional response to heat shock, while strains with high levels of trehalose have an enhanced transcriptional response to heat shock. The enhanced transcriptional response does not require the other heat-responsive transcription factors Msn2/4 but is dependent upon heat and Hsf1. In addition, the phosphorylation levels of Hsf1 correlate with both transcriptional activity and the presence of trehalose. These in vivo results support a new role for trehalose, where trehalose directly modifies the dynamic range of Hsf1 activity and therefore influences heat shock protein mRNA levels in response to stress.

Trehalose is a disaccharide of glucose that is found predominantly in bacteria, fungi (including yeasts), plants, and invertebrates. This natural osmolyte was initially characterized as a storage carbohydrate due to the high intracellular concentrations observed during these organisms' resting and anhydrobiotic states (reviewed in reference 55). Since these states involve the ability to survive stressful conditions, trehalose levels were then linked to thermotolerance, the ability of an organism to survive an otherwise lethal heat shock (reviewed in reference 47). Trehalose has been shown to stabilize the structures and enzymatic activities of proteins against thermal denaturation in vitro (15, 27, 63). In addition, trehalose can prevent the aggregation of misfolded proteins, including amyloidogenic proteins (3, 11, 27, 33, 46, 52), and is being considered for clinical trials for Huntington's disease (53). Trehalose is more effective than other sugars in protecting proteins against thermal denaturation and aggregation because of its unusual ability to alter the water environment surrounding a protein, stabilizing the protein in its native conformation (1, 30, 34, 48).

In the yeast *Saccharomyces cerevisiae*, trehalose levels vary depending on the environment of the cell. The levels are almost undetectable during normal exponential growth. After heat shock, trehalose levels increase rapidly and dramatically, along with the accumulation of heat shock proteins (HSPs) (26, 28). This rapid increase in trehalose has been attributed to the increase in both the translation of the genes involved in the synthesis of trehalose (*TPS1* and *TPS2*) (4, 41, 58) and the substrates required for trehalose synthesis (1, 61). In addition, the enzymatic activity of *TPS1* increases during the heat shock response (1, 38). High trehalose levels can stabilize enzymatic activity and can prevent the aggregation of exogenous proteins in vivo during heat shock (45, 46). Trehalose is degraded in the cytoplasm by neutral trehalase, encoded by the gene *NTH1* (26, 31, 38, 40, 59). The transcription of *NTH1* is stress responsive (62); however, the activity of Nth1 is greater during recovery from stress (39), allowing Nth1 to successfully compete with the biosynthetic pathway and reduce intracellular trehalose levels (28). This degradation of trehalose is critical for recovery from heat shock (59) as very high levels of trehalose can interfere with normal protein activity by stabilizing proteins in nonnative conformations and inhibiting the refolding of these denatured proteins by HSPs (12, 43, 44, 46, 56). Taken together, these data have led to a temporal model of trehalose function as a cochaperone during the heat shock response: trehalose functions to protect proteins at the initial stages of the heat shock response before HSPs have been fully induced, but trehalose must be degraded in order for the HSPs to fully assist the cell in recovery from heat shock (47).

In *S. cerevisiae*, the transcriptional response to heat shock is controlled by two sets of transcription factors, the heat shock transcription factor (Hsf1) and the partially redundant transcription factors Msn2 and Msn4 (Msn2/4). The Msn2/4 transcription factors, which are found only in yeast, bind to stress response elements found in the promoters of many heat shock genes (35). The activity of Msn2/4 is regulated by nuclear localization and phosphorylation (20, 29). Hsf1 is part of a family of conserved transcription factors critical to the heat shock response in all eukaryotes (57). All heat shock transcription factors (HSFs) share a conserved central core, consisting of a DNA-binding domain, a flexible linker, and a trimerization domain, which are essential for binding to heat shock elements (42). Stress response elements and heat shock elements are found in overlapping sets of promoters, allowing Msn2/4 and

* Corresponding author. Mailing address: University of Pennsylvania School of Medicine, Department of Biochemistry and Biophysics, 813A Stellar-Chance, 422 Curie Blvd., Philadelphia, PA 19104-6059. Phone: (215) 573-7473. Fax: (215) 253-2085. E-mail: hnelson@mail.med.upenn.edu.

▽ Published ahead of print on 4 December 2006.

Hsf1 to provide distinct contributions to the heat shock response (2, 7).

The mechanism by which Hsf1's transcriptional activity is regulated in response to heat shock is not well understood. In yeast, Hsf1 is localized to the nucleus, with a low level of constitutive transcriptional activity that is necessary for normal cellular processes (50, 60). Following a mild stress, such as heat shock, Hsf1 becomes transcriptionally active and the expression of HSP mRNA dramatically increases. During a prolonged stress, Hsf1 activity gradually decreases to a new plateau, while removal of stress causes Hsf1 activity to return rapidly to near-constitutive levels. The dramatic changes in Hsf1 activity during heat shock involve several components, including hyperphosphorylation (24, 50) and conformational changes (9, 32). Hsf1 is also thought to be negatively regulated through interactions with several heat shock proteins, including Hsp82 and members of the Hsp70 family, under both constitutive and heat shock conditions (6, 10, 13, 23, 51). To date, no specific signal that positively regulates Hsf1 activity during a heat shock has been identified.

We have previously shown that high concentrations of trehalose can increase the structure of the *S. cerevisiae* Hsf1 C-terminal activation domain in vitro, and this structural change is enhanced by temperature (8). We hypothesized that the effects of trehalose and elevated temperature on Hsf1's structure in vitro were linked to its dramatic increase in transcriptional activity after heat shock. In this paper, we show that trehalose is required for the robust increase in transcription of heat shock protein genes by Hsf1 during the initial response to heat shock. In addition, a high trehalose level maintains Hsf1 in a highly active state, preventing the decrease in activity of Hsf1 that occurs during a sustained heat shock response. The enhanced transcriptional response does not require the other heat-responsive transcription factors Msn2/4 but is dependent upon heat and Hsf1. Despite the structural enhancement of the C-terminal activation domain (CAD) observed in vitro, the enhanced transcriptional response does not require the presence of the C-terminal activation domain, suggesting that any structural changes in vivo must be more global. In addition, the increase in transcriptional activity is correlated with an increase in Hsf1 phosphorylation. By showing that trehalose modifies the dynamic range of Hsf1's heat-induced transcriptional activity, we have identified a novel and physiologically relevant function of trehalose as a positive regulator of the transcriptional response to heat shock.

MATERIALS AND METHODS

Plasmid construction. The *NTH1* and *TPS1* open reading frames (ORFs) were generated by PCR amplification from the W303-1A strain by using primers LC13 (GGATCCATGAGTCAAGTTAATACAAGCC) and LC14 (GTCGACCTAT AGTCCATAGAGGTTTC), which add BamHI and SalI restriction sites to the 5′ and 3′ ends of the *NTH1* ORF, respectively, and primers LC18 (ACTAGTA TGACTACGGATAACGCTAAGGCG) and LC19 (GGATCCTCAGTTTTTG GTGGCAGAGGAGC), which add SpeI and BamHI restriction sites to the 5′ and 3′ ends of the *TPS1* ORF, respectively. These fragments were then cloned into the yeast expression vector p42XADH (37), allowing for overexpression of these genes from the promoter fusions P_{ADH1}-*TPS1* and P_{ADH1}-*NTH1*. The strains that did not use the overexpressed version of *NTH1* or *TPS1* were transformed with appropriately marked empty vectors to ensure that all strains within a given experiment had the same auxotrophies and could be grown in the same synthetic media.

Yeast strains and media. All yeast strains were derived from the *S. cerevisiae* W303-1A (*MAT***a** *ade2-1 trp1-1 can1-100 leu2,3-112 his3-11,15 ura3-1*) strains YHN963, YHN1172, and YHN1189, which have been described previously (17). The YHN963 strain contains both the *msn2*Δ and *msn4*Δ alleles. YHN1172 was derived from YHN963, in which native *HSF1* is under a dual tetracycline repression system, allowing for strong repression of *HSF1* transcription upon the addition of 20 μg/ml doxycycline. YHN1189, also derived from YHN963, contains a *13Myc* tag at the 3′ end of the gene encoding Hsf1 protein. Deletion of the C-terminal end of the Hsf1 protein was done as previously described (14). Briefly, a *URA3*-marked version of the *hsf1*(*1-583*) allele was used to replace the *HSF1* allele from YHN963 to create YHN2018.

Gene knockouts of *NTH1* or *TPS1* in YHN963, YHN1172, YHN1189, and YHN2018 were performed using drug-resistant-gene disruption cassettes, as previously described (19, 22). Briefly, primers were used to amplify the *hphMX4* cassette from the plasmid pAG32. For the *nth1*Δ strains, primers LC9 (ATAAACAAAAA AAGAAAAATTAACAAAAAAAATCAGTAGAGCATAGGCCACTAGTGG ATCTG) and LC10 (TACCTGGAGTATATATATATATATATATATTATC TCAACAGCTGAAGCTTCGTACGC) were used. For the *tps1*Δ strains, primers LC1 (AACTAGGTACTCACATACAGACTTATTAAGACATAGAACTGCAT AGGCCACTAGTGGATCTG) and LC2 (GGACCAGGAATAGACGATCGTC TCATTTGCATCGGGTTCACAGCTGAAGCTTCGTACGC) were used. The various yeast strains were then transformed with the amplified cassettes, and proper integration of the reporter was verified by PCR. The deletion strains were then transformed with the appropriate plasmids, either empty vectors or vectors that overexpressed *NTH1* and *TPS1*, in order to create the set of strains used for experiments (Table 1).

