

The Skn7 Response Regulator of *Saccharomyces cerevisiae* Interacts with Hsf1 In Vivo and Is Required for the Induction of Heat Shock Genes by Oxidative Stress

Desmond C. Raitt,^{*†} Anthony L. Johnson,^{*} Alexander M. Erkiner,[‡]
Kozo Makino,[§] Brian Morgan,^{||} David S. Gross,[‡] and Leland H. Johnston^{*}

^{*}Division of Yeast Genetics, National Institute for Medical Research, The Ridgeway, London NW7 1AA, United Kingdom; [‡]Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, Shreveport, Louisiana 71130; [§]Department of Molecular Microbiology, Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan; and ^{||}Department of Biochemistry and Genetics, Medical School, University of Newcastle, Newcastle-Upon-Tyne NE2 4HH, United Kingdom

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The Skn7 response regulator has previously been shown to play a role in the induction of stress-responsive genes in yeast, e.g., in the induction of the thioredoxin gene in response to hydrogen peroxide. The yeast Heat Shock Factor, Hsf1, is central to the induction of another set of stress-inducible genes, namely the heat shock genes. These two regulatory *trans*-activators, Hsf1 and Skn7, share certain structural homologies, particularly in their DNA-binding domains and the presence of adjacent regions of coiled-coil structure, which are known to mediate protein–protein interactions. Here, we provide evidence that Hsf1 and Skn7 interact in vitro and in vivo and we show that Skn7 can bind to the same regulatory sequences as Hsf1, namely heat shock elements. Furthermore, we demonstrate that a strain deleted for the *SKN7* gene and containing a temperature-sensitive mutation in Hsf1 is hypersensitive to oxidative stress. Our data suggest that Skn7 and Hsf1 cooperate to achieve maximal induction of heat shock genes in response specifically to oxidative stress. We further show that, like Hsf1, Skn7 can interact with itself and is localized to the nucleus under normal growth conditions as well as during oxidative stress.

INTRODUCTION

Cells must survive challenges from the environment with regard to heat, UV radiation, and heavy metals as well as tolerate the endogenous generation of reactive oxygen intermediates during respiration. Oxygen, in the form of superoxide anion (O_2^-), hydroxyl ion (OH^-), and hydrogen peroxide (H_2O_2), causes damage to nucleic acids, cell membranes, and proteins (reviewed by Halliwell, 1994). Yeast, in common with all other organisms, has evolved protective mechanisms to survive in the presence of these by-products of aerobic metabolism and can mount distinct adaptive responses to different sources of oxidative stress (Jamieson, 1992; Ruis and Schüller, 1995). For example, the Cu,Zn-linked superoxide dismutase, encoded by the *SOD1* gene, detoxifies superoxide anion to hydrogen peroxide;

catalase, encoded by the cytosolic *CTT1* gene, can catalyze the breakdown of hydrogen peroxide. Other free radical scavengers in the cell include glutathione, ascorbic acid, and thioredoxin. The *SKN7* gene was initially isolated as a multicopy suppressor of a *kre9Δ* mutation, which results in defective cell wall biosynthesis (Brown *et al.*, 1993). The *SKN7* gene was also cloned as *POS9* in a screen for mutants resulting in sensitivity to hydrogen peroxide (Krems *et al.*, 1995), suggesting a role for *SKN7* in the yeast oxidative stress response. The oxidative stress induction of the small antioxidant molecule thioredoxin, encoded by the *TRX2* gene, was subsequently shown to be regulated by *SKN7* and the yeast AP-1 homologue *YAP1*, both of which bind to distinct sites within the *TRX2* promoter (Kuge and Jones, 1994; Morgan *et al.*, 1997).

Of the many proteins that are induced under adverse environmental conditions, perhaps the best understood are the heat shock proteins (Lindquist and Craig, 1988; Mager and De Kruijff, 1995). The major heat shock proteins have

[†] Corresponding author. E-mail address: desmond_raitt@dfci.harvard.edu.

been classified according to their molecular weight: Hsp104, the Hsp90 and Hsp70 families, Hsp60, Hsp26, and Hsp12 (Craig, 1992; Mager and Ferreira, 1993). The Hsp70 family of heat shock proteins act as molecular chaperones; this family contains at least five heat-inducible isoforms and others that are expressed constitutively at high levels (Craig, 1992; Ras-sow *et al.*, 1997). Their principal role includes the transport and folding of polypeptides and the solubilization of denatured proteins (Craig *et al.*, 1994). Hsp104 is thought to protect the cell during exposure to lethal heat shock and is required for cross-protection against a variety of stress conditions (Sanchez *et al.*, 1992), although its exact function remains unclear.

