Regulation of chaperone gene expression by heat shock transcription factor in *Saccharomyces cerevisiae*: Importance in normal cell growth, stress resistance, and longevity

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**A B S T R A C T**

Heat shock transcription factor (HSF), a key regulator in the expression of heat shock protein (HSP) chaperones, is involved in the maintenance of protein homeostasis. However, the impact of HSF-mediated transcription of each HSP gene on this process is not fully understood. We show that *Saccharomyces cerevisiae* cells containing mutations in the HSP90 promoters exhibit various phenotypes, including slow growth, proteotoxic stress sensitivity, and reduced chronological lifespan. Similar phenotypes were observed when HSF-binding sequences in five mitochondrial HSP promoters were mutated. Therefore, HSF-regulated changes in expression of these chaperone genes are necessary to maintain cell viability under various growth conditions.

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1. Introduction

The heat shock response, an evolutionarily conserved stress response system, is triggered by proteotoxic stress, including heat, ethanol, reactive oxygen species, heavy metals, and other toxic substances. Eukaryotic heat shock transcription factor (HSF), a central player in this system, induces the expression of a group of proteins called heat shock proteins (HSPs), many of which function as molecular chaperones [1]. HSF in its trimeric form regulates the transcription of target genes, via binding to heat shock elements (HSEs) that consist of at least three inverted repeats of the 5-bp sequence NGAAN. Recent investigations have revealed that HSF participates not only in the heat shock response but also in the processes of cell differentiation, development, and aging [2].

Molecular chaperone proteins prevent the formation of non-specific protein aggregates and assist proteins in the acquisition of their native structures [1,3]. Chaperonin encapsulates non-native proteins of molecular sizes ranging up to 60 kDa and mediates the folding of proteins into the native state. Hsp70 participates in a large number of protein-folding processes. Under physiological conditions, Hsp70 is involved in the de novo folding of proteins and the translocation of proteins across membranes, and under stress conditions, it solubilizes protein aggregates and refolds aggregated proteins. Hsp90 has a function similar to those of chaperonin and Hsp70; however, it appears to be necessary for the conformational regulation of only a limited set of substrates. Hsp100 is involved in solubilization of protein aggregates and degradation of proteins. In addition, a family of small HSPs displays further variation in the regulation of protein homeostasis.

In the yeast *Saccharomyces cerevisiae*, Hsf1 regulates the transcription of approximately 3.0% of the loci in the yeast genome [4,5]. The target genes encode molecular chaperones and other proteins implicated in a broad range of biological functions, including maintenance of cell integrity, energy generation, carbohydrate metabolism, cell signaling, and transcription. Constitutive and stress-induced activity of Hsf1 is essential for growth under physiological conditions as well as thermal- and oxidative-stress conditions [6–8]. Cells containing mutations in *HSF1* exhibit phenotypes defective in mitochondrial function, cell wall integrity, and cell cycle progression [9–12].

Although Hsf1 is involved in many diverse cellular processes in yeast, the impact of Hsf1-mediated transcription of each target gene on each of these processes is unknown. To explore this issue, we constructed cells containing mutations in the HSEs of chromo-
2. Materials and methods

2.1. Yeast strains and media

The yeast strains used in this study are listed in Table 1. Strains NOY396, HS126, HS170T, and YAY22 were described previously [5,12]. Cells containing nucleotide substitutions in the HSEs of Hsf1-target promoters were constructed using the pop-in/pop-out replacement method [13]. Nucleotide sequences of the HSEs are shown in Fig. 1A. Media used were YPD (1% yeast extract, 2% polypeptone, and 2% glucose) and SD (0.67% yeast nitrogen base) medium. 

Cells grown in SD medium were inoculated into fresh SD medium. The inoculation time point was considered day 0. Total RNA was prepared from cells grown as described in the figure legends and was subjected to RT-PCR analysis as described previously [7,12]. The experiments were performed at least three times with similar results.

2.2. RT-PCR analysis

Total RNA was prepared from cells grown as described in the figure legends and was subjected to RT-PCR analysis as described previously [7,12]. The experiments were performed at least three times with similar results.

2.3. Determination of chronological lifespan

Cells grown in SD medium were inoculated into fresh SD medium. The inoculation time point was considered day −1 (minus 1). Cells were cultured at 30 °C, and aliquots from the culture were plated onto YPD plates to determine cell viability. Viability at day 0 was considered the initial percent survival [14]. For calorie-restriction experiments, cells were inoculated into water. The experiments were performed at least four times, and statistical analysis was performed using a paired t-test (Supplementary Table S1).