Because *tps1*Δ strains cannot grow with glucose as a carbon source (4, 54, 58), all strains were grown in synthetic complete media with 2% galactose as a carbon source. Amino acids were supplemented as necessary. Aminoglycosides were used as necessary at concentrations of 200 μg/ml Geneticin G418 (Invitrogen), 200 μg/ml hygromycin B (Roche), and 100 μg/ml CloNAT (Warner). Cells were grown at 30°C to mid-logarithmic phase (optical density at 600 nm of 0.3 to 0.5). Cells were then aliquoted into prewarmed flasks at 40°C for the times indicated in the figures. For experiments concerning recovery from heat shock, the flasks of cells that were allowed to recover from heat shock were transferred to a 30°C water bath for the indicated times. To test for viability, heat-shocked and recovering cells were serially diluted into sterile water, spotted onto agar plates, and grown at 30°C for 2 days.

Determination of trehalose levels. Trehalose was isolated from yeast cells as previously described (62). Briefly, yeast cells were subjected to heat as described above and were harvested by pelleting. Cells were washed twice in ice-cold water, the cell pellets were resuspended in a volume of ice-cold water equal to the measured wet weight, and a small aliquot was taken for determining cell counts. The remaining cells were placed in a boiling water bath for 20 min. Cells were then spun down at maximum speed in a tabletop microcentrifuge, and 3 μl of the supernatant was applied to a Silica Gel 60 plate (Merck). Standard dilutions of trehalose (Sigma) were also applied to the plate. Thin-layer chromatography (TLC) was performed by using a solution of 5:3:2 butanol-ethanol-water. The trehalose was visualized by spraying the plate with a 20% H_2SO_4 solution, followed by charring treatment in a 95°C oven. The trehalose was then quantitated using spot densitometry on the Fluorchem 8800 cooled-charge-coupled-device (CCD) detection system (Alpha Innotech). All graphical representations of trehalose levels represent data averaged from at least three independent experiments.

RNA preparation and Northern blot analysis. Total RNA isolation and Northern blot analysis were performed as previously described (17). Briefly, total RNA was isolated from yeast by using the hot acidic phenol method. RNA samples were quantified, and 10 μg of total RNA was run on a 1% agarose denaturing gel containing 18% formaldehyde. The RNA was transferred onto ZetaProbe nylon membranes (Bio-Rad) by capillary action and UV cross-linked. The membranes were probed with biotin-labeled DNA probes generated by the incorporation of the biotin-16-dUTP nucleotide (Pierce) into PCR products from plasmids containing the ORFs *HSP12*, *HSP26*, *HSP82*, *HSP104*, *SSA4*, and *SSA3*. The membranes were then developed using the North2South chemiluminescent detection kit (Pierce), and the signal was quantitated using a Fluorchem 8800 cooled-CCD detection system (Alpha Innotech). All Northern blot analyses were repeated at least three times, with representative gels shown in the figures. Graphical representations of *HSP* mRNA levels represent data averaged from at least three independent experiments.

Phosphorylation analysis. Cells containing *Myc*-tagged *HSF1* were collected by centrifugation after the indicated length of heat shock, frozen in liquid N_2, and stored at −70°C until further use. Cell pellets were resuspended in 2× LDS buffer (Invitrogen) containing yeast-specific protease inhibitor cocktail and

TABLE 1. Strains used in this study

Strain	Relevant genotype[a]	Experimental designation
YHN963[b]	*MAT***a** *msn2*Δ::*loxP msn4*Δ::*kanMX*	
YHN1172[b]	*MAT***a** *msn2*Δ::*loxP msn4*Δ::*kanMX* P*tetO*$_2$-*HSF1 adh*$_P$-*tetR-VP16 natMX4 adh*$_P$-*tetR′-SSN6*::*LEU2*	
YHN1189[b]	*MAT***a** *msn2*Δ::*loxP msn4*Δ::*kanMX HSF1-13Myc*::*TRP1*	
YHN450	YHN963 p424GPD p425ADH	Control
YHN431	YHN963 *tps1*Δ::*hphMX*	
YHN455	YHN963 *tps1*Δ::*hphMX* p424GPD p425ADH	LT
YHN433	YHN963 *nth1*Δ::*hphMX*	
YHN437	YHN963 *nth1*Δ::*hphMX* p424ADH-*TPS1* (P_{ADH1}-*TPS1*) p425ADH-*NTH1* (P_{ADH1}-NTH1)	HT
YHN454	YHN963 *nth1*Δ::*hphMX* p424GPD p425ADH	VHT
YHN456	YHN1172 p424ADH p426ADH	Control Tet[c] shutoff
YHN452	YHN1172 *nth1*Δ::*hphMX*	
YHN457	YHN1172 *nth1*Δ::*hphMX* p424ADH p426ADH	VHT Tet shutoff
YHN459	YHN1172 *nth1*Δ::*hphMX* p424ADH-*TPS1* (P_{ADH1}-*TPS1*) p426ADH-*NTH1* (P_{ADH1}-*NTH1*)	HT Tet shutoff
YHN2018	YHN963 *hsf1(1-583)*	
YHN2063	YHN963 *hsf1(1-583)* p424ADH p425ADH	Control HSFΔCAD
YHN2019	YHN963 *nth1*Δ::*hphMX hsf1(1-583)*	
YHN2066	YHN963 *nth1*Δ::*hphMX hsf1(1-583)* p424ADH-*TPS1* (P_{ADH1}-*TPS1*) p425ADH-*NTH1* (P_{ADH1}-*NTH1*)	HT HSFΔCAD
YHN2020	YHN1189 pRS425ADH p426GPD	Control Myc tag
YHN2057	YHN1189 *tps1*Δ::*hphMX*	
YHN2058	YHN1189 *tps1*Δ::*hphMX* p425ADH p426ADH	LT Myc tag
YHN484	YHN1189 *nth1*Δ::*hphMX*	
YHN2022	YHN1189 *nth1*Δ::*hphMX* p425ADH p426ADH	VHT Myc tag
YHN2023	YHN1189 *nth1*Δ::*hphMX* p426ADH-*TPS1* (P_{ADH1}-*TPS1*) p425ADH-*NTH1* (P_{ADH1}-*NTH1*)	HT Myc tag

[a] All strains are derivatives of W303-1A.
[b] Ferguson et al. (17).
[c] Tet, tetracycline.

serine/threonine phosphate inhibitor cocktail, with a final concentration of cells at an optical density at 600 nm of 7.5 units per 100 μl of buffer. Cells were heated at 100°C for 5 min and then placed into an ice bath for 5 min. Approximately 80 μl of glass beads was added, and the samples were vortexed for 10 min at 4°C. Samples were clarified by centrifugation, supernatants were transferred into fresh tubes, and the pellets were treated to a second round of lysis. Proteins were resuspended in 2× LDS buffer (Invitrogen) and were resolved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto an Immobilon P polyvinylidene difluoride membrane (Millipore). Hsf1 was detected by Western blotting using anti-Myc(9B11) (Cell Signaling) and horseradish peroxidase-conjugated anti-mouse immunoglobulin G (Amersham). Membranes were developed using Immobilon Western chemiluminescent horseradish peroxidase substrate (Millipore). The signal was quantitated by using a Fluorchem 8800 cooled-CCD detection system (Alpha Innotech). All Western blot analyses were repeated at least three times with independent samples; for representative gels, see Fig. 8.

For phosphatase treatment, cell protein extracts were prepared as described above. Equal volumes of sample were then diluted with FA lysis buffer (50 mM HEPES, pH 7.5; 150 mM NaCl; 1 mM EDTA; 1% Triton X-100; 0.1% sodium deoxycholate; 0.1% sodium dodecyl sulfate) containing yeast-specific protease inhibitor cocktail and serine/threonine phosphate inhibitor cocktail. Samples were precleared for 1 h using protein A-agarose beads (Life Technologies, Inc.) at 4°C. Proteins were then incubated with anti-Myc(9B11) antibody for 1.5 h and then with protein A-agarose beads overnight. The beads were washed three times in FA lysis buffer. The beads were then split, with one half incubated with 25 units of calf intestinal alkaline phosphatase (New England Biolabs). Beads were then diluted in 2× LDS sample buffer, and Western blotting was performed as described above.

