Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5301–5306, May 1996
Microbiology

Heat-shock protein 104 expression is sufficient for thermotolerance in yeast

(chaperones/stress tolerance/heat-shock factor/prion)

SUSAN LINDQUIST* AND GINA KIM

Howard Hughes Medical Institute and Department of Molecular Genetics and Cell Biology, The University of Chicago, 5841 South Maryland Avenue, MC 1028, Chicago, IL 60637

Communicated by Dan L. Lindsley, University of California, San Diego, La Jolla, CA, January 11, 1996 (received for review September 29, 1995)

ABSTRACT In all organisms, mild heat pretreatments induce tolerance to high temperatures. In the yeast *Saccharomyces cerevisiae*, such pretreatments strongly induce heat-shock protein (Hsp) 104, and *hsp104* mutations greatly reduce high-temperature survival, indicating Hsp104 plays a critical role in induced thermotolerance. Surprisingly, however, a heat-shock transcription factor mutation (*hsfl-m3*) that blocks the induction of Hsps does not block induced thermotolerance. To resolve these apparent contradictions, we reexamined Hsp expression in *hsfl-m3* cells. Hsp104 was expressed at a higher basal level in this strain than in other *S. cerevisiae* strains. Moreover, whereas the *hsfl-m3* mutation completely blocked the induction of Hsp26 by heat, it did not block the induction of Hsp104. *HSP104* could not be deleted in *hsfl-m3* cells because the expression of heat-shock factor (and the viability of the strain) requires nonsense suppression mediated by the yeast prion [*PSI*$^+$], which in turn depends upon Hsp104. To determine whether the level of Hsp104 expressed in *hsfl-m3* cells is sufficient for thermotolerance, we used heterologous promoters to regulate Hsp104 expression in other strains. In the presence of other inducible factors (with a conditioning pretreatment), low levels of Hsp104 are sufficient to provide full thermotolerance. More remarkably, in the absence of other inducible factors (without a pretreatment), high levels of Hsp104 are sufficient. We conclude that Hsp104 plays a central role in ameliorating heat toxicity. Because Hsp104 is nontoxic and highly conserved, manipulating the expression of Hsp100 proteins provides an excellent prospect for manipulating thermotolerance in other species.

When organisms are exposed to moderately elevated temperatures, a small group of proteins, known as the heat-shock proteins (Hsps), are induced (1). These proteins play important roles in helping cells cope with the toxic effects of high temperatures, but their individual patterns of expression and their specific molecular functions are diverse. Some Hsps are expressed at normal temperatures and are only modestly induced by heat. In the yeast *Saccharomyces cerevisiae*, two such proteins, Hsp90 and Hsp70, are essential at all temperatures and are required at higher concentrations for growth at higher temperatures (2, 3). In contrast, Hsp104 is expressed at a very low level at normal temperatures and is very strongly induced by heat. It is not required for growth at any temperature, but it plays a vital role in helping cells survive short exposures to extreme temperatures.

When *hsp104* mutants are given a short pretreatment (30 min at 37°C) followed by a severe heat shock (50°C), they initially exhibit some induced thermotolerance, but after just a few minutes, they begin to die at 100 to 1000 times the rate of wild-type cells (4). Hsp104 is required for survival after heat shock to promote the resolubilization and reactivation of proteins that have unfolded and aggregated after exposure to high temperatures (5). Other Hsps have different but related functions. Hsp70 and Hsp90, for example, bind to unfolded proteins, maintain them in a soluble state, and prevent aggregation (1, 6, 7). Yet other Hsps help to usher unfolded proteins along the degradation pathway (1).

The common signal for Hsp induction is believed to be the presence of unfolded proteins, so that the trigger that induces the expression of Hsps is directly related to the function of Hsps (8, 9). In eukaryotic cells, conditions that cause proteins to unfold activate a preexisting transcription factor known as heat-shock factor (HSF; ref. 10), which binds to heat-shock elements in the 5′ regions of Hsp genes. Multiple HSF genes occur in humans, mice, and chickens (9), but in the yeast *S. cerevisiae*, HSF is encoded by a single, essential gene. Yeast HSF is required for the constitutive expression of Hsps, as well as for their induction by heat (11, 12).

Although many studies have established a relationship between the induction of Hsps and the acquisition of thermotolerance (1), several contradictions remain. Perhaps the most compelling contradiction involves an HSF mutant in *S. cerevisiae*, *hsfl-m3*. Using an heat-shock element-regulated *lacZ* gene to monitor the heat-shock response, no increase in β-galactosidase activity was detected with heat treatment in this mutant. Furthermore, when radiolabeled amino acids were employed to monitor protein synthesis, no induction of Hsps was observed. Surprisingly, however, the induction of thermotolerance was unimpaired in *hsfl-m3* cells. These results led to the suggestion that acquired thermotolerance is mediated by factors other than the induction of Hsps (13).