3. Results and discussion

3.1. Construction of cells containing mutations in HSEs of chromosomal chaperone genes

Yeast Hsp90 exists as two isoforms, encoded by the genes HSC82 and HSP82, and the presence of at least one of the genes is essential for viability [15]. We created hsc82-hse and hsp82-hse

cells, which contained mutations in the HSEs of the chromosomal HSC82 and HSP82 promoters, respectively. In addition, we created hsp90-hse cells, which contained mutations in both the HSC82 and HSP82 promoters (Fig. 1A). The results of RT-PCR analysis showed that hsc82-hse, hsp82-hse, and hsp90-hse cells were defective in heat-induced accumulation of transcripts of HSF1, HSC82, and HSP82, and both genes, respectively, although accumulation of transcripts of Hsf1 target genes SSA1 and SSA2 (encoding members of the Hsp70 family) was observed (Fig. 1B).

Five mitochondrial chaperone genes, HSP10, HSP60, HSP78, MDJ1, and SSC1, are suggested to be targets of Hsf1 [4,5]. The products encoded by HSP10 and HSP60 constitute mitochondrial chaperonin; HSP78 encodes an Hsp100-family chaperone; and SSC1 and MDJ1 encode Hsp70 and its co-chaperone, respectively [16]. When putative HSEs were mutated, heat-induced accumulation

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>NOY396</td>
<td>MATa ade2 ura3 leu2 his3 trp1 can1</td>
</tr>
<tr>
<td>HS231</td>
<td>hsp10-hse of NOY396</td>
</tr>
<tr>
<td>HS232</td>
<td>hsp60-hse of NOY396</td>
</tr>
<tr>
<td>HS233</td>
<td>hsp78-hse of NOY396</td>
</tr>
<tr>
<td>HS234</td>
<td>ssc1-hse of NOY396</td>
</tr>
<tr>
<td>HS235</td>
<td>mdj1-hse of NOY396</td>
</tr>
<tr>
<td>HS236</td>
<td>hsp10-hse hsp60-hse of NOY396</td>
</tr>
<tr>
<td>HS237</td>
<td>ssc1-hse mdj1-hse of NOY396</td>
</tr>
<tr>
<td>HS238</td>
<td>hsp10-hse hsp60-hse hsp78-hse ssc1-hse mdj1-hse of NOY396</td>
</tr>
<tr>
<td>HS270</td>
<td>hsp82-hse of NOY396</td>
</tr>
<tr>
<td>HS271</td>
<td>hsc82-hse of NOY396</td>
</tr>
<tr>
<td>HS272</td>
<td>hsp82-hse hsc82-hse of NOY396</td>
</tr>
<tr>
<td>HS126</td>
<td>MATa ade2 ura3 leu2 his3 trp1 can1 hsf1::HIS3 Ycp-uRA3-HSF1</td>
</tr>
<tr>
<td>HS170T</td>
<td>YCP-TRP1-HSF1 of HS126</td>
</tr>
<tr>
<td>YAY22</td>
<td>YCP-TRP1-hsf1-K206S-F256S of HS126</td>
</tr>
<tr>
<td>HS172</td>
<td>rim15::ADE2 YCP-TRP1-HSF1 of HS126</td>
</tr>
<tr>
<td>HS313</td>
<td>rim15::ADE2 YCP-TRP1-hsf1-K206S-F256S of HS126</td>
</tr>
</tbody>
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Fig. 1. HSE mutations in chromosomal HSP promoters. (A) Mutations in HSEs. Nucleotide sequences of the HSEs and the GAA and TTC repeats (bold uppercase letters) are shown. Mutations introduced are shown below the sequences. (B) RT-PCR analysis of HSP genes in cells exposed to heat. Cells (upper panel: strains NOY396, HS270, HS271, and HS272; lower panel: strains NOY396, HS231, HS232, HS234, HS235, and HS238) grown in SD medium at 28 °C (C) were heat shocked at 39 °C for 15 min (HS). Total RNA was prepared form the cells and subjected to RT-PCR analysis of HSP genes and control gene ACT1 (encoding actin). Right panels show constitutive mRNA levels in cells (strains NOY396, HS238, and HS272) grown in YPD medium at 30 °C.
of the corresponding HSP mRNA was diminished (Fig. 1A and B). The mRNA levels of all five genes were not heat-induced in mit-hse cells, which contained all five of the HSE mutations. The binding of Hsf1 to the mutated promoters was significantly reduced in hsp90-hse and mit-hse cells, as judged by chromatin immunoprecipitation assays (Supplementary Fig. S1). These results show that Hsf1 plays a key role in the heat shock response of these seven genes via binding to their HSEs. These cells are useful for the analysis of the impact of Hsf1-dependent transcription of each gene on growth under various conditions.