RESULTS

Manipulation of trehalose levels in vivo. In yeast, the intracellular concentration of trehalose is quite low during normal conditions and rapidly increases during heat shock and under many other stress-inducing conditions (18). Trehalose levels are controlled in yeast through regulation of both the activities and the expression of several metabolic enzymes, including Nth1 and Tps1. Nth1 is the neutral trehalase, which is responsible for the degradation of trehalose (31). Tps1, or trehalose-6-phosphate synthase, is the first enzyme in the major trehalose biosynthetic pathway and is responsible for converting glucose into trehalose-6-phosphate (4). To determine the role of trehalose in HSP mRNA expression and Hsf1 activity in vivo, we first needed to construct strains that synthesized different trehalose levels. We accomplished this by deleting and/or overexpressing *NTH1* and *TPS1*. The heat-inducible transcription of HSP genes is regulated by the Hsf1 and Msn2/4 transcription factors. Since we wanted to focus on the contributions of Hsf1 to HSP mRNA transcription, all strains were derivative of an *msn2/4*Δ strain which has previously been shown to have a robust stress response (2, 14). In addition, we chose a 40°C heat shock because Hsf1 is more highly activated than Msn2/4 at that temperature (41, 49, 50). After analyzing a number of strains, we chose three strains for further study based on their significant differences in intracellular trehalose concentrations under constitutive and heat shock conditions (Table 1). These strains included a *tps1*Δ strain and two *nth1* knockout strains, one containing only the *nth1*Δ allele and the other containing the *nth1*Δ allele and vectors overexpressing *TPS1* and *NTH1* from the heterologous *ADH1* promoter (e.g., *nth1*Δ P_{ADH1}-*TPS1* P_{ADH1}-*NTH1*).

The *tps1*Δ strain had significantly lower levels of trehalose than the control strain at all time points prior to and during the heat shock (Fig. 1A). In the control strain, trehalose levels increased after 10 min of heat shock and continued to increase during the heat shock. In contrast, the trehalose levels in the *tps1*Δ strain did not increase significantly and never even reached the constitutive level of trehalose found in the control strain. The lower trehalose levels in the *tps1*Δ strain were not due to increased cell death during heat shock, as no change in cell viability was observed until after 40 min of heat shock (data

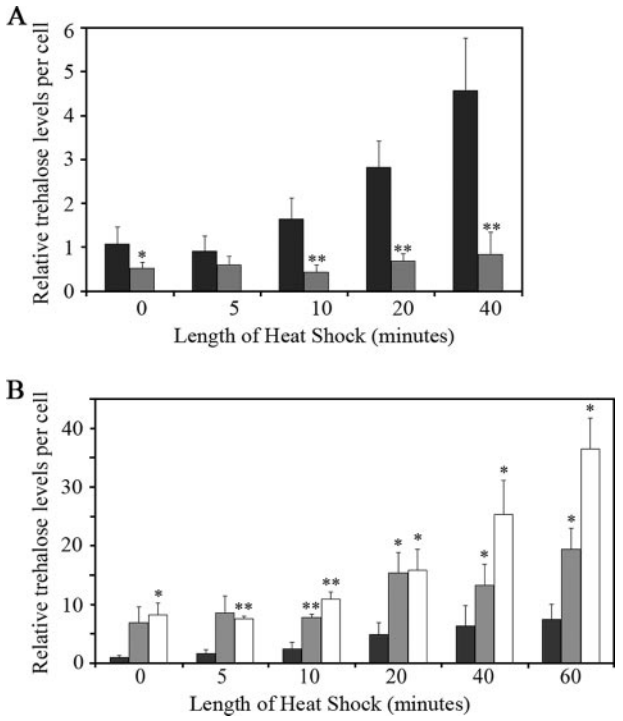

FIG. 1. Changes in trehalose levels during the heat shock response. Relative trehalose levels, determined by thin-layer chromatography and normalized to cell counts, are shown over the course of a 40°C heat shock for the control (YHN450), LT (YHN455), HT (YHN437), and VHT (YHN454) strains. (A) The LT strain (gray bars), which harbors a *TPS1* deletion, has decreased levels of trehalose compared to the control strain (black bars). (B) The HT (gray bars) and VHT (white bars) strains, which harbor *NTH1* deletions (*nth1*Δ P_{ADH1}-*TPS1* P_{ADH1}-*NTH1* and *nth1*Δ, respectively), have increased levels of trehalose compared to the control strain (black bars). Error bars show standard errors of the mean (SEM). *, $P < 0.05$; **, $P < 0.01$.

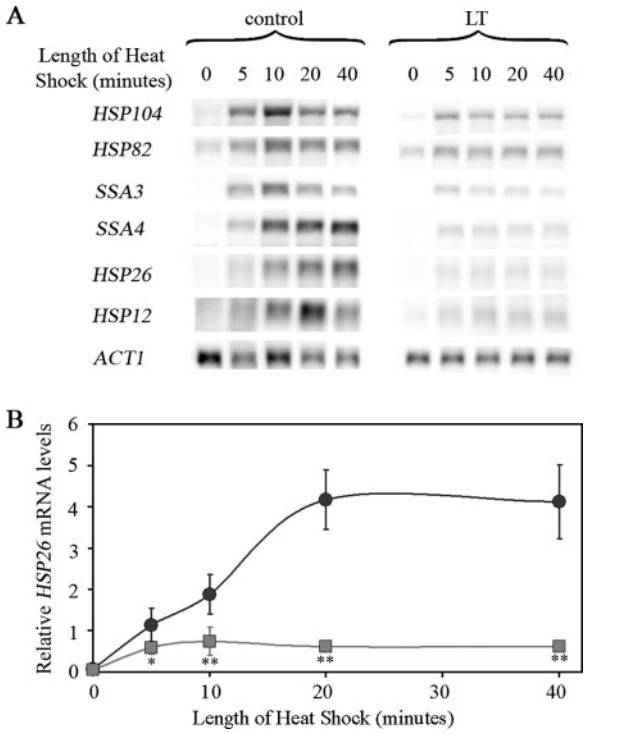

FIG. 2. Reduced HSP mRNA levels are correlated with decreased trehalose levels. (A) Northern blot analysis showing HSP mRNA levels over the course of a 40°C heat shock for the LT (YHN455) and control (YHN450) strains. (B) Graphical representation of *HSP26* mRNA levels, normalized to *ACT1*, shows that the LT strain (gray squares) has lower heat-induced transcript levels than the control strain (black circles). Error bars show SEM. *, $P < 0.05$; **, $P < 0.01$.

not shown). Therefore, this strain will be referred to hereinafter as the low-trehalose (LT) strain.

The two *nth1* knockout strains had significantly higher levels of trehalose at all time points prior to and during the heat shock (Fig. 1B). Prior to the heat shock, the constitutive levels of trehalose in both of the *nth1* knockout strains were approximately the same as the level of trehalose observed in the control strain after a 60-min heat shock. No differences were observed between the *nth1*Δ and *nth1*Δ P_{ADH1}-*TPS1* P_{ADH1}-*NTH1*strains until after 40 min of heat shock. At these later heat shock times, the *nth1*Δ strain had approximately three to four times the trehalose levels of the control strain, while the *nth1*Δ P_{ADH1}-*TPS1* P_{ADH1}-*NTH1* strain had approximately two to three times as much trehalose as the control strain. The high levels of trehalose did not appear to affect cell growth and viability (data not shown). The *nth1*Δ and *nth1*Δ P_{ADH1}-*TPS1* P_{ADH1}-*NTH1* strains will be referred to hereinafter as the very-high-trehalose (VHT) and high-trehalose (HT) strains, respectively. To summarize, we have been able to manipulate yeast to synthesize different trehalose levels under both constitutive and heat shock conditions.

Low trehalose levels correspond to reduced HSP mRNA levels. Trehalose levels increase during a normal heat shock, along with HSP mRNA and protein levels (21, 26). To determine if trehalose is necessary for HSP gene transcription, we measured HSP mRNA levels in our LT and control strains throughout the heat shock response (Fig. 2). The *msn2/4*Δ control strain had a typical response to a 40°C heat shock, with low HSP mRNA levels prior to heat shock, a peak level reached after 5 to 20 min, and then a gradual decrease to a new plateau level. The LT strain had a more diminished response. Prior to heat shock, the LT strain had low HSP mRNA levels, similar to the control strain. During the first 5 to 10 min of the heat shock response, the HSP mRNA levels rose slightly in the LT strain but significantly less than in the control strain. As the heat shock progressed, the LT strain continued to have significantly lower levels of HSP mRNAs. Depending on the HSP gene, maximal levels in the LT strain occurred 5 to 10 min earlier and were 1.5- to 6-fold lower than the levels reached in the control strain. In addition, the plateaus were 1.4- to 6.75-fold lower than those in the control strain. Thus, trehalose is critical for the heat-induced expression of HSP mRNAs.