Recent work on trehalose provides an example of such a factor. Trehalose is a disaccharide that changes the solvent properties of the fluid phase and reduces the denaturation of proteins at high temperatures (14, 15). Its synthesis is stimulated by heat shock, primarily through the activation of preexisting trehalose synthase (16, 17). Mutations that block trehalose synthesis sharply reduce thermotolerance (18), demonstrating that trehalose plays an important role in acquired thermotolerance in *S. cerevisiae*. Because the synthesis of trehalose does not depend upon the induction of new proteins, this thermotolerance mechanism should still be operating in *hsfl-m3* cells.

Nevertheless, although trehalose plays an important role in thermotolerance, it does not substitute for Hsp104. Hsp104-deficient cells are fully capable of synthesizing trehalose, yet have 100- to 1000-fold less thermotolerance than wild-type cells (4, 17, 19). Thus, the puzzling observation with *hsfl-m3* cells is not that they are able to acquire some thermotolerance, but that they are able to acquire the same level of thermotolerance as wild-type cells.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Hsp, heat-shock protein; HSF, heat-shock factor.

*To whom reprint requests should be addressed. e-mail: S-Lindquist@uchicago.edu.

To achieve a better understanding of the relationship between thermotolerance and the induction of Hsps, in particular of Hsp104, we first examined Hsp expression in the *hsf1-m3* mutant and found that Hsp104 is expressed at an unexpectedly high level. We then asked whether enhanced expression of Hsp104 alone is sufficient to provide thermotolerance and determined the threshold levels of Hsp104 required for its acquisition. Finally, we found that the thermoprotective effects of Hsp104 were greatly enhanced by conditioning pretreatments at elevated temperatures.

MATERIALS AND METHODS

Yeast Strains and Media. The strains used in this study are listed in Table 1. MYY290 (wild-type) and MYY385 (the *hsf1-m3* mutant) were kindly provided by M. Yaffe (13). Strains W303 (wild-type) and W303Δ104 (*hsp104* deletion) were described (4, 19). To produce the strain that expresses Hsp104 in response to estrogen, ER-gal104/W303Δ104 cells were transformed by electroporation with pYSGal104 and pHCA/GAL4(1–93)ER.VP16. The control strain, ER-gal, was transformed with pYSGal and pHCA/GAL4(1–93)ER.VP16. pYSGal was constructed by inserting the *Bam*HI–*Hin*dIII fragment of the *GAL1–10* promoter into pRS316 (URA3, CEN4, ARS6; ref. 20). To produce pYSGal104, a fragment containing 58 bp of the sequence 5′ of the *HSP104* translation initiation site, the entire *HSP104* coding sequence, and 1.2 kb of downstream sequence was inserted into the *Bam*HI site of pYSGal. Plasmid pHCA/GAL4(1–93)ER.VP16 encodes a chimeric transcription factor consisting of the DNA-binding domain of Gal4 fused to the hormone-binding domain of the human estrogen receptor and the transcription-activation domain of VP16 (21).

Cells were grown in rich yeast extract/peptone/dextrose medium (YPD; containing 1% yeast extract, 2% bactopeptone, 2% glucose, and 40 mg of adenine sulfate per liter) or in synthetic glucose (S-Glu; containing 0.7% yeast nitrogen base without amino acids, 0.5% ammonium sulfate, and 2% glucose with essential amino acids and nucleotides) to mid-logarithmic phase ($3–7 \times 10^6$ cells per ml for YPD and $1–3 \times 10^6$ cells per ml for S-Glu). Because thermotolerance, trehalose, and Hsp104 are induced as cells approach stationary phase, all cultures were maintained in early- or mid-logarithmic phase for at least 15 hr before each experiment by diluting fresh overnight cultures to the appropriate density and growing them overnight into mid-logarithmic phase. β-Estradiol (Sigma) was added at the indicated concentrations from a 0.1 mM stock in ethanol.

Protein Extraction and Immunological Analysis. For heat pretreatments, a portion of each culture was transferred to a 37°C water bath in a preheated Erlenmeyer flask and maintained at that temperature, with vigorous aeration, for the indicated period. Control and hormone-induced cultures were maintained at 25°C with vigorous aeration. Cells were collected by centrifugation, and proteins from equal numbers of cells were extracted by glass bead lysis in ethanol. After electrophoretic separation on SDS/polyacrylamide gels (7.5% or 12.5%), proteins were transferred to Immobilon membranes (Amersham) and stained with Coomassie blue to assess equal loading. Membranes were blocked, washed, and incubated with antisera specific for the Hsp70 Ssa subfamily (gift of E. A. Craig, University of Wisconsin), Hsp26 (22), or Hsp104 (23). Immune complexes were visualized by reaction with protein A–peroxidase conjugate (Boehringer Mannheim) followed by enhanced chemiluminescense (ECL; Amersham). Between each antigenic reaction, preexisting immune complexes were removed by incubating the membranes for 30 min in 2% SDS with 0.05% 2-mercaptoethanol in 1× PBS (180 g of NaCl per liter/23 g of Na_2HPO_4 (dibasic anhydrous) per liter/4 g of KH_2PO_4 (monobasic) per liter/4 g of KCl per liter/0.02% thimerosol).