The induction of these genes in response to heat shock is mediated by Heat Shock Factor, encoded by the essential *HSF1* gene (Sorger and Pelham, 1988). The Hsf1 protein binds to heat shock elements (HSEs) consisting of tandem inverted repeats of the sequence AGAAn (where n is any nucleotide) found in the promoters of many heat shock genes (Fernandes *et al.*, 1995). In *Saccharomyces cerevisiae*, Hsf1 binds HSEs as a trimeric complex: constitutively to high-affinity sites (Gross *et al.*, 1990) and inducibly to low-affinity sites (Giardina and Lis, 1995; Erkin *et al.*, 1999). The protein undergoes extensive phosphorylation on serine and threonine residues upon heat shock. This posttranslational modification has been correlated with its transcriptional activation (Sorger and Pelham, 1988; Sorger, 1990). However, evidence has also been presented to suggest that hyperphosphorylation of serine residues located between the trimerization and C-terminal activation domains is involved in the deactivation of Hsf1 (Høj and Jakobsen, 1994). In addition to HSEs, a number of stress-responsive genes also contain stress-responsive elements (STREs) within their promoters, through which the Zn-finger transcription factors Msn2 and Msn4 can activate stress gene expression in response to a variety of stress conditions independent of Hsf1 (Schüller *et al.*, 1994; Martínez-Pastor *et al.*, 1996).

In addition to its role in the heat shock response, Hsf1 has also been shown to protect the cell against heavy metals, such as copper and cadmium, through its activation of the copper metallothionein gene *CUP1* (Silar *et al.*, 1991; Sewell *et al.*, 1995). Hsf1 becomes phosphorylated in response to the superoxide generator menadione. This modification correlates with transcriptional activation of *CUP1* by oxidative stress (Liu and Thiele, 1996). Hence, Hsf1 also plays a critical role in the cell's defense against oxidative stress. Because our previous work had established a role for the Skn7 response regulator in oxidative stress protection (Morgan *et al.*, 1997), it seemed possible that Skn7 and Hsf1 share overlapping functions.

The Skn7 protein contains a region with a high degree of homology to the receiver domain of bacterial two-component systems, a class of proteins involved in signal transduction in bacteria and lower eukaryotes (Stock *et al.*, 1989; Parkinson, 1993). Thus, a membrane-bound sensor histidine kinase can phosphorylate a conserved aspartate residue within the receiver domain of its cognate response regulator. This phospho-aspartate form of the response regulator can then carry out a function appropriate to the incoming signal, usually the transcriptional activation of a specific set of genes. A conserved aspartate, residue D427 in Skn7, has been shown to be required for the function of the protein in

cell wall biosynthesis (Brown *et al.*, 1993) and the activation of G1 cyclin expression (Morgan *et al.*, 1995). However, phosphorylation of this residue does not appear necessary for the role of SKN7 in the oxidative stress response (Morgan *et al.*, 1997).

The Skn7 protein also contains a C-terminal glutamine-rich region consistent with a possible role in transcriptional activation (Brown *et al.*, 1993; Morgan *et al.*, 1995). Toward the N terminus there is a region of extensive homology to the helix-turn-helix DNA-binding domain of Hsf1 (Figure 1). This domain is separated from the receiver motif by a region of coiled-coil structure, again similar to the leucine zipper domain of the yeast Hsf1. Given the degree of conservation in the structures of both the DNA-binding domain and the leucine zipper region of the Skn7 and Hsf1 proteins (Figure 1), it was of interest to determine whether Skn7 interacted with Hsf1 and to establish the significance of these interactions in the yeast stress response.

We present evidence here for both genetic and direct biochemical interaction between Hsf1 and the Skn7 response regulator. Furthermore, we show that a protein other than Heat Shock Factor, Skn7, can bind to HSEs *in vitro*, is localized to the nucleus under normal and oxidative stress growth conditions, and is required for the full induction of heat shock genes in response to oxidative stress.

MATERIALS AND METHODS

Yeast Strains and Growth Conditions

The yeast strains used were as follows: W303-1a (a *ade2-1 trp1-1 can1-100 leu2-3112 his3-11 ura3*); W303-1a *skn7Δ* (a *ade2-1 trp1-1 can1-100 leu2-3112 his3-11 ura3 skn7::HIS3*); MYY290, a *ura3* derivative of strain AH216 (a *leu2 his3 phoC phoE*); MYY385 (a *leu2 his3 ura3 phoC phoE hsf1-m3*) (Smith and Yaffe, 1991); and DR20-2b, which was obtained as a haploid *HIS⁺* spore clone from a cross of MYY385 and W303 *skn7Δ*. Minimal and rich media for yeast propagation have been described previously (Sherman *et al.*, 1996).

β-Galactosidase Assays

The vector pZJHSE2-137 (a gift from E. Craig, University of Wisconsin, Madison, WI) containing a region of the *SSA1* promoter responsible for heat shock activation of HSP70 cloned into the 2 μ -based lacZ fusion plasmid pLG660 was transformed into W303-1a and the isogenic *skn7Δ* strain. Transformants were grown to midlog phase in selective medium at 30°C and harvested before or after the addition of *t*-butyl hydrogen peroxide to 0.6 mM for 1 h. For the heat shock experiment, cells were initially grown in selective minimal medium at 25°C, and cells were harvested before and 1 h after the culture was shifted to a 37°C water bath. Cell extracts were prepared as described previously (Guarente and Mason, 1983). Units of activity are given as the change in OD₄₂₀ per minute per milligram of total protein. Values represent the average of duplicate samples in two independent experiments.