High trehalose levels correspond to elevated HSP mRNA levels. Decreased trehalose levels result in a reduction of HSP gene transcription (Fig. 2). To determine if increased levels of trehalose can enhance the transcriptional response to heat shock, we compared HSP mRNA levels between the control strain and the two *nth1* knockout strains, which had higher trehalose levels (Fig. 3). Prior to heat shock, both strains with increased trehalose levels had HSP mRNA levels that were

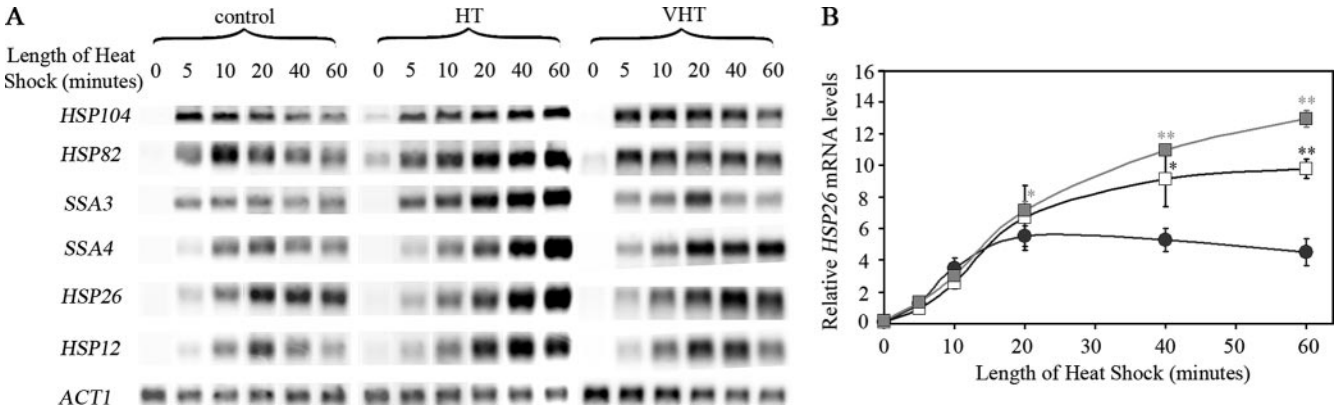

FIG. 3. Elevated HSP mRNA levels are correlated with increased trehalose levels. (A) Northern blot analysis showing HSP mRNA levels over the course of a 40°C heat shock for the HT (YHN437), VHT (YHN454), and control (YHN450) strains. (B) Graphical representation of *HSP26* mRNA levels, normalized to *ACT1*, shows that the HT (gray squares) and VHT (white squares) strains have higher heat-induced transcript levels than the control strain (black circles). Error bars show SEM. *, $P < 0.05$; **, $P < 0.01$.

similar to those of the control strain. Indeed, this held true for the first 5 to 20 min of the heat shock response. Therefore, increased trehalose has no effect on constitutive levels of HSP mRNAs or on the initial response to heat shock. However, as the heat shock progressed, the *nth1* knockout strains had significantly higher HSP mRNA levels than the control strain (Fig. 3 and 4). While HSP mRNA levels in the control strain reached a plateau or decreased between 5 and 20 min, the levels in the *nth1* knockout strains continued to increase, with plateaus occurring from 20 to beyond 150 min. Depending on the HSP gene, the transcript levels in the *nth1* knockout strains were elevated between 1.7- and 4.2-fold over those in the control strain at the 1-h time point, with maximal differences in transcript levels of 1.8- to 9.0-fold. Thus, trehalose enhances the prolonged or sustained response to heat shock.

Surprisingly, the strain with the very high levels of trehalose did not have the highest levels of HSP mRNAs. For example, levels of *HSP26* mRNA after a 1-h heat shock were significantly higher in the HT strain than in the VHT strain (Fig. 3B). This held true even for prolonged response to stress. When the length of the heat shock was increased to 2.5 h, the HT strain still did not reach a plateau in *HSP26* expression levels, even though the VHT strain reached its plateau at 60 min (Fig. 4). Despite these differences, the VHT strain maintained higher levels of HSP mRNAs than the control strain. These results show that the correlation between trehalose levels and HSP mRNA levels is complex and suggest that maximal HSP transcription occurs within an optimal range of trehalose levels.

Trehalose and heat are required for elevated HSP mRNA levels. Increased trehalose levels enhance the sustained response to heat shock; however, increased levels of trehalose prior to heat treatment have no effect on the constitutive levels of HSP mRNAs (Fig. 3). Recovery from heat stress is normally associated with a reduction in both HSP mRNA and trehalose levels (21), but trehalose levels in the HT strain remained high during recovery (Fig. 5A). To determine if high levels of trehalose alone are sufficient to maintain elevated HSP mRNA levels in the absence of heat, the control and HT strains were exposed to a 1-h heat shock, followed by recovery at a constitutive temperature (30°C) for up to 1.5 h. In both strains, the HSP mRNA levels decreased, beginning at the first recovery time point (Fig. 5B and C). Despite the increased levels of trehalose in the HT strain, the extents of reduction in HSP mRNA levels were similar in both strains, suggesting that continuous exposure to heat is critical for the trehalose-dependent effect on transcription. These results confirm that both heat and trehalose are required for the elevated levels of HSP mRNA.

Hsf1 is required for trehalose-dependent elevation of HSP mRNA levels. We have shown that trehalose and heat are required for the heat-induced transcription of HSP mRNAs. Since all our strains are *msn2/4*Δ, we wanted to confirm that the elevated HSP mRNA levels in the *nth1* knockout strains require Hsf1. Because *HSF1* is an essential gene, we used a tetracycline-regulated dual-expression system to repress *HSF1* transcription (5, 17). In this system, the addition of doxycycline results in a loss of *HSF1* mRNA (16) but has no effect on the trehalose levels (data not shown). After a 60-min heat shock, the doxycycline-treated control and HT strains showed little or no heat-induced increase in HSP mRNA levels (Fig. 6). In fact, there was no detectable difference in HSP mRNA levels between the control and HT strains. The same held true for the VHT strain (data not shown). These results confirm that the elevated HSP mRNA levels in response to high trehalose levels require the transcriptional activator Hsf1, suggesting that trehalose affects Hsf1's transcriptional activity.

The C-terminal activation domain is not required for trehalose-dependent elevation of HSP mRNA levels. Yeast Hsf1 has two activation domains, located amino and carboxy terminal to the central conserved core. Both activation domains play important roles during the heat shock response, with each activation domain being critical for the expression of different subsets of HSPs (14). We have shown that trehalose increases both the secondary and tertiary structures of the CAD in vitro (8). To determine whether the CAD is required for the trehalose-dependent elevation in HSP mRNA levels, we deleted the CAD in our control and HT strains (Table 1). Deletion of the CAD did not affect trehalose levels or viability during the 90-min heat shock (data not shown). For all

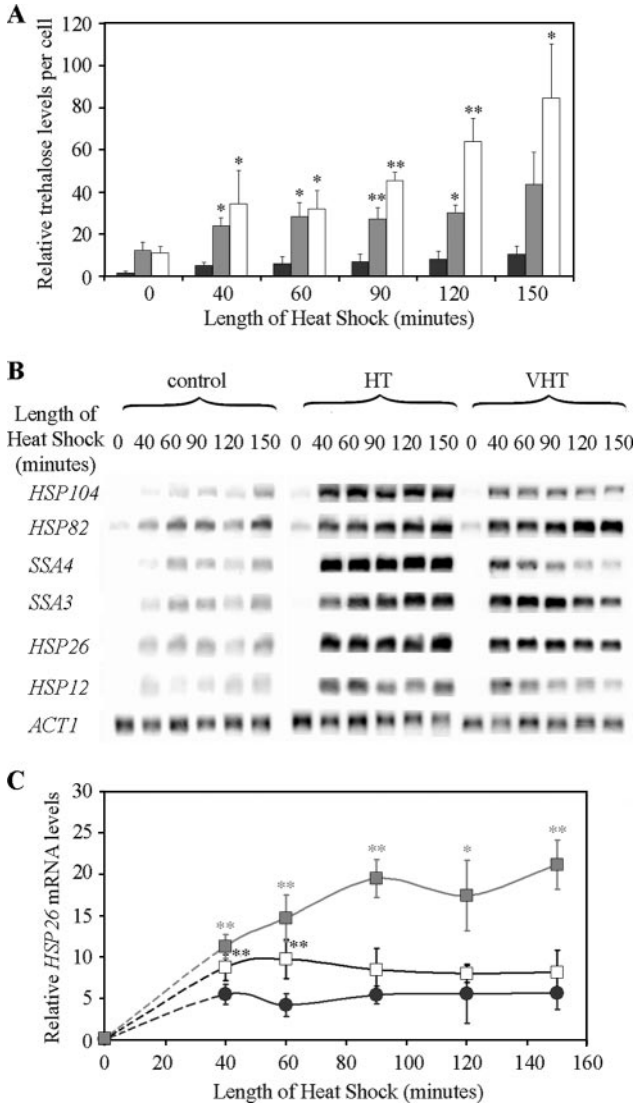

FIG. 4. The *nth1* knockout strains behave differently during prolonged exposure to heat stress. The HT (YHN437), VHT (YHN454), and control (YHN450) strains were subjected to heat shocks of up to 2.5 h. (A) Trehalose levels of both the HT (gray bars) and VHT (white bars) strains are significantly increased over that of the control strain (black bars) at every time point. (B) Northern blot showing the levels of several HSP mRNAs during a prolonged heat shock. (C) Graphical representation of Northern blot of *HSP26* mRNA normalized to *ACT1* reveals strain differences between the HT (gray squares), VHT (white squares), and control (black circles) strains. Error bars show SEM. *, $P < 0.05$; **, $P < 0.01$.