Thermotolerance Assays. Cultures incubated as described above were sonicated briefly to dissociate any cell aggregates. Equal portions of the culture were transferred to glass tubes and incubated in a 50°C water bath for the indicated periods as described (24). After incubation at 50°C, tubes were transferred to ice, diluted serially (5-fold at each step) into a microtiter plate, and transferred to YPD agar plates with a 48-pin replicator (V & P Scientific, San Diego). Plates were photographed after 2 days of incubation at 25°C.

Table 1. Strains used in this study

Strain	Relevant Genotype
MYY290	*MAT***a** *his3 leu2 ura3*
MYY385	*MAT***a** *leu2 ura3 hsf1-m3*
W303	*MAT***a** *can1-100 ade2-1 his3-11, 15 LEU2 trp1-1 ura3-1*
W303Δ104	*MAT***a** *can1-100 ade2-1 his3-11, 15 leu2-3, 12 trp1-1 ura3-1 Δhsp104::LEU2*
ER-gal	*MAT***a** *can1-100 ade2-1 his3-11, 15 leu2-3 trp1-1 ura3-1 Δhsp104::LEU2* (pYSGal)(pHCA/GAL4(1-93) ER.VP16)
ER-gal104	*MAT***a** *can1-100 ade2-1 his3-11, 15 leu2-3 trp1-1 ura3-1 Δhsp104::LEU2* (pYSGal104)(pHCA/GAL4 (1-93)ER.VP16)

RESULTS

Hsp104 Expression in *hsf1-m3* Cells. Hsp expression was examined during logarithmic phase growth at 25°C and after a tolerance-inducing heat treatment (30 min at 37°C) in the same mutant HSF strain (*hsf1-m3*, MYY385; ref. 13) that had been the subject of previous thermotolerance studies. Its wild-type parent (MYY290), our standard lab strain (W303), and an isogenic *hsp104* deletion strain (W303Δ104) provided a basis of comparison. When total cellular proteins were electrophoretically separated, transferred to filters, and stained with Coomassie blue (Fig. 1*A*), all samples exhibited similar quantities of protein. The proteins were then reacted with antiserum specific for the major Hsps of *S. cerevisiae*.

With an Hsp26 antibody, strong reaction was observed in heat-shocked samples for each of the control strains but not for the *hsf1-m3* mutant (Fig. 1*B*). Thus, as expected, heat-induced Hsp expression in this strain is severely curtailed. Surprisingly, however, the HSF mutant exhibited a substantial level of Hsp104 expression after heat shock (Fig. 1*C*). Several features of the strain seem to contribute to this expression. First, in the MYY290 background (Fig. 1*C*, *Right*), Hsp104 was constitutively expressed at a slightly higher level than in the W303 background (Fig. 1*C*, *Left*). Note that the heat-inducible cytosolic Hsp70 proteins of *S. cerevisiae* (Ssa proteins) were, if anything, expressed at a lower level in the MYY290 background. Second, constitutive expression of Hsp104 was higher in the HSF mutant derivative (MYY385) than in its wild-type parent (MYY290). Third, the induction of Hsp104 at 37°C was reduced in *hsf1-m3* cells, but it was not completely eliminated. Indeed, after a full hour at 37°C, the length of pretreatment employed in previous thermotolerance studies with this mutant (13), Hsp104 was expressed at a level approaching that of our standard wild-type strain under identical conditions (W303; Fig. 1*C*). We do not know why radiolabeling of *hsf1-m3* cells previously failed to detect the expression of Hsp104 (13), but our observations on the accumulation of Hsp104 in these cells were clear and reproducible. Expression of Hsp104 in *hsf1-m3* cells must bypass some aspect of the regulatory pathway that dominates the expression of Hsp26.

We hypothesized that the unexpectedly high levels of Hsp104 synthesis in the *hsf1-m3* mutant could account for this strain's ability to acquire normal levels of thermotolerance. To provide a direct test, we attempted to delete the Hsp104 gene in this strain. Repeated attempts to obtain the transformants