Hydrogen Peroxide Sensitivity Assays

Strains were tested for sensitivity to hydrogen peroxide by taking a suspension of cells in water and making a single streak of the suspension from the edge to the center of the plate, which contained a disk of 3MM paper onto which was placed 0.5–2 μ l of *t*-butyl hydrogen peroxide (Sigma Chemical, St. Louis, MO). The *t*-butyl hydrogen peroxide was allowed to diffuse freely throughout the agar, and the extent of growth inhibition from the center point of the plate was taken as a measure of the sensitivity of a given strain to oxidative stress.

(Adobe Systems, Mountain View, CA). Nuclei were visualized by DAPI staining.

RNA Analysis

Northern hybridization was performed as described previously (White *et al.*, 1986). In all cases, probes for hybridization to heat shock genes used in this study were derived from PCR amplification of an internal fragment of the coding sequence of the gene, either from genomic DNA or from plasmids containing the gene of interest. The internal control used for mRNA quantitation in hydrogen peroxide- and heat shock-treated cells was *ACT1*, the abundance of which was found to be relatively insensitive to these conditions.

Protein Extraction and Pull-Down Experiments

Yeast cell breakage was achieved through repeated vortexing with glass beads for 5 × 30 s with 30-s rests on ice in breakage buffer (150 mM NaCl, 1% NP40, 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM NaF, 50 mM β-glycerol phosphate). At time of use, a protease inhibitor mixture was added to a final concentration of 100 μg/ml PMSF, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, 50 μg/ml Nα-p-tosyl-L-Lysine chloromethyl ketone, and 100 μg/ml L-1-tosylamide-2-phenylethylchloromethyl. Cleared lysates were prepared by centrifugation for 20 min at 18,000 rpm (Beckman [Fullerton, CA] SS34 rotor), and 1 mg of whole cell extract was incubated at 4°C with 50 μl of a 50% suspension of glutathione-Sepharose beads (Pharmacia, Piscataway, NJ) equilibrated in breakage buffer in a total volume of 250 μl. The beads were then harvested and washed four times in breakage buffer containing 200 mM NaCl, followed by one wash in the same buffer with 50 mM NaCl, and finally resuspended in an equal volume of 2× SDS sample buffer. Coimmunoprecipitations with 9E10 mAb were performed by incubating 500 μg of cell extract with 2 μg of anti-myc mAb (Berkeley Antibody, Berkeley, CA) for 1 h, and precipitates were recovered by incubation with continuous mixing at 4°C with 50 μl of a 50% solution of protein G-Sepharose (Gammabind, Pharmacia) equilibrated in breakage buffer. Beads were washed six times in breakage buffer containing 200 mM NaCl, and once in buffer containing 50 mM NaCl, before being resuspended in 2× SDS sample buffer. Proteins were separated by SDS-PAGE through 6% acrylamide gels and transferred to nitrocellulose membranes via semidry transfer before ECL (Amersham, Arlington Heights, IL). Western analysis was performed in accordance with manufacturers' guidelines (Amersham), and membranes were exposed to X-ograph XB-200 film (Eastman Kodak, Rochester, NY) for between 30 s and 5 min.

For *in vitro* association assays, 1 mg of cell extract prepared as described above (with the omission of EDTA from the breakage buffer) was added to 200 μl of Ni²⁺ nitrilotriacetic acid (NTA) resin (50% slurry) equilibrated in breakage buffer. After incubation with mixing at 4°C for 1 h, the resin was washed four times in wash buffer (200 mM NaCl, 50 mM Tris-HCl, 1% NP40) and once in wash buffer containing 50 mM NaCl. Beads were then boiled for 2 min in 2× sample buffer, and the supernatant was subjected to SDS-PAGE as described above.

Purification of 6His-Skn7 and Mobility Shift Assays

A 2-kb *Bam*HI fragment of the original *SKN7* genomic clone in YEp24, which contains the entire *SKN7* ORF (Morgan *et al.*, 1995), was inserted into plasmid pQE-30 (Qiagen, Chatsworth, CA). Transformed DH-5α (minimum 2 l) was grown to OD₆₀₀ = 0.5 at 37°C and then brought to 25°C by brief incubation on ice before induction by addition of isopropyl-1-thio-β-D-galactoside to 1 mM for 5 h at 25°C. Cells were harvested by centrifugation (5 min, 5000 rpm, GSA Sorval rotor, Kendro Laboratory Products, Newtown, CT), washed in cold distilled water, and resuspended in 2–5 ml of breakage buffer (150 mM NaCl, 25 mM Tris, pH 7.5, 10% glycerol, 0.5% NP40).