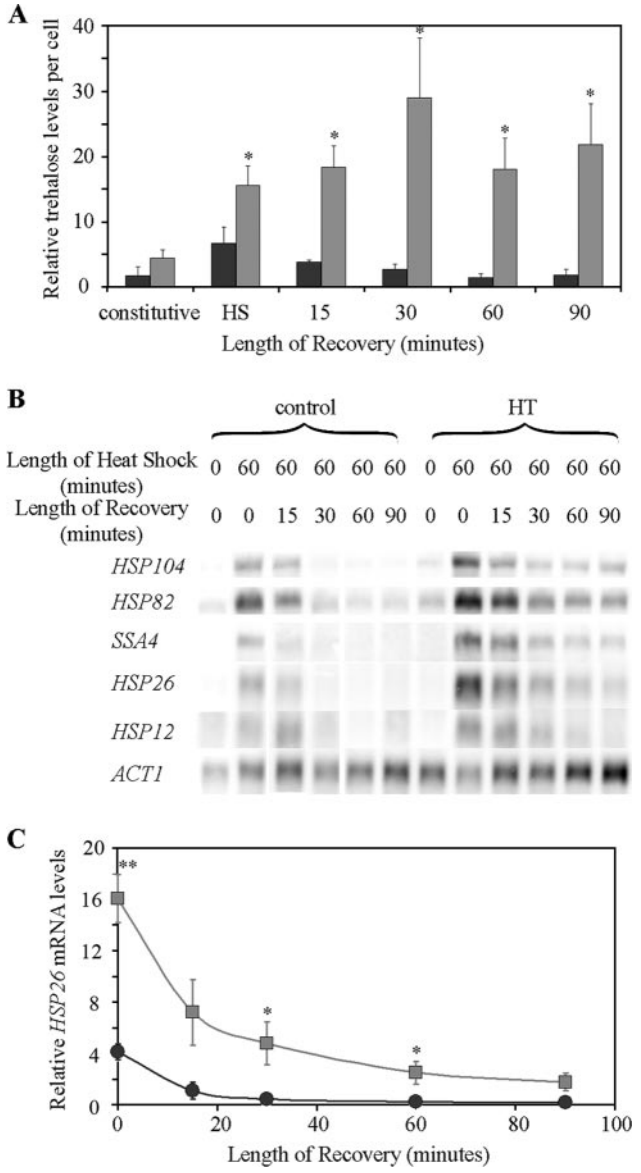

FIG. 5. Increased trehalose levels are unable to prevent recovery from heat stress. After a 60-min heat shock (HS) at 40°C, the HT (YHN437) and control (YHN450) strains were allowed to recover for up to 90 min at a constitutive temperature (30°C). (A) Trehalose levels remain high during recovery in the HT strain (gray bars), while trehalose levels decrease in the control strain (black bars). (B) Northern blot analysis showing HSP mRNA levels upon recovery from heat shock. (C) Graphical representation of *HSP26* mRNA levels, normalized to *ACT1*, shows that the HT (gray squares) and control (black circles) strains have similar decreases in HSP mRNA levels. Error bars show SEM. *, $P < 0.05$; **, $P < 0.01$.

HSPs tested, the HSFΔCAD strain with high trehalose levels had enhanced HSP mRNA levels compared to the HSFΔCAD control strain (Fig. 7), similar to what was seen with the HT and control strains containing the wild-type *HSF1* allele (Fig. 3). This enhanced transcription occurred even for those HSP genes, such as *HSP26* and *HSP82*, which are dependent primarily on the CAD for maximal heat shock induction (14). These results suggest that the mechanism by which trehalose increases activity is not specific to the C-terminal activation domain.

Trehalose-dependent heat-induced transcription correlates with changes in Hsf1 phosphorylation. Changes in the transcriptional activity of Hsf1 during heat shock are correlated with changes in the phosphorylation state of the protein, with activated Hsf1 being hyperphosphorylated during heat shock (50). Since trehalose affects the heat-induced transcriptional activity of Hsf1 (Fig. 5), we determined whether trehalose levels are correlated with changes in Hsf1 modi-

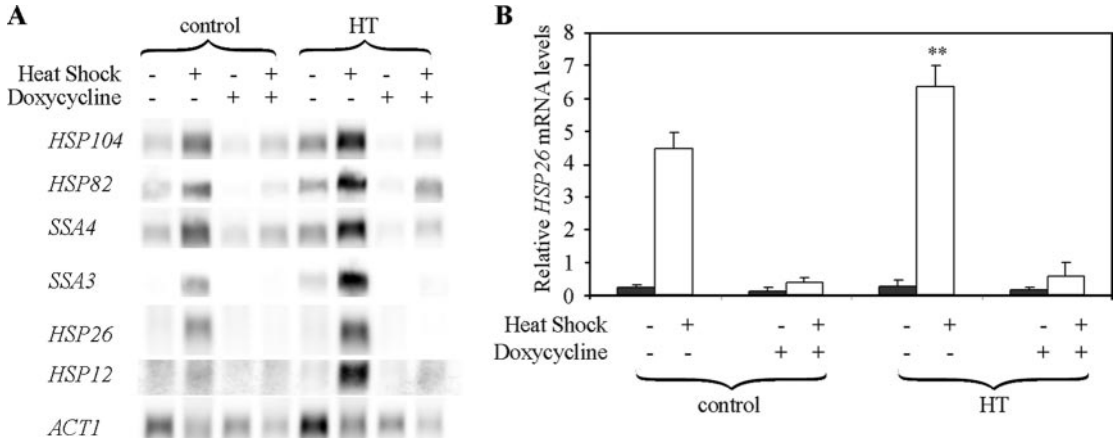

FIG. 6. *HSF1* is required for the trehalose-dependent increase in HSP mRNA levels during heat shock. Strains containing a tetracycline-repressible *HSF1* allele were grown for 16 h in medium containing doxycycline, followed by heat shock for 60 min at 40°C. (A) Northern blot analysis showing HSP mRNA levels for the HT (YHN459) and control (YHN456) strains with the conditional tetracycline-repressible Hsf1 allele. (B) Graphical representation of *HSP26* mRNA levels, normalized to *ACT1*, shows that the HT and control strains had a loss in heat-induced expression upon depletion of *HSF1*. Error bars show SEM. **, $P < 0.01$; +, present; −, absent.

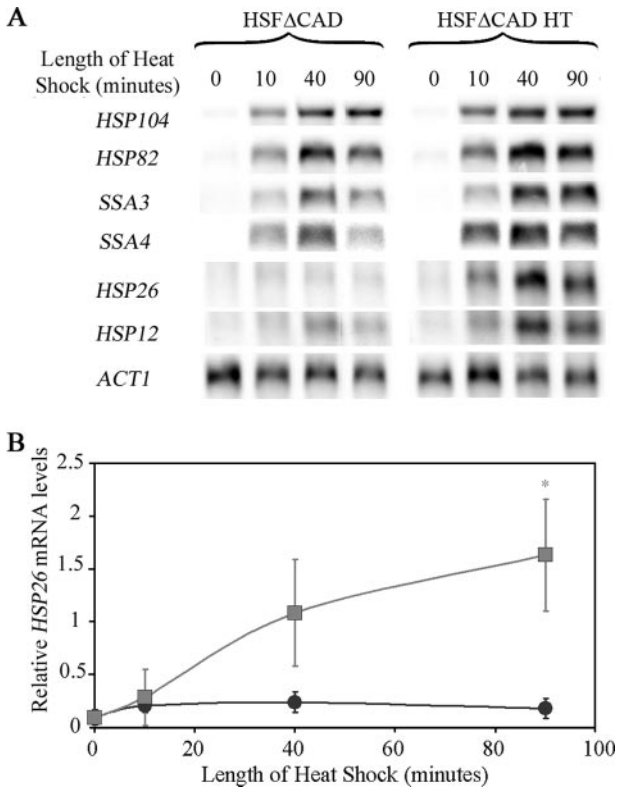

FIG. 7. The C-terminal activation domain is not required for a trehalose-dependent increase in HSP mRNA levels during heat shock. (A) Northern blot analysis showing HSP mRNA levels over the course of a 40°C heat shock for the HSFΔCAD HT (YHN2066) and HSFΔCAD control (YHN2063) strains containing an *hsf1* (*1-583*) allele. (B) Graphical representation of *HSP26* mRNA levels, normalized to *ACT1*, shows that the HSFΔCAD HT strain (gray squares) has higher heat-induced transcript levels than the HSFΔCAD control strain (black circles). Error bars show SEM. *, $P < 0.05$.

fications. Hsf1 hyperphosphorylation can be detected as a decrease in its electrophoretic mobility on a sodium dodecyl sulfate-polyacrylamide gel and can be confirmed by phosphatase treatment, which removes the change in electrophoretic mobility (50). Not only are changes in phosphorylation associated with the initial response to heat stress, but phosphorylation also increases even more during the sustained heat shock response (49, 50).