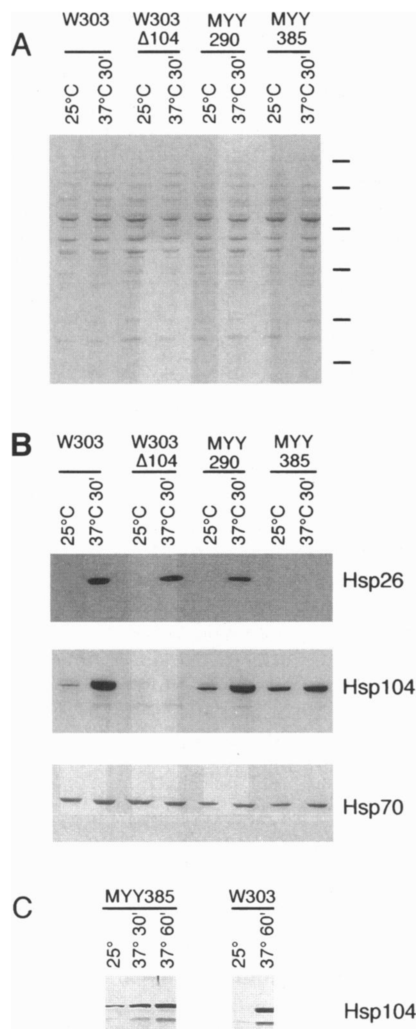

FIG. 1. Hsp104 is constitutively expressed and heat-inducible in the *hsf1-m3* mutant. (*A*) W303 (wild-type), W303Δ104, MYY290 (wild-type), and MYY385 (*hsf1-m3* mutant) cells grown to mid-logarithmic phase at 25°C were maintained at 25°C or shifted to 37°C for 30 min. Total proteins separated by SDS/PAGE were transferred to an Immobilon membrane and stained with Coomassie blue. Positions of molecular mass markers are indicated (–), and the positions from the top are as follows: 97.4, 69, 46, 30, 21.5, and 14.3 kDa. (*B*) Proteins on the blot depicted in *A* were reacted with antisera specific for Hsp26, Hsp104, and Hsp70(Ssa). Immune complexes were visualized with the ECL reagent (Amersham). (*C*) Cells were grown as in *A*, except that additional samples were maintained at 37°C for 60 min. Proteins were reacted with Hsp104 antiserum only.

failed, while control transformations with other strains succeeded. Attempts to introduce the *hsp104* mutation into these cells by mating were also problematic. We were able to introduce the *hsp104* mutation into the parental background and to change its mating type with the HO plasmid (25), but when this modified parental strain was mated to the HSF mutant, sporulation efficiency and spore viability in the resulting diploid were very low. Finally, to examine the possibility that the *HSP104* gene had become essential in the *hsf1-m3* background, we attempted to transform the strain with an extrachromosomal copy of the *HSP104* to provide a protected background in which to produce an *hsp104* chromosomal disruption. However, we were unable to recover *hsf1-m3* transformants with an extrachromosomal copy of *HSP104*, whereas control transformants with the plasmid alone were readily obtained. A possible explanation for the baffling behavior of the *hsf1-m3* strain in these experiments is provided in the *Discussion*.

Hsp104 Provides Thermotolerance in the Absence of a Heat Pretreatment. To determine whether the increased levels of Hsp104 expressed by *hsf1-m3* cells could account for their normal ability to acquire thermotolerance, we asked whether similar quantities of Hsp104 were sufficient for thermotolerance in another strain. To this end, *HSP104* coding sequences were placed under the control of a hormone-regulated expression system. This system allowed us to precisely vary the expression of Hsp104 over a broad range and to avoid the complications of other induction systems that involve changes in carbon source (19). It also allowed us to determine if Hsp104 is able to provide thermotolerance in the absence of other heat-induced tolerance factors.

W303 cells carrying a deletion of *HSP104* were transformed with (*i*) a constitutive expression plasmid encoding a chimeric transcription factor that is activated by estrogen and binds to the *GAL1* promoter and (*ii*) an inducible plasmid with *HSP104* coding sequences under the control of the *GAL1* promoter (21). To control for the possible effects of estrogen and of the chimeric transcription factor on thermotolerance, a control strain was constructed with the same plasmids, except that the *GAL1* plasmid did not contain Hsp104 coding sequences.

As shown in Fig. 2, when β-estradiol (100 nM) was added to the medium, Hsp104 was detected in cells containing the full expression system (ER-gal104) but not in cells containing the control constructs (ER-gal). On longer exposures, a low level of Hsp104 expression could be detected in ER-gal104 cells in the absence of hormone, but none could be detected in ER-gal cells. In separate experiments (data not shown), total protein