At the time of use, lysozyme was added at 1 mg/ml and PMSF at 1 mM. The cell suspension was incubated on ice for 30 min and then passed twice through a chilled French press chamber. The clarified supernatant was then incubated with 5 ml of a 50% slurry of Ni²⁺-NTA resin, equilibrated in binding buffer (250 mM NaCl, 50 mM Tris-HCl, pH 7.5, 15 mM imidazole), and allowed to mix at 4°C for 1 h before the material was packed into a 5-ml column. After washing in binding buffer, tagged protein was eluted by a step gradient of binding buffer containing 50, 100, and 250 mM imidazole. Bradford protein assays (Bio-Rad, Richmond, CA) were carried out on 0.5-ml fractions, and DNA-binding activity was assayed by gel mobility shift assay.

Mobility shift assays have been described elsewhere (Lowndes *et al.*, 1991). 6His-Skn7 protein was incubated with 0.5 ng (1 × 10⁵ cpm) of ³²P 5'-end-labeled double-stranded oligonucleotides of the following sequences: HSE2, 5' tcgaTTTTCCAGAACGTTCCATCGGC; MUT HSE, 5' tcgaTTTTCCA~~AA~~AACGTTTCATCGGC. Binding reactions in 25 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 7 mM MgCl₂, 10% glycerol, protease mixture as described above, and 1 μg of poly(dI.dC) nonspecific competitor DNA were incubated at room temperature for 15 min and on ice for another 20 min. Protein-DNA complexes were resolved on a 4% nondenaturing polyacrylamide gel (37.5:1) by electrophoresis at 200 V in 0.5× TBE buffer (89 mM tris base, 89 mM boric acid, 2 mM EDTA) for 2 h. Gels were dried onto Whatman (Clifton, NJ) 3MM paper and exposed to Kodak (Rochester, NY) X-Omat AR film overnight at -20°C.

RESULTS

SKN7Δ Cells Are Sensitive to Acute Heat Stress

Deletion of the *SKN7* gene does not confer a temperature-sensitive phenotype when cells are shifted from 25 to 37°C (Morgan *et al.*, 1995). However, given the high degree of homology between the DNA-binding domains of Skn7 and Hsf1, we further investigated the effect of a *skn7Δ* mutation on cell viability under acute heat shock at 51°C. Cells deleted for the *SKN7* gene were found to be some 10 times more sensitive to the lethal effects of acute heat stress than the isogenic wild-type strain (Figure 2). It has been reported that the main cause of cell death under these conditions is the generation of toxic intermediates of oxygen metabolism (Davidson *et al.*, 1996). Because *SKN7* has been shown to be required for cell survival under conditions of oxidative stress (Krems *et al.*, 1996; Morgan *et al.*, 1997), it was of interest to determine whether *SKN7* has a role, together with *HSF1*, in the induction of heat shock gene expression in response to oxidative stress.

SSA1-LacZ Induction by Hydrogen Peroxide Requires *SKN7*

To investigate the potential role of the *SKN7* gene in the induction of heat shock protein expression, we assessed the expression of an Hsp70-*LacZ* reporter construct in wild-type and *skn7Δ* cells. The *SSA1* gene encodes a major isoform of the yeast Hsp70 protein that is abundant under nonstressed conditions and is strongly induced by heat shock (Craig, 1992). The plasmid pZJHSE2-137 contains an HSE, HSE2, from the *SSA1* promoter fused to the β-galactosidase coding sequence (Slater and Craig, 1987; Park and Craig, 1989). The HSE2 sequence is responsible for the majority of both basal and heat shock-induced expression of *SSA1* (Slater and Craig, 1987). β-Galactosidase assays were carried out on wild-type W303-1a and isogenic *skn7Δ* cells containing the reporter plasmid after treatment for 1 h with hydrogen

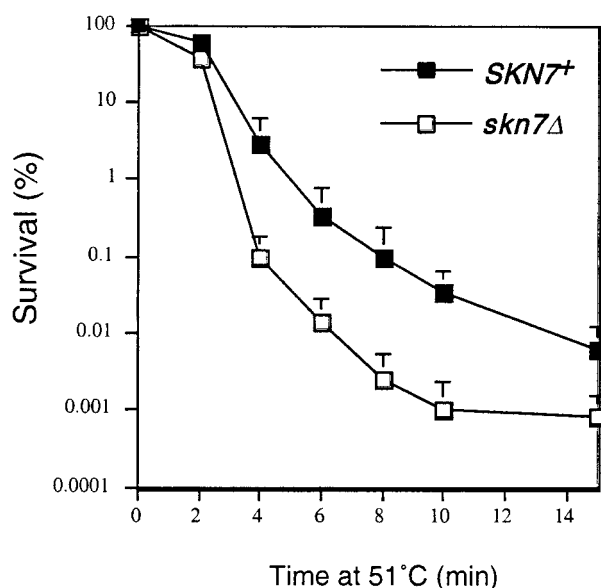


Figure 2. *skn7*Δ cells are sensitive to acute heat stress. Midlog-phase cultures of W303-1a and isogenic *skn7*Δ cells were grown in YPD at 25°C, and an aliquot was shifted to a test tube placed in a 51°C water bath. Samples were taken at the times indicated, diluted into ice-cold YPD, and immediately plated onto YPD agar to assess cell viability. Survival at 51°C was expressed as a percentage of viable cells relative to cells grown at 25°C. Because the vertical axis is logarithmic, only positive errors are included for clarity.