We used Western blot analysis to determine the phosphorylation status of Myc-tagged Hsf1 in the control, LT, HT, and VHT strains (Fig. 8). In all strains, Hsf1 had the same electrophoretic mobility prior to heat shock (compare lanes 1 and 2 in Fig. 8A, C, and E,), showing that constitutive Hsf1 phosphorylation levels were the same in all strains despite differences in intracellular trehalose levels. However, the electrophoretic mobility of heat-induced Hsf1 differed quantitatively and temporally in response to heat shock, depending on the strain and its levels of trehalose. Hsf1 from the LT strain had lower levels of hyperphosphorylation than that from the control strain, and differences were evident after 5 min of heat shock (Fig. 8A), the same time at which the HSP mRNA levels in these strains differed. The HT and VHT strains showed even greater differences from the control strain in their levels of Hsf1 hyperphosphorylation (Fig. 8C and E). The enhanced mobility of Hsf1 from the HT and VHT strains was observed after 40 min of heat shock, the same time at which significant differences in HSP mRNA levels were observed (Fig. 3). Hsf1 from the VHT strain had a level of mobility between those of Hsf1 proteins from the control and HT strains (Fig. 8G). The changes in the mobility of Hsf1 in all strains were no longer observed when the lysates were treated with phosphatase (Fig. 8B, D, and F), confirming that the mobility differences were due to differences in phosphorylation. Correlation between Hsf1 modification and activity was also verified during recovery from heat shock, as both Hsf1 transcriptional activity and phosphorylation decreased during this time (Fig. 5 and 8H). Taken together, these data indicate that the trehalose-dependent changes in HSP mRNA levels correlate temporally with changes in Hsf1 phos-

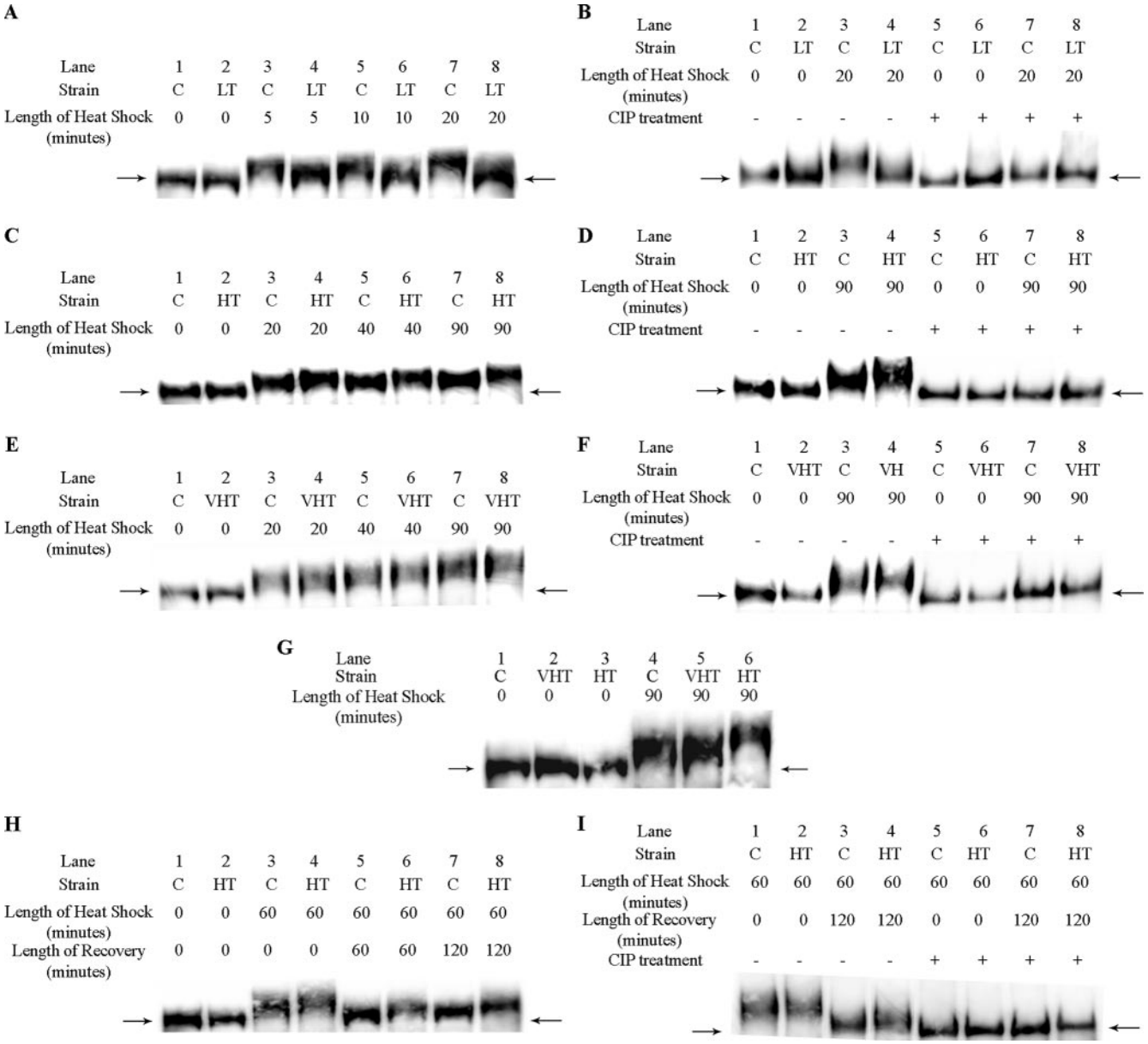

FIG. 8. Changes in Hsf1 phosphorylation correlate with changes in trehalose-dependent transcriptional activity. Western blot analysis (A, C, E, G, and H) or immunoprecipitation-Western blot analysis (B, D, F, and I) was performed on the control strain (labeled C; YHN2020) or LT (YHN2058), HT (YHN2023), and VHT (YHN2022) strains carrying *Myc*-tagged *HSF1.* Cells were heat shocked and allowed to recover for the indicated times. Calf intestinal alkaline phosphatase (CIP) treatment (B, D, F, and I) confirmed that the shift in mobility of the *Myc-HSF1* gene product was due to phosphorylation. +, present; −, absent.

phorylation during the heat shock response, confirming that trehalose levels influence heat-induced Hsf1 activity.

DISCUSSION

Trehalose is known to play an important role during the heat shock response as a chemical cochaperone. Trehalose increases the thermal stability of proteins, thus preserving enzymatic activity and even preventing native proteins from denaturing during elevated temperatures (27, 30, 46). It also works alongside HSPs to suppress the aggregation of denatured proteins (27, 46). In this paper, we show that trehalose plays an additional role: trehalose promotes the transcriptional response during a heat shock by functioning as a positive regulator to modify the dynamic range of Hsf1 activity. As a consequence, trehalose works at the transcriptional level to increase HSP mRNAs. This expands the model in which trehalose cooperates with HSPs to maintain and protect proteins during the response to heat shock (47).

Trehalose is required for the heat-induced increase of HSP mRNAs in the absence of Msn2/4 (Fig. 2). At very low levels of trehalose, HSP mRNA levels increased only nominally within the first 5 min of heat shock and then decreased or reached a plateau. Our results are in general agreement with previous

studies of HSP levels in *tps1*Δ strains with wild-type *MSN2/4* alleles. *HSP26*, *HSP104*, and *SSA4* mRNA levels reached in the *tps1*Δ *MSN2/4* strain were two- to eightfold lower than those reached in the parent *MSN2/4* strain after a 1-h, 39°C heat shock (25), similar to what was seen at the 40-min time point for *msn2/4*Δ strains (Fig. 2). However, a milder 35°C heat shock produced different results, with no difference in Hsp104 or Hsp26 protein levels between the *tps1*Δ *MSN2/4* strain and the parent *MSN2/4* strain (46). We suspect that Msn2/4 was a major contributor to *HSP104* and *HSP26* expression in this experiment, as Msn2/4 is more highly activated than Hsf1 at a lower heat shock temperature (41, 49, 50).

The role of trehalose in enhancing HSP gene transcription is dependent on the continued presence of heat. At constitutive temperatures, the *nth1* knockout strains have significantly increased levels of trehalose compared to the control strain, but the strains all have the same constitutive levels of HSP mRNA (Fig. 3). During recovery from heat shock, HSP mRNA levels in the HT strain decreased despite the retention of high trehalose levels (Fig. 5), showing that trehalose alone is not sufficient to maintain an elevated level of HSP expression. If trehalose merely prevented the degradation of HSP mRNA during heat shock, the mRNA levels would not have decreased during recovery while high trehalose levels were maintained. Instead, trehalose increases the normal transcriptional response to heat shock.

The trehalose-dependent increase in HSP gene transcription is mediated through Hsf1. When cells are depleted of Hsf1, the heat-induced HSP mRNA levels are suppressed to the same extent in both the control and HT strains, despite the continued high levels of trehalose in the HT strain (Fig. 6). In support of Hsf1's dependence on trehalose, the phosphorylation of Hsf1 correlates with the trehalose-dependent changes in HSP mRNA levels (Fig. 8). However, this dependence is complex, with trehalose requirements varying throughout the heat shock response. A minimum level of trehalose is required for the initial response to heat shock, as the LT strain has a severely reduced level of heat-induced Hsf1 activity (Fig. 2). The control, HT, and VHT strains all have similar HSP profiles for the first 15 to 20 min, confirming that trehalose levels above a certain threshold are required for the initial response of Hsf1 to heat shock (Fig. 3). During a prolonged heat shock, a higher threshold of trehalose is necessary to maintain and even amplify Hsf1's activity, but even this level is tightly controlled, as the VHT strain does not have as much Hsf1 activity as the HT strain (Fig. 4). Previous studies have shown that excess trehalose can interfere with protein activity by stabilizing proteins in nonnative conformations and may interfere with HSP function on unfolded proteins (46). The trehalose-dependent increase in HSP gene transcription may be diminished when trehalose levels become so high that they interfere with the specific transcriptional process, perhaps by inhibiting the ability of Hsf1 to be hyperphosphorylated. It is unlikely that very high trehalose levels generally inhibit transcription, as *ACT1* mRNA and rRNA levels are not affected and total RNA levels per cell volume are the same regardless of trehalose levels (data not shown). Thus, the levels of trehalose serve to modify the dynamic range of Hsf1 activity in specific ways: trehalose is required for maximal Hsf1 activity at the initiation of heat shock, while a higher, yet limited, threshold of trehalose is required for sustained transcriptional activity.