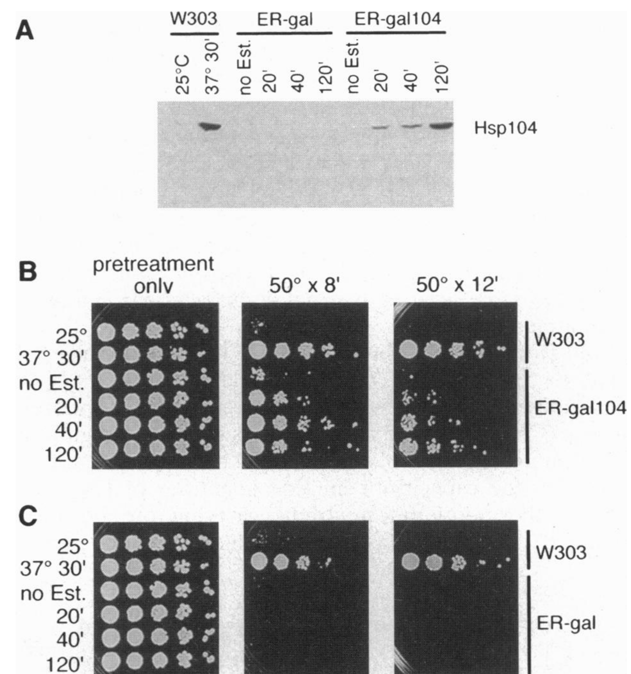

FIG. 2. Hsp104 expressed from an estrogen-regulated plasmid provides thermotolerance in the absence of a conditioning heat treatment. (*A*) The induction of Hsp104 in response to β-estradiol. W303Δ104 cells carrying the complete estrogen-regulated Hsp104 expression system (ER-gal104) or matching control constructs lacking Hsp104 coding sequences (ER-gal) were grown at 25°C to mid-logarithmic phase and treated with β-estradiol (100 nM) at 25°C for 0, 20, 40, or 120 min. Wild-type W303 cells were grown to mid-logarithmic phase at 25°C and maintained at that temperature or shifted to 37°C for 30 min. Proteins were analyzed as in Fig. 1*A*. (*B* and *C*) The induction of thermotolerance in response to β-estradiol. Cultures treated as in *A* were transferred to β-estradiol-free medium, and aliquots were shifted to 50°C for 0, 8, or 12 min. Cells were placed on ice, serially diluted (5-fold at each step), and spotted onto agar plates to measure colony-forming units.

concentrations were measured, and Hsp104 levels were analyzed on gels that also contained serial dilutions of purified Hsp104 (26). After 120 min, Hsp104 accounted for ≈0.2% of total protein, approximately the same concentration observed in wild-type cells after a 30-min heat treatment at 37°C (Fig. 2*A*).

To assay thermotolerance, cells were resuspended in hormone-free medium, and the number of colony-forming units in cultures maintained at 25°C was compared with those in cultures heat-shocked at 50°C (Fig. 2 *B* and *C*). Wild-type W303 cells, with or without a conditioning pretreatment (37°C at 30 min), provided a thermotolerance standard. Because small fluctuations in water bath temperatures can produce substantial changes in survival, all cultures in a given experiment were subjected to heat shocks in the same water bath at the same time. Although absolute levels of survival varied somewhat from experiment to experiment, the relative levels of survival obtained with different strains and different treatments were extremely reproducible.

All cultures that were not exposed to 50°C (Fig. 2 *B* and *C*, *Left*) exhibited the same number of colony-forming units. Thus, neither the heat pretreatment nor the hormone pretreatment caused any loss of viability. After exposure to 50°C, wild-type cells showed much higher levels of survival with the heat pretreatment than without it. Cells that had been induced with estradiol (ER-gal104) to express Hsp104 and had not been given a pretreatment also showed increased survival. In this case, levels of survival depended upon the length of exposure to estradiol and correlated with the quantity of Hsp104 induced. No increase in survival was observed in cells carrying the control plasmids (ER-gal). A similar dose dependence of survival on Hsp104 expression was observed when all cells were exposed to hormone for 2 hr but at varying concentrations (Fig. 3).

Elsewhere we have shown that even very high levels of Hsp104 expression cause no detectable change in the expression of any other cellular proteins, including other Hsps (27). Thus, increased survival in ER-gal104 cells compared to ER-gal cells can be attributed to the increased expression of Hsp104. Remarkably, induction of Hsp104 alone provided nearly the same level of thermotolerance as a conditioning pretreatment.

Mild Heat Pretreatments Potentiate Thermotolerance Provided by Hsp104. The *hsfl-m3* mutant expresses Hsp104 constitutively at a modest level, yet a conditioning pretreatment at 37°C is required for these cells to achieve high levels of thermotolerance. To determine if mild heat treatments potentiate the capacity of limiting quantities of Hsp104 to provide thermotolerance, strains carrying the hormone-regulated expression plasmids were induced with estradiol (100 nM) for 40 min, to provide partial thermotolerance and a low level of Hsp104 expression (Fig. 4). One portion of the culture was maintained at 25°C. Other portions were given a 30-min conditioning pretreatment at 37°C, either during the hormone treatment (D) or immediately thereafter (A).