peroxide. In wild-type cells, an eightfold induction of β -galactosidase activity was observed 1 h after the addition of *t*-butyl hydrogen peroxide (Table 1). In the *skn7*Δ strain, basal expression of HSE2-*LacZ* activity was reduced and induction in response to oxidative stress was abolished. However, induction of HSE2-*LacZ* activity in response to a temperature shift from 25 to 37°C was unaffected in the *skn7*Δ strain, although the overall level of expression in the *skn7*Δ cells was reduced. The decreased basal expression of HSE2-*LacZ* in the *skn7*Δ strain extends previous results that

Table 1. Induction of *SSA1-LacZ* in response to *t*-butyl hydrogen peroxide requires Skn7

Strain	Hydrogen peroxide (0.6 mM)		Heat shock	
	-	+	25°C	37°C
W303-1a	0.08	0.61	0.15	1.33
W303-1a <i>skn7</i> Δ	0.04	0.03	0.02	0.23

Wild type and isogenic *skn7*Δ cells containing pZJHSE2-137 were assayed for β -galactosidase levels before (-) and after (+) the addition of *t*-butyl hydrogen peroxide to 0.6 mM for 1 h. For the heat shock experiment, cells were grown at 25°C and transferred to 37°C for 1 h. β -Galactosidase activity is expressed as change in OD₄₂₀ per minute per milligram of protein. Values are averages of duplicate samples from two independent experiments.

indicated a similar reduction in basal expression levels of an *SSA1-LacZ* reporter construct in response to an HSE2 double point mutation (Park and Craig, 1989). Thus, HSE2 appears to be critical for both basal and stress-induced expression of the *SSA1* gene. It has been proposed that Yap1, which has been shown to interact with Skn7 at the *TRX2* promoter (Morgan *et al.*, 1997), is also required for the induction of HSE2-*LacZ* activity in response to hydrogen peroxide (Stevens *et al.*, 1995). However, in our genetic background, we found no evidence that *yap1*Δ affects the hydrogen peroxide induction of the *SSA1* HSE2-*LacZ* reporter construct (D.C. Raitt, unpublished observations). Although dispensable for heat shock induction, our data suggest that Skn7 may be required for full induction of HSE-driven gene expression in response to free radical stress.

Skn7 Can Specifically Bind the HSE2 Element from the SSA1 Promoter

The DNA-binding domain of Skn7 is highly homologous to that of Hsf1 (Figure 1B). To determine whether Skn7 can recognize and bind specifically to the same recognition sequence as Hsf1, we performed electrophoretic mobility shift assays (EMSA) with *Escherichia coli*-expressed 6His-Skn7. The *SKN7* gene was inserted into plasmid pQE-30, and the 6His-Skn7 fusion protein was subsequently purified on an Ni²⁺-NTA agarose affinity column (see MATERIALS AND METHODS). As demonstrated by EMSA, the 6His-Skn7 fusion protein binds specifically to the 26-base pair sequence encompassing the HSE2 region of the *SSA1* promoter (Figure 3). To confirm the presence of the 6His-Skn7 protein in the retarded complex, polyclonal antiserum to the protein was added to the band shift reaction mixture. The retarded complex formed with the HSE2 probe and the 6His-Skn7 protein was super-shifted by the anti-Skn7 antibody, whereas no effect was observed with the addition of preimmune serum at the same concentration.

To determine the specificity of this binding, competitive binding assays were performed with the native HSE2 oligonucleotide and a mutated probe, MUT-HSE2, in which the G and C positions of the consensus HSE sequence AGAAnnTTCn were changed to A and T, respectively. These mutations within the consensus have previously been shown to abolish binding of Hsf1 (Park and Craig, 1989). The native 26-mer HSE competes efficiently for the binding of Skn7 at a 10-fold molar excess (Figure 3). However, the mutated version of the HSE, MUT-HSE2, does not compete for binding of the 6His-Skn7 protein at up to 100-fold molar excess. Similar results have been seen with an *E. coli*-expressed Skn7 derivative consisting of the DNA-binding domain alone fused in frame to GST and also with full-length 6His-Skn7 protein purified from yeast (our unpublished results). These results demonstrate that Skn7 binds to HSEs with a specificity similar to that of Hsf1.