Given that trehalose is known to modify protein structure and activity in vivo, there are three likely models for the mechanism of its action on Hsf1 during heat shock. First, trehalose might act directly on Hsf1 by promoting and maintaining the structure of Hsf1 activation domains in a highly active state and thus increasing HSP mRNA transcription during heat shock. In support of this idea, we have shown that trehalose changes the conformation of the C-terminal activation domain in vitro, with increases in both the secondary and tertiary structures (8). However, the trehalose-dependent increase in Hsf1 activity does not depend on the presence of this domain (Fig. 7). In a strain lacking the C-terminal activation domain, all HSP mRNAs are enhanced with high trehalose (Fig. 7), regardless of whether the heat-induced expression of the HSP is dependent primarily on either the N- or the C-terminal activation domain (14). Therefore, if trehalose influences Hsf1 structure in vivo, it likely affects the global structure. Any trehalose-induced conformational change might be responsible for the increased phosphorylation of Hsf1, as trehalose could maintain Hsf1 in a structurally accessible state for a kinase or a structurally inaccessible state for a phosphatase. Second, trehalose could act more indirectly on Hsf1 by stabilizing a kinase or destabilizing a phosphatase, altering their activities during a sustained heat shock response. Hsf1 would therefore be maintained in a hyperphosphorylated, and presumably more transcriptionally active, state. In support of this mechanism, trehalose is known to stabilize enzymatic activity at high temperatures (27, 30, 63) and has been correlated with PKC1 activity (36). Finally, the known role of trehalose as a chemical cochaperone may affect Hsf1 activity by interfering with the feedback regulation of Hsf1 by HSPs. The high levels of trehalose, which are known to prevent refolding of proteins by HSPs, could maintain the high levels of misfolded proteins necessary to continue the stimulation of the heat shock response (47). By functioning as a positive regulator of Hsf1, trehalose may effectively balance the levels between trehalose and HSPs during the heat shock response. Alternatively, trehalose might protect Hsf1 from specific HSPs that function as negative regulators during the heat shock response and prevent the deactivation of Hsf1 (10, 13, 23, 51). We are currently investigating all of these possibilities to determine how trehalose promotes Hsf1 activity and enhances the expression of HSPs during the heat shock response.

ACKNOWLEDGMENTS

This work was supported by NIH grant GM44086.

We thank Amanda Bulman and Scott Ferguson for the critical initial discussions about this project and Sara Achenbach and Dawn Eastmond for continued discussions about this project.

REFERENCES

1. **Alexandre, H., L. Plourde, C. Charpentier, and J. Francois.** 1998. Lack of correlation between trehalose accumulation, cell viability and intracellular acidification as induced by various stresses in Saccharomyces cerevisiae. Microbiology **144:**1103–1111.
2. **Amorós, M., and F. Estruch.** 2001. Hsf1p and Msn2/4p cooperate in the expression of Saccharomyces cerevisiae genes HSP26 and HSP104 in a gene- and stress type-dependent manner. Mol. Microbiol. **39:**1523–1532.
3. **Arora, A., C. Ha, and C. B. Park.** 2004. Inhibition of insulin amyloid formation by small stress molecules. FEBS Lett. **564:**121–125.
4. **Bell, W., P. Klaassen, M. Ohnacker, T. Boller, M. Herweijer, P. Schoppink,**