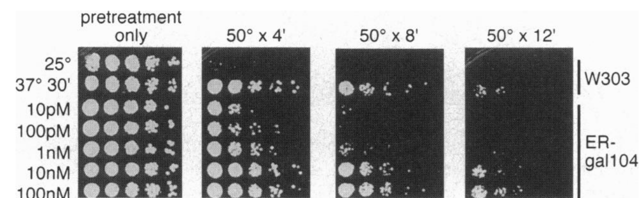

FIG. 3. In cells containing the estrogen-regulated Hsp104 expression system, thermotolerance depends upon the concentration of β-estradiol. W303Δ104 cells carrying the complete estrogen-regulated Hsp104 expression system (ER-gal104) were grown to mid-logarithmic phase at 25°C and maintained at 25°C with the addition of β-estradiol at a concentration of 10 pM, 100 pM, 1 nM, 10 nM, or 100 nM for 2 hr. Wild-type W303 cells were grown to mid-logarithmic phase at 25°C and maintained at that temperature or shifted to 37°C for 30 min to induce thermotolerance. Cells treated with β-estradiol were transferred to β-estradiol-free medium and thermotolerance assays were performed as in Fig. 2 *B* and *C*).

As expected, the heat pretreatments produced a low but reproducible increase in thermotolerance in cells that did not express Hsp104 (ER-gal). In cells expressing low levels of Hsp104 (ER-gal104), the same pretreatments increased survival by ≈500-fold. Thus, in the presence of other heat-inducible factors, even a low concentration of Hsp104 (less than the concentration observed in the *hsfl-m3* mutant) is sufficient to provide high levels of thermotolerance.

DISCUSSION

Our data demonstrate that Hsp104 plays a central role in helping yeast cells survive short exposures to extreme heat (1). Previously, one of the most compelling arguments against this hypothesis had been the ability of cells carrying a mutation in the heat-shock transcription factor, *hsfl-m3*, to acquire wild-type levels of induced thermotolerance (13). Contrary to expectation, we find that this mutant constitutively expresses Hsp104 at higher levels than wild-type cells. Furthermore, the mutation reduces, but does not block, the induction of Hsp104 by heat. Using a heterologous promoter to express Hsp104 at different levels, we find that even a low level of Hsp104 is sufficient to provide wild-type levels of thermotolerance when cells are given conditioning pretreatments. Thus, the expression of Hsp104 in *hsfl-m3* cells is sufficient to account for the strain's ability to acquire wild-type levels of thermotolerance.

Repeated attempts to delete the *HSP104* gene in *hsfl-m3* cells and to directly test the role of Hsp104 in the thermotolerance of this strain failed. Surprisingly, we were also unable to obtain *hsfl-m3* transformants that simply carried an extrachromosomal copy of *HSP104*. Recent information on the nature of the *hsfl-m3* mutation (M. Yaffe, personal communication) and the functions of Hsp104 (28) provide an interesting explanation. The *hsfl-m3* mutation is a single nucleotide substitution that introduces a stop codon in the middle of HSF. Because HSF is an essential protein, this mutation should be lethal. However, the mutant is derived from a strain that carries [*PSI*$^+$], an extrachromosomal suppressor that causes translational misreading at all three nonsense codons. This results in the production of a full-length HSF, with a missense mutation, that provides sufficient function for normal growth but does not permit transcription from heat-shock elements at high temperatures. [*PSI*$^+$] has recently been shown to be a yeast prion protein—specifically, an altered from of the translation termination factor Sup35. Remarkably, the maintenance of this altered form of Sup35 depends upon an intermediate level of Hsp104 expression. Deletion of *HSP104* eliminates [*PSI*$^+$]-mediated suppression as does the presence of even a single-copy extrachromosomal plasmid of *HSP104* (28). Thus, in *hsfl-m3* cells, which appear to depend upon PSI-mediated suppression for viability, Hsp104 must be maintained at a specific, intermediate level. Both deletion of *HSP104* and transformation with an extrachromosomal plasmid of *HSP104* should be lethal.

Not only is Hsp104 constitutively expressed at a higher-than-normal level in *hsfl-m3* cells, its induction by heat is only partially inhibited. In contrast, the induction of Hsp26 was completely blocked. Thus, the induction of individual Hsps does not depend upon HSF to the same extent. New findings also provide an explanation for this phenomenon. Yeast cells contain a distinct stress transcription factor, MSN2, that induces transcription from STRE promoter elements in response to heat, heavy metal ions, and UV light (ref. 29; A. Schmitt and K. McEntee, personal communication). The region upstream of the *HSP104* coding sequence contains