Induction of Heat Shock Gene Expression by Hydrogen Peroxide Requires Skn7

We have previously shown that Skn7 cooperates with the yeast AP-1 homologue Yap1 in the oxidative stress induction of the *TRX2* gene (Morgan *et al.*, 1997). Given the defect in hydrogen peroxide-mediated induction of HSE-*LacZ* expression in *skn7*Δ cells relative to isogenic wild-type cells

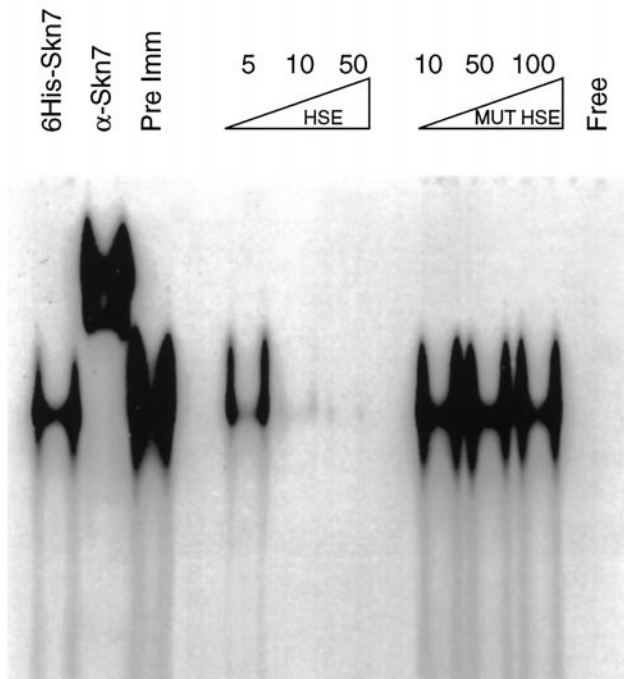


Figure 3. Purified 6His-Skn7p can specifically bind HSEs in vitro. EMSA was performed with the use of *E. coli*-expressed affinity-purified 6His-Skn7 protein and a probe comprising a double-stranded oligonucleotide corresponding to the 26-base pair HSE2 region of the *SSA1* gene. The specificity of binding was assessed by the addition of cold HSE2 probe (HSE: tcgaTTTCCAGAACGTTCATCGGC) at 5-, 10-, and 50-fold molar excess, compared with the addition of a mutated HSE (MUT HSE: tcgaTTTCCAAAACGTTTCATCGGC) at 10-, 50-, and 100-fold molar excess. Mutation of the underlined nucleotides in the consensus HSE abolishes Hsf1 binding to the sequence. Polyclonal anti-Skn7 antibody (α -Skn7) at a 1:100 dilution or preimmune serum at the same concentration (Pre Imm) was added to the binding reaction 15 min before the addition of labeled probe. Free probe without the addition of protein migrated off the gel and is indicated (Free).

described above, we explored the possibility that Skn7 could have a role in the induction of heat shock genes in response to oxidative stress. Therefore, Northern analysis was carried out on a number of heat shock genes in W303-1a and W303-1a *skn7* Δ . In wild-type cells, the genes encoding *HSP12*, *HSP26*, and *HSP104* were found to be strongly induced by *t*-butyl hydrogen peroxide (Figure 4A). However, the 5-fold induction of *HSP12* in response to hydrogen peroxide was practically abolished by the *skn7* Δ deletion. Similarly, the 18-fold induction of *HSP26* was reduced significantly in *skn7* Δ cells, and the 9-fold induction of *HSP104* was again reduced by the *skn7* Δ mutation. These heat shock genes, therefore, appear to be dependent on the Skn7 response regulator for their full induction in response to hydrogen peroxide-mediated oxidative stress. Furthermore, evidence for a role of Skn7 in the oxidative stress induction of other heat shock proteins (*SSA1* and *HSP82*) has recently been presented (Lee *et al.*, 1999); however, in the W303-1a genetic background, we found no significant induction of any Hsp70 gene (*SSA1*, *SSA2*, *SSA3*, or *SSA4*) or of *HSP82* in

response to 0.6 mM *t*-butyl hydrogen peroxide (our unpublished results).

To determine whether Skn7 also contributed to heat shock-induced expression, we performed Northern analysis of heat-shocked W303-1a and isogenic *skn7* Δ cells. The kinetics of induction of seven genes (*HSP12*, *HSP26*, *SSA1*, *SSA3*, *SSA4*, *HSP82*, and *HSP104*) were found to be virtually indistinguishable between wild-type and *skn7* Δ cells (Figure 4B; our unpublished results). *SKN7*, therefore, is specifically required for the oxidative stress induction of heat shock genes. This is in accord with our observations that *skn7* Δ cells show no increased sensitivity upon a temperature shift from 25 to 37°C compared with the isogenic wild-type strain (D.C. Raitt, unpublished observations).