3.2. Growth of hsp90-hse and mit-hse cells at varying temperatures

Although cells containing either hsc82-hse or hsp82-hse mutations were able to grow at 37 °C, a combination of both mutations (hsp90-hse cells) caused a growth defect at this temperature (Fig. 2). This result suggests that an increase in HSP90 mRNA levels induced by Hsf1, and most likely a consequent increase in Hsp90 protein levels, is necessary for protein folding at elevated temperatures [15]. Growth of mit-hse cells was also inhibited at 37 °C; however, cells containing HSE mutations in chaperonin genes (hsp10-hse hsp60-hse), the Hsp100-family chaperone gene (hsp78-hse), or the genes for Hsp70 and its co-chaperone (ssc1-hse mdj1-hse) did not show temperature sensitivity (Fig. 2). This may reflect overlapping functions of these chaperones in the maintenance of mitochondrial protein homeostasis [16]. These results show that Hsf1 supports cell growth, in part, by inducing expression of HSP90 and mitochondrial HSP genes at elevated temperatures.

Under control growth conditions (in YPD medium at 30 °C), hsp90-hse and mit-hse cells grew slightly more slowly than wild-type cells, with doubling times of 125 and 110 min, respectively, compared with 90 min for wild-type cells. It has been shown that Hsf1 binds constitutively to target promoters and maintains basal-level transcription [4,5,17]. Compared with wild-type cells, constitutive levels of both HSC82 and HSP82 mRNAs were lower in hsp90-hse cells (see Fig. 1B). An increase in SSA1 and SSA2 mRNA levels was observed, which suggests negative regulation of Hsf1 by Hsp90 chaperones [6]. Constitutive levels of all five mitochondrial HSP mRNAs were lower in mit-hse cells. It should be noted that HSP10, HSP60, and SSC1 are essential genes. These results suggest that constitutive expression of HSP90 and mitochondrial HSP genes by Hsf1 is necessary for the maintenance of the normal growth rate.

3.3. Roles of Hsf1-regulated genes in the response to chemical stressors

The presence of azetidine-2-carboxylic acid (AZC), an amino acid analog, in the culture medium leads to the accumulation of misfolded proteins in cells [7,18]. Ethanol affects various cellular processes, including protein stability, membrane fluidity, and energy status [19]. Diamide is a thiol-oxidizing agent, and menadione is a superoxide generator [20].

As shown in Fig. 3A, the mRNA levels from HSP90 and mitochondrial HSP genes were increased with the treatment of cells with either AZC, ethanol, diamide, or menadione, or with heat. In contrast, cells with HSE mutations did not show mRNA accumulation. Therefore, Hsf1 is the major activator of stress-induced transcription of these genes.

We have shown that cells containing R206S and F256S mutations in the Hsf1 DNA-binding domain (hsf1-RF/S) are defective in the heat shock response of most Hsf1 target genes [6]. The hsf1-RI/S mutation caused a growth defect of cells on medium containing AZC or ethanol (Fig. 3B). Growth of hsp90-hse cells was severely inhibited by AZC and moderately inhibited by ethanol, while growth of mit-hse cells was moderately inhibited by AZC and severely by ethanol. Therefore, Hsf1-induced expression of HSP90 is needed to cope with proteotoxic stress caused by AZC treatment. Under ethanol-stress conditions, cells maintain energy production, leading to activation of glycolysis and mitochondrial function [19].
which suggests the importance of increased expression of mitochondrial HSP genes by Hsf1.

Growth of hsf1-RF/S cells was severely inhibited by diamide and moderately inhibited by menadione (Fig. 3B). Both hsp90-hse and mit-hse mutations caused slight sensitivity only to diamide. These results suggest that stress-induced transcription of HSP90 and mitochondrial HSP genes has a slight effect on growth under oxidative stress conditions. This also suggests that other Hsf1 target genes may be more important for managing oxidative stress.

The candidate genes are AHP1 (thioredoxin peroxidase), GTT1 (glutathione S-transferase), and CUP1 (copper metallothionein), whose products are involved in defense against oxidative stress [4,5,8].

3.4. Involvement of Hsf1-regulated genes in chronological lifespan

Yeast chronological lifespan (CLS), defined as the survival of non-dividing cells, is regulated by nutrient availability and genetic factors [21,22]. It has been shown that glucose limitation at entry into stationary phase induces gene transcription by Hsf1; in this pathway Snf1, a protein kinase playing a central role in cellular metabolism under low glucose conditions, phosphorylates Hsf1 and enhances transcription of target genes [23]. When changes in the mRNA levels were monitored along with growth, CUP1 mRNA levels, which have been shown to increase under glucose starvation conditions [23], were slightly elevated at entry into stationary phase (1.5- and 2.0-day-old cultures of Fig. 4A). HSP90 and mitochondrial HSP genes were also induced at this phase in wild-type cells; however, constitutive mRNA levels of the respective genes were lower in the hsp90-hse and mit-hse cells, and induction was not evident.