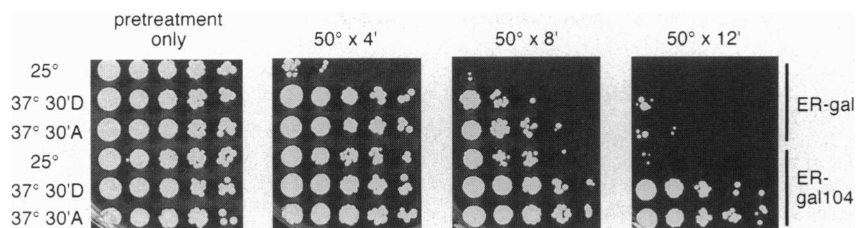

FIG. 4. Conditioning heat treatments enhance the thermotolerance provided by a limiting concentration of Hsp104. W303Δ104 cells carrying the complete estrogen-regulated Hsp104 expression system (ER-gal104) or matching control constructs (ER-gal) were grown to mid-logarithmic phase at 25°C and treated with β-estradiol at a concentration of 100 nM for 40 min. One portion of the culture was maintained at 25°C, one was shifted to 37°C during (D) the last 30 min of hormone treatment, and a third was maintained at 25°C during hormone treatment and transferred to 37°C for 30 min after (A) being transferred to hormone-free medium. Thermotolerance assays were performed as in Fig. 2 *B* and *C*.

three matches to the STRE element (AGGGG) within ≈250 nucleotides of upstream sequence (30). The upstream region of *HSP26* contains only one match, ≈350 nucleotides from the coding sequence, and this lies within the coding sequence of another gene. Hsp104, but not Hsp26, is induced by UV light, indicating STRE elements are likely to contribute to Hsp104 expression (T. McGhee and K. McEntee, personal communication).

Using a heterologous promoter to express Hsp104, we found that the thermotolerance provided by low levels of Hsp104 is enhanced by moderate heat pretreatments. These treatments might affect Hsp104's activity in many ways, including a change in its structure, its interaction with partner proteins or substrates, and its localization within the cell. The pretreatments probably also enhance thermotolerance in a manner that is completely extrinsic to Hsp104. The induction of other Hsps and of trehalose, for example, would be expected to reduce heat damage to levels where limited quantities of Hsp104 are sufficient to cope with it.

Perhaps our most surprising finding is that high levels of Hsp104 expression (from a heterologous promoter) can provide thermotolerance in the absence of a conditioning pretreatment. This thermotolerance is likely to result from a direct effect of Hsp104 on heat-sensitive targets. Others have shown that Hsp104 has no effect on the accumulation of trehalose (17). And we have found that even very high constitutive expression of Hsp104, from a glyceraldehyde-3-phosphate dehydrogenase promoter on a 2-μ vector, has no detectable effect on the expression of other yeast proteins, including other Hsps (27). High constitutive expression-Hsp104 also increased survival in cells shifted directly to high temperatures (unpublished data), albeit not as much as β-estradiol-induced expression of Hsp104 (50-fold rather than 500-fold higher survival than wild-type cells; data not shown). It may be that constitutively expressed Hsp104 is less active than newly synthesized protein. Alternatively, other factors induced by the chimeric ER-gal transcription factor may enhance Hsp104's activity in thermotolerance. Note, however, that control cells expressing the same transcription factor, without an *HSP104* expression plasmid, exhibited no thermotolerance when exposed to β-estradiol. Thus, the increased thermotolerance of cells expressing Hsp104 can be attributed specifically to Hsp104. This leads to two important conclusions concerning the role of Hsp104 in stress tolerance.

First, the ability of Hsp104 to increase survival in the absence of other heat-induced thermotolerance factors indicates this protein must act on the most critical heat-induced lesions. Because heat treatments perturb a multitude of cellular processes, the critical lethal lesions induced by heat are not known (1, 31, 32). However, if Hsp104 protected or rescued only a small subset of the critical targets, deletion of Hsp104 might reduce thermotolerance, but overexpression would not provide it. Second, because the primary function of Hsp104 is to act on proteins damaged by heat (5), our observations indicate that under those conditions where Hsp104 alone can provide tolerance, the primary lethal lesions must involve protein damage. A similar conclusion based on theoretical, thermodynamic arguments was reached previously (33).

High levels of Hsp104 expression have little, if any, toxic effect on the growth of yeast cells (26). Moreover, the thermotolerance functions of Hsp104 are highly conserved; expression of the Hsp100 protein of *Arabadopsis thaliana*, AT101, provides thermotolerance in ΔHsp104 yeast cells (34), and the Hsp100 proteins of bacteria are important in both heat and salt tolerance (35, 36). We suggest that increasing the expression of Hsp100 proteins may provide a simple, general mechanism for increasing basal thermotolerance in many species.

We thank Dr. M. Yaffe for the gift of strains MYY290 and MYY385, Drs. M. Yaffe and K. McEntee for their generosity in sharing unpublished data, John Taulien for technical assistance, Dr. Y. Sanchez for construction of pYS-Gal104, Dr. E. Craig for the gift of Ssa antiserum, Drs. D. Nathan, E. Schirmer, and J. Chang for assistance and advice on procedures, and Dr. J. Glover for comments on the manuscript. This work was supported by the Howard Hughes Medical Institute and National Institutes of Health Grant GM25874.