Genetic Interactions between *SKN7* and *HSF1*

To explore the possibility that *SKN7* and *HSF1* may interact in vivo, we took a genetic approach and determined whether deletion of the *SKN7* gene combined with a conditional mutation in *HSF1* would result in a synthetic phenotype. Although the gene encoding yeast heat shock factor is essential, an *hsf1* temperature-sensitive allele, *hsf1-m3*, has been isolated (Smith and Yaffe, 1991). Thus, an *skn7* Δ derivative of W303-1a was crossed with the *hsf1*^{ts} strain MYY385, and an *hsf1*^{ts} spore clone containing the *HIS3*⁺-marked *skn7* Δ deletion, strain DR20-2b, was selected for further study. Both the *hsf1*^{ts} strain and strain DR20-2b were then tested for growth at various temperatures. As expected, both strains grew at the permissive temperature of 25°C and neither grew at the restrictive temperature of 37°C (our unpublished results). However, at an intermediate temperature of 33°C, the *hsf1*^{ts} strain formed colonies but the double mutant, DR20-2b, failed to grow (Figure 5A). Because these strains were not isogenic, strain DR20-2b was transformed with a CEN version of the *SKN7* gene. This restored growth at 33°C (Figure 5A), indicating that the increased temperature sensitivity of DR20-2b is due specifically to the deletion of the *SKN7* gene rather than to genetic background effects. Thus, deletion of *SKN7* exacerbates the growth defect of the *hsf1*^{ts} strain.

We then assessed whether overexpression of the *SKN7* gene could suppress the growth defect associated with the *hsf1*^{ts} allele at higher temperatures. The *hsf1*^{ts} strain was transformed with the high-copy vector Yep24-*SKN7* or the empty vector alone. The *hsf1*^{ts} cells expressing high levels of Skn7 displayed strong growth at a temperature of 35°C, whereas the *hsf1*^{ts} strain containing the empty vector alone could not form colonies at this temperature (Figure 5B). However, the high-copy expression of *SKN7* failed to rescue the *hsf1*^{ts} strain at 37°C (our unpublished results). Given the pleiotropic nature of the *hsf1-m3* allele, which causes defects in mitochondrial protein import, reduces heat shock gene induction in response to increased temperatures, and leads to specific defects in cell cycle progression (Smith and Yaffe, 1991), the absence of suppression at 37°C is perhaps not surprising. Although there is clearly some overlap between the functions of *SKN7* and *HSF1* in the cell, the Skn7 response regulator cannot completely substitute for Hsf1 and thus fails to rescue the *hsf1-m3* mutation at 37°C.

Both *HSF1* and *SKN7* have been shown to play a role in the activation of stress-responsive gene expression under conditions of free radical stress (Krems *et al.*, 1996; Liu and