Viability of hsp90-hse cells after entry into the stationary phase decreased compared with wild-type cells, showing that constitutive and/or induced transcription of HSP90 genes by Hsf1 is necessary for CLS extension (Fig. 4B). In Caenorhabditis elegans, networks of chaperones, including Hsp90, are suggested to be important in the regulation of longevity [24]. The mit-hse mutations also caused a decrease in longevity (Fig. 4B). This is consistent with observations that mitochondrial metabolism directly controls aging [25] and suggests the importance of the mitochondrial protein homeostasis maintained by chaperones.

3.5. Regulation of chronological lifespan by Hsf1

The above results demonstrated the involvement of Hsf1 in CLS extension. Previous observations also suggested the involvement of Hsf1 in the regulation of longevity [26,27]. Consistently, hsf1-RF/S cells exhibited a reduced-CLS phenotype under normal culture conditions (Fig. 4C). The lifespan of wild-type cells was extended under calorie restriction, where cultures were switched to water [14,22]; however, the hsf1-RF/S mutation again caused a reduced lifespan (Fig. 4D).

Previously, we have screened multicopy suppressor genes of a temperature-sensitive hsf1 mutation and identified RIM15 and PDE2 genes [12]. RIM15 encodes a protein kinase that is negatively regulated by cAMP-dependent protein kinase (PKA), PDE2 encodes a cAMP phosphodiesterase, thereby functioning as a negative regulator of the PKA pathway and a positive regulator of Rim15. The activity of Rim15 is also inhibited by TOR and Sch9 kinases [14]. PKA, TOR, and Sch9 are components of nutrient-sensing pathways, and nutrient depletion activates Rim15, which is involved in establishment of stationary phase [14]. It has been shown that Hsf1 activation inhibits TOR signaling [28]. In light of these observations, it is interesting to analyze the relationship between Hsf1 and Rim15.

![Fig. 4. Longevity of HSF and hsf1 mutant cells. (A) RT-PCR analysis of HSP genes in cells grown to stationary phase. Cells (strains NOY396, HS238, and HS272) were grown for 0.5 day (OD600 = 3.1–4.1), 1.0 day (OD600 = 5.5–6.7), 1.5 days (OD600 = 6.0–7.2), 2.0 days (OD600 = 5.8–6.9), 2.5 days (OD600 = 5.5–6.5), and 3.0 days (OD600 = 5.4–6.5). Total RNA prepared from the cells was analyzed by RT-PCR. Ethidium-bromide stained gels are shown as control for the amounts of RNA. (B) CLS of hsp90-hse and mit-hse cells under normal culture conditions. Viability of cells (strains NOY396, HS238, and HS272) cultured in SD medium was determined. (C) CLS of hsf1-RF/S and rim15A cells under normal culture conditions. Viability of cells (strains HS170T, HS172, HS313, and YAY22) cultured in SD medium was determined. (D) CLS of hsf1-RF/S and rim15A cells under calorie-restriction conditions. Viability of cells (strains HS170T, HS172, HS313, and YAY22) cultured in water was determined.](image)
in the regulation of lifespan. Under normal culture conditions, CLS of rim15Δ cells was significantly reduced, and the effects of a combination with the hsf1-Δ ranged were not evident (Fig. 4C). Under calorie-restriction conditions, however, lifespan of cells containing both hsf1-Δ and rim15Δ mutations was notably reduced compared with that of cells containing either mutation alone (Fig. 4D). Therefore, Hsf1 regulates longevity through mechanisms that are, at least in part, separate from Rim15.

The ECLI gene (previously known as VGR146c), one of the multicopy suppressor genes we previously identified [12], also regulates CLS [29]. It was suggested that, in Schizosaccharomyces pombe, hsf1Δ is a transcriptional activator of an ECLI homolog ecc2Δ and lifespan extension by hsf1Δ over-expression mainly depends on ecc2Δ [30]. In S. cerevisiae, Hsf1 regulates longevity through transcription of HSP90 genes, mitochondrial HSP genes, and other target genes such as ECLI. The observation that Rim15, PDE2, and ECLI are multicopy suppressor genes of an hsf1Δ mutation provides an important clue to understand the roles of Hsf1 in the regulation of longevity.

As shown here, Hsf1 is a constitutive and stress-inducible activator of two HSP90 genes and five mitochondrial HSP genes. We observed that the essential roles of Hsf1 under normal physiological conditions, various proteotoxic (heat-, A2C-, and ethanol-treatments) conditions, and aging are partially mediated by the expression of these seven genes. These results suggest that Hsf1-regulated changes in chaperone levels are necessary for maintenance of protein folding and cellular homeostasis.

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Appendix A. Supplementary data


References