1. Parsell, D. A. & Lindquist, S. (1993) *Annu. Rev. Genet.* **27**, 437–496.
2. Borkovich, K. A., Farrelly, F. W., Finkelstein, D. B., Taulien, J. & Lindquist, S. (1989) *Mol. Cell. Biol.* **9**, 3919–3930.
3. Craig, E. A. & Jacobsen, K. (1984) *Cell* **38**, 841–849.
4. Sanchez, Y. & Lindquist, S. L. (1990) *Science* **248**, 1112–1115.
5. Parsell, D. A., Kowal, A. S., Singer, M. A. & Lindquist, S. (1994) *Nature (London)* **372**, 475–478.
6. Jakob, U., Lilie, H., Meyer, I. & Buchner, J. (1995) *J. Biol. Chem.* **270**, 7288–7294.
7. Craig, E. A., Baxter, B. K., Becker, J., Halladay, J. & Ziegelhoffer, T. (1994) in *The Biology of Heat Shock Proteins and Molecular Chaperones*, eds. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab. Press, New York), pp. 31–52.
8. Craig, E. A. & Gross, C. A. (1991) *Trends Biochem. Sci.* **16**, 135–140.
9. Morimoto, R.I., Jaravich, D. A., Kroeger, P. E., Mathur, S. K., Murphy, S. P., Nakai, A., Sarge, K., Abravaya, K. & Sistoren, L. T. (1994) in *The Biology of Heat Shock Proteins and Molecular Chaperones*, eds. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab. Press, New York), pp. 417–456.
10. Lis, J. & Wu, C. (1993) *Cell* **74**, 1–4.
11. Sorger, P. K. & Pelham, H. R. (1988) *Cell* **54**, 855–864.
12. Wiederrecht, G., Seto, D. & Parker, C. S. (1988) *Cell* **54**, 841–853.
13. Smith, B. J. & Yaffe, M. P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11091–11094.
14. Hottiger, T., De, V. C., Hall, M. N., Boller, T. & Wiemken, A. (1994) *Eur. J. Biochem.* **219**, 187–193.
15. Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D. & Somero, G. N. (1982) *Science* **217**, 1214–1222.
16. Beck, C. A. & Edwards, D. P. (1992) *Biochem. J.* **288**, 859–864.
17. Winkler, K., Kienle, I., Burgert, M., Wagner, J. C. & Holzer, H. (1991) *FEBS Lett.* **291**, 269–272.
18. De Virgilio, C., Hottiger, T., Dominguez, J., Boller, T. & Wiemken, A. (1994) *Eur. J. Biochem.* **219**, 179–186.
19. Sanchez, Y., Taulien, J., Borkovich, K. A. & Lindquist, S. (1992) *EMBO J.* **11**, 2357–2364.
20. Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122**, 19–27.

21. Louvion, J.-F., Havaux-Copf, B. & Picard, D. (1993) *Gene* **131,** 129–134.
22. Rossi, J. M. & Lindquist, S. (1989) *J. Cell. Biol.* **108,** 425–439.
23. Parsell, D. A., Sanchez, Y., Stitzel, J. D. & Lindquist, S. (1991) *Nature (London)* **353,** 270–273.
24. Petko, L. & Lindquist, S. (1986) *Cell* **45,** 885–894.
25. Herskowitz, I. & Jensen, R. E. (1991) in *Guide to Yeast Genetics and Molecular Biology: Methods in Enzymology*, eds. Guthrie, C. & Fink, G. R. (Academic, San Diego), pp. 132–146.
26. Parsell, D. A., Kowal, A. S. & Lindquist, S. (1994) *J. Biol. Chem.* **269,** 4480–4487.
27. Vogel, J. L., Parsell, D. A. & Lindquist, S. (1995) *Curr. Biol.* **5,** 306–317.
28. Chernoff, Y. O., Lindquist, S. L., Ono-B-i., Inge-Vechtomov, G. & Liebman, S. W. (1995) *Science* **268,** 880–883.
29. Kobayashi, N. & McEntee, K. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6550–6554.
30. Mager, W. H. & Kruijff, A. J. J. (1995) *Microbiol. Rev.* **59,** 506–531.
31. Nover, L., ed. (1991) *Heat Shock Response.* (CRC, Boca Raton, FL).
32. Laszlo, A. (1992) *Cell Prolif.* **25,** 59–87.
33. Rosenberg, B., Kemeny, G., Switzer, R. C. & Hamilton, T. C. (1971) *Nature (London)* **232,** 471–473.
34. Schirmer, E. C., Lindquist, S. & Vierling, E. (1994) *Plant Cell* **6,** 1899–1909.
35. Squires, C. L., Pedersen, S., Ross, B. M. & Squires, C. (1991) *J. Bacteriol.* **173,** 4254–4262.
36. Kruger, E., Volker, U. & Hecker, M. (1994) *J. Bacteriol.* **176,** 3360–3367.