P. Van der Zee, and A. Wiemken. 1992. Characterization of the 56-kDa subunit of yeast trehalose-6-phosphate synthase and cloning of its gene reveal its identity with the product of CIF1, a regulator of carbon catabolite inactivation. Eur. J. Biochem. **209:**951–959.
5. **Bellí, G., E. Gari, L. Piedrafita, M. Aldea, and E. Herrero.** 1998. An activator/repressor dual system allows tight tetracycline-regulated gene expression in budding yeast. Nucleic Acids Res. **26:**942–947.
6. **Bonner, J. J., T. Carlson, D. L. Fackenthal, D. Paddock, K. Storey, and K. Lea.** 2000. Complex regulation of the yeast heat shock transcription factor. Mol. Biol. Cell **11:**1739–1751.
7. **Boy-Marcotte, E., G. Lagniel, M. Perrot, F. Bussereau, A. Boudsocq, M. Jacquet, and J. Labarre.** 1999. The heat shock response in yeast: differential regulations and contributions of the Msn2p/Msn4p and Hsf1p regulons. Mol. Microbiol. **33:**274–283.
8. **Bulman, A. L., and H. C. Nelson.** 2005. Role of trehalose and heat in the structure of the C-terminal activation domain of the heat shock transcription factor. Proteins **58:**826–835.
9. **Chen, T., and C. S. Parker.** 2002. Dynamic association of transcriptional activation domains and regulatory regions in Saccharomyces cerevisiae heat shock factor. Proc. Natl. Acad. Sci. USA **99:**1200–1205.
10. **Cheng, L., N. Kirk, and P. W. Piper.** 1993. A small influence of HSP90 levels on the trehalose and heat shock element inductions of the yeast heat shock response. Biochem. Biophys. Res. Commun. **195:**201–207.
11. **Davies, J. E., S. Sarkar, and D. C. Rubinsztein.** 2006. Trehalose reduces aggregate formation and delays pathology in a transgenic mouse model of oculopharyngeal muscular dystrophy. Hum. Mol. Genet. **15:**23–31.
12. **Diamant, S., N. Eliahu, D. Rosenthal, and P. Goloubinoff.** 2001. Chemical chaperones regulate molecular chaperones in vitro and in cells under combined salt and heat stresses. J. Biol. Chem. **276:**39586–39591.
13. **Duina, A. A., H. M. Kalton, and R. F. Gaber.** 1998. Requirement for Hsp90 and a CyP-40-type cyclophilin in negative regulation of the heat shock response. J. Biol. Chem. **273:**18974–18978.
14. **Eastmond, D. L., and H. C. Nelson.** 2006. Genome-wide analysis reveals new roles for the activation domains of the Saccharomyces cerevisiae heat shock transcription factor (Hsf1) during the transient heat shock response. J. Biol. Chem. **281:**32909–32921.
15. **Felix, C. F., C. C. Moreira, M. S. Oliveira, M. Sola-Penna, J. R. Meyer-Fernandes, H. M. Scofano, and A. Ferreira-Pereira.** 1999. Protection against thermal denaturation by trehalose on the plasma membrane H+-ATPase from yeast. Synergetic effect between trehalose and phospholipid environment. Eur. J. Biochem. **266:**660–664.
16. **Ferguson, S. B.** 2005. Negative regulation of the heat shock transcription factor by protein kinase A in *Saccharomyces cerevisiae.* Ph.D. thesis. University of Pennsylvania, Philadelphia.
17. **Ferguson, S. B., E. S. Anderson, R. B. Harshaw, T. Thate, N. L. Craig, and H. C. Nelson.** 2005. Protein kinase A regulates constitutive expression of small heat-shock genes in an Msn2/4p-independent and Hsf1p-dependent manner in Saccharomyces cerevisiae. Genetics **169:**1203–1214.
18. **François, J., and J. L. Parrou.** 2001. Reserve carbohydrates metabolism in the yeast Saccharomyces cerevisiae. FEMS Microbiol. Rev. **25:**125–145.
19. **Goldstein, A. L., and J. H. McCusker.** 1999. Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast **15:**1541–1553.
20. **Görner, W., E. Durchschlag, M. T. Martinez-Pastor, F. Estruch, G. Ammerer, B. Hamilton, H. Ruis, and C. Schuller.** 1998. Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. Genes Dev. **12:**586–597.
21. **Gross, C., and K. Watson.** 1998. Transcriptional and translational regulation of major heat shock proteins and patterns of trehalose mobilization during hyperthermic recovery in repressed and derepressed Saccharomyces cerevisiae. Can. J. Microbiol. **44:**341–350.
22. **Güldener, U., S. Heck, T. Fielder, J. Beinhauer, and J. H. Hegemann.** 1996. A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. **24:**2519–2524.
23. **Halladay, J. T., and E. A. Craig.** 1995. A heat shock transcription factor with reduced activity suppresses a yeast HSP70 mutant. Mol. Cell. Biol. **15:**4890–4897.
24. **Hashikawa, N., and H. Sakurai.** 2004. Phosphorylation of the yeast heat shock transcription factor is implicated in gene-specific activation dependent on the architecture of the heat shock element. Mol. Cell. Biol. **24:**3648–3659.
25. **Hazell, B. W., H. Nevalainen, and P. V. Attfield.** 1995. Evidence that the Saccharomyces cerevisiae CIF1 (GGS1/TPS1) gene modulates heat shock response positively. FEBS Lett. **377:**457–460.
26. **Hottiger, T., T. Boller, and A. Wiemken.** 1987. Rapid changes of heat and desiccation tolerance correlated with changes of trehalose content in Saccharomyces cerevisiae cells subjected to temperature shifts. FEBS Lett. **220:**113–115.
27. **Hottiger, T., C. De Virgilio, M. N. Hall, T. Boller, and A. Wiemken.** 1994. The role of trehalose synthesis for the acquisition of thermotolerance in yeast. II. Physiological concentrations of trehalose increase the thermal stability of proteins in vitro. Eur. J. Biochem. **219:**187–193.
28. **Hottiger, T., P. Schmutz, and A. Wiemken.** 1987. Heat-induced accumulation and futile cycling of trehalose in *Saccharomyces cerevisiae*. J. Bacteriol. **169:**5518–5522.
29. **Jacquet, M., G. Renault, S. Lallet, J. De Mey, and A. Goldbeter.** 2003. Oscillatory nucleocytoplasmic shuttling of the general stress response transcriptional activators Msn2 and Msn4 in Saccharomyces cerevisiae. J. Cell Biol. **161:**497–505.
30. **Kaushik, J. K., and R. Bhat.** 2003. Why is trehalose an exceptional protein stabilizer? An analysis of the thermal stability of proteins in the presence of the compatible osmolyte trehalose. J. Biol. Chem. **278:**26458–26465.
31. **Kopp, M., H. Muller, and H. Holzer.** 1993. Molecular analysis of the neutral trehalase gene from Saccharomyces cerevisiae. J. Biol. Chem. **268:**4766–4774.
32. **Lee, S., T. Carlson, N. Christian, K. Lea, J. Kedzie, J. P. Reilly, and J. J. Bonner.** 2000. The yeast heat shock transcription factor changes conformation in response to superoxide and temperature. Mol. Biol. Cell **11:**1753–1764.
33. **Liu, R., H. Barkhordarian, S. Emadi, C. B. Park, and M. R. Sierks.** 2005. Trehalose differentially inhibits aggregation and neurotoxicity of beta-amyloid 40 and 42. Neurobiol. Dis. **20:**74–81.
34. **Magazù, S., F. Migliardo, C. Mondelli, and M. Vadala.** 2005. Correlation between bioprotective effectiveness and dynamic properties of trehalose-water, maltose-water and sucrose-water mixtures. Carbohydr. Res. **340:**2796–2801.
35. **Martínez-Pastor, M. T., G. Marchler, C. Schuller, A. Marchler-Bauer, H. Ruis, and F. Estruch.** 1996. The Saccharomyces cerevisiae zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE). EMBO J. **15:**2227–2235.
36. **Mensonides, F. I., S. Brul, F. M. Klis, K. J. Hellingwerf, and M. J. Teixeira de Mattos.** 2005. Activation of the protein kinase C1 pathway upon continuous heat stress in *Saccharomyces cerevisiae* is triggered by an intracellular increase in osmolarity due to trehalose accumulation. Appl. Environ. Microbiol. **71:**4531–4538.
37. **Mumberg, D., R. Muller, and M. Funk.** 1995. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene **156:**119–122.
38. **Neves, M. J., and J. Francois.** 1992. On the mechanism by which a heat shock induces trehalose accumulation in Saccharomyces cerevisiae. Biochem. J. **288:**859–864.
39. **Nwaka, S., and H. Holzer.** 1998. Molecular biology of trehalose and the trehalases in the yeast Saccharomyces cerevisiae. Prog. Nucleic Acid Res. Mol. Biol. **58:**197–237.
40. **Nwaka, S., M. Kopp, and H. Holzer.** 1995. Expression and function of the trehalase genes NTH1 and YBR0106 in Saccharomyces cerevisiae. J. Biol. Chem. **270:**10193–10198.
41. **Parrou, J. L., M. A. Teste, and J. Francois.** 1997. Effects of various types of stress on the metabolism of reserve carbohydrates in Saccharomyces cerevisiae: genetic evidence for a stress-induced recycling of glycogen and trehalose. Microbiology **143:**1891–1900.
42. **Pirkkala, L., P. Nykanen, and L. Sistonen.** 2001. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J. **15:**1118–1131.
43. **Sampedro, J. G., R. A. Munoz-Clares, and S. Uribe.** 2002. Trehalose-mediated inhibition of the plasma membrane H^+-ATPase from *Kluyveromyces lactis*: dependence on viscosity and temperature. J. Bacteriol. **184:**4384–4391.
44. **Sebollela, A., P. R. Louzada, M. Sola-Penna, V. Sarone-Williams, T. Coelho-Sampaio, and S. T. Ferreira.** 2004. Inhibition of yeast glutathione reductase by trehalose: possible implications in yeast survival and recovery from stress. Int. J. Biochem. Cell Biol. **36:**900–908.
45. **Simola, M., A. L. Hanninen, S. M. Stranius, and M. Makarow.** 2000. Trehalose is required for conformational repair of heat-denatured proteins in the yeast endoplasmic reticulum but not for maintenance of membrane traffic functions after severe heat stress. Mol. Microbiol. **37:**42–53.
46. **Singer, M. A., and S. Lindquist.** 1998. Multiple effects of trehalose on protein folding in vitro and in vivo. Mol. Cell **1:**639–648.
47. **Singer, M. A., and S. Lindquist.** 1998. Thermotolerance in Saccharomyces cerevisiae: the Yin and Yang of trehalose. Trends Biotechnol. **16:**460–468.
48. **Sola-Penna, M., and J. R. Meyer-Fernandes.** 1998. Stabilization against thermal inactivation promoted by sugars on enzyme structure and function: why is trehalose more effective than other sugars? Arch. Biochem. Biophys. **360:**10–14.
49. **Sorger, P. K.** 1990. Yeast heat shock factor contains separable transient and sustained response transcriptional activators. Cell **62:**793–805.
50. **Sorger, P. K., and H. R. Pelham.** 1988. Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. Cell **54:**855–864.
51. **Stone, D. E., and E. A. Craig.** 1990. Self-regulation of 70-kilodalton heat shock proteins in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**1622–1632.
52. **Tanaka, M., Y. Machida, S. Niu, T. Ikeda, N. R. Jana, H. Doi, M. Kurosawa, M. Nekooki, and N. Nukina.** 2004. Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. Nat. Med. **10:**148–154.
53. **Tanaka, M., Y. Machida, and N. Nukina.** 2005. A novel therapeutic strategy

for polyglutamine diseases by stabilizing aggregation-prone proteins with small molecules. J. Mol. Med. **83:**343–352.

54. **Van Aelst, L., S. Hohmann, B. Bulaya, W. de Koning, L. Sierkstra, M. J. Neves, K. Luyten, R. Alijo, J. Ramos, and P. Coccetti.** 1993. Molecular cloning of a gene involved in glucose sensing in the yeast Saccharomyces cerevisiae. Mol. Microbiol. **8:**927–943.
55. **VanLaere, A.** 1989. Trehalose, reserve and/or stress metabolite? FEMS Microbiol. Rev. **63:**201–210.
56. **Viner, R. I., and J. S. Clegg.** 2001. Influence of trehalose on the molecular chaperone activity of p26, a small heat shock/alpha-crystallin protein. Cell Stress Chaperones **6:**126–135.
57. **Voellmy, R.** 2004. On mechanisms that control heat shock transcription factor activity in metazoan cells. Cell Stress Chaperones **9:**122–133.
58. **Vuorio, O. E., N. Kalkkinen, and J. Londesborough.** 1993. Cloning of two related genes encoding the 56-kDa and 123-kDa subunits of trehalose synthase from the yeast Saccharomyces cerevisiae. Eur. J. Biochem. **216:**849–861.
59. **Wera, S., E. De Schrijver, I. Geyskens, S. Nwaka, and J. M. Thevelein.** 1999. Opposite roles of trehalase activity in heat-shock recovery and heat-shock survival in Saccharomyces cerevisiae. Biochem. J. **343:**621–626.
60. **Wiederrecht, G., D. Seto, and C. S. Parker.** 1988. Isolation of the gene encoding the S. cerevisiae heat shock transcription factor. Cell **54:**841–853.
61. **Winkler, K., I. Kienle, M. Burgert, J. C. Wagner, and H. Holzer.** 1991. Metabolic regulation of the trehalose content of vegetative yeast. FEBS Lett. **291:**269–272.
62. **Zähringer, H., M. Burgert, H. Holzer, and S. Nwaka.** 1997. Neutral trehalase Nth1p of Saccharomyces cerevisiae encoded by the NTH1 gene is a multiple stress responsive protein. FEBS Lett. **412:**615–620.
63. **Zancan, P., and M. Sola-Penna.** 2005. Trehalose and glycerol stabilize and renature yeast inorganic pyrophosphatase inactivated by very high temperatures. Arch. Biochem. Biophys. **444:**52–60.