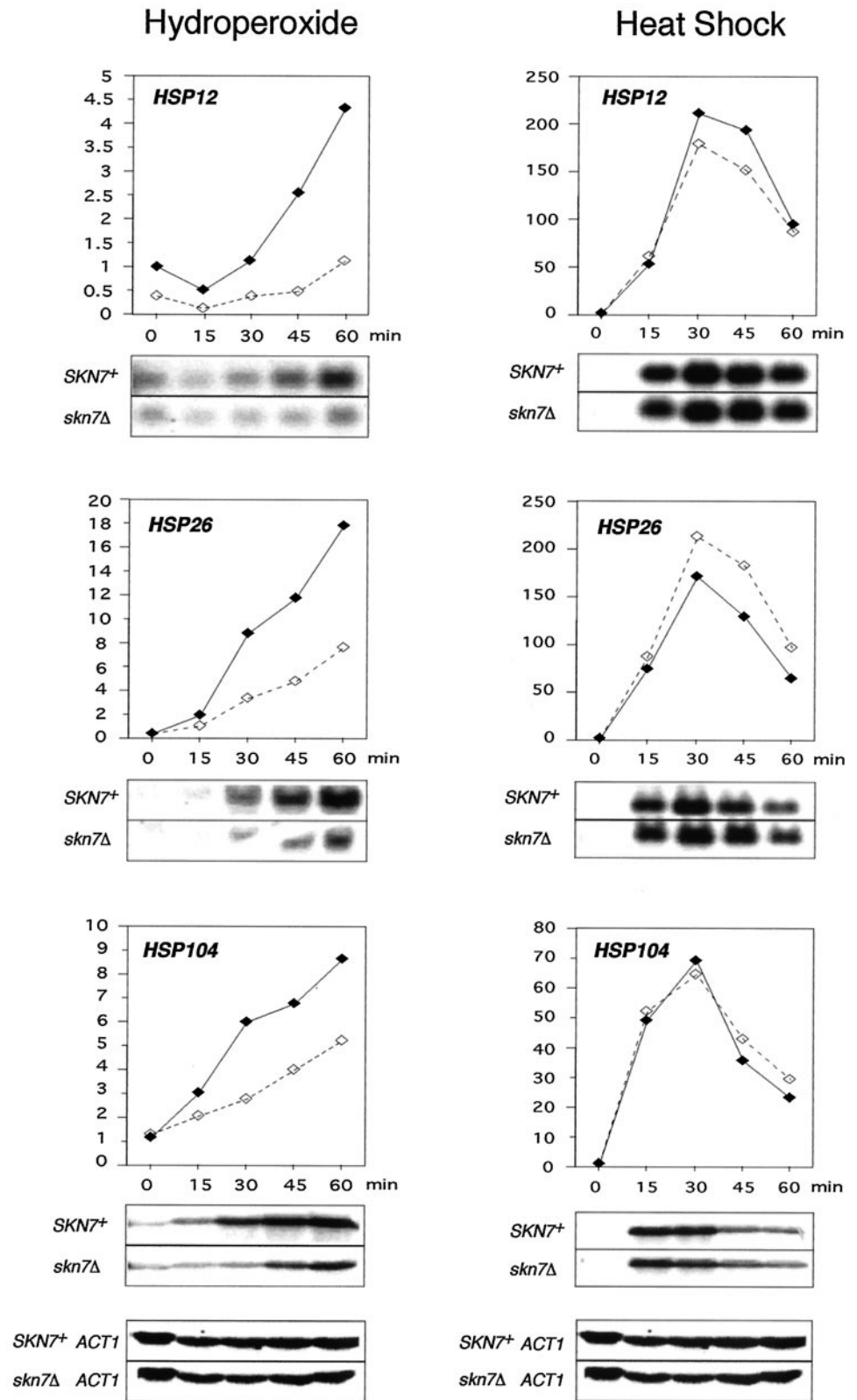


Figure 4. Skn7 is required for the induction of heat shock gene expression specifically in response to hydrogen peroxide. (A) Northern blot analysis of the effect of *skn7Δ* mutations on heat shock gene induction by oxidative stress. Total RNA was prepared from midlog-phase cultures of W303-1a and W303-1a *skn7Δ* grown at 30°C in YPD. Samples for RNA extraction were taken before (time 0) and at the times indicated after the addition of 0.6 mM *t*-butyl hydrogen peroxide. Northern blots were prepared as described (see MATERIALS AND METHODS) and hybridized to probes specific for *HSP12*, *HSP26*, and *HSP104*. (B) Skn7 is not required for the heat shock induction of heat shock gene expression. W303-1a and *skn7Δ* cells were grown to midlog phase at 25°C, and a portion of the culture was transferred to a 39°C water bath. Cells were harvested at the times indicated, and RNA was extracted for Northern hybridization with the probes specified in A. Quantitation of mRNA was by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA) and was expressed relative to the *ACT1* transcript, the abundance of which appeared to be unaffected by oxidative stress.

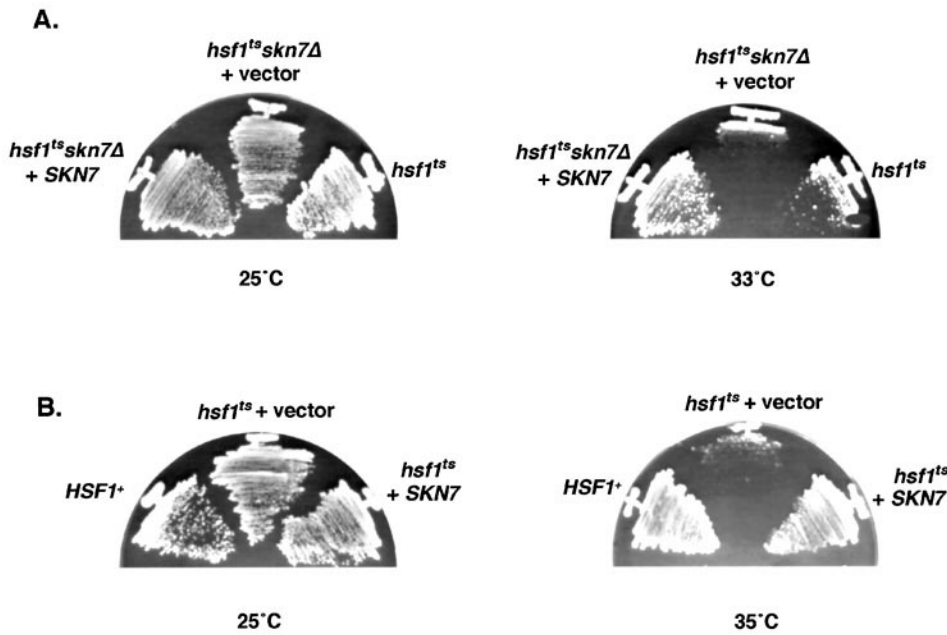


Figure 5. Genetic interactions between *SKN7* and *HSF1*. (A) Single-copy expression of *SKN7* rescues the growth defect of DR20-2b (*hsf1^{ts} skn7Δ*) at 33°C. DR20-2b (*hsf1^{ts} skn7Δ*) was transformed with the CEN vector YCplac111 (vector) or with this vector containing the *SKN7* gene (+ *SKN7*) and was incubated at permissive (25°C) and intermediate (33°C) temperatures on rich medium. (B) High-copy expression of *SKN7* rescues the growth defect of the *hsf1^{ts}* strain, MYY385, at 35°C. The *hsf1^{ts}* strain MYY385 was transformed with the 2 μ -based plasmid YEp24 containing the *SKN7* gene or with the vector alone (vector) and was streaked onto selective medium and incubated at 35°C for 4 d. The wild-type strain MYY290 (*HSF1⁺*) was included as a positive growth control.

Thiele, 1996; Morgan *et al.*, 1997). Therefore, we wished to establish whether they might somehow cooperate in the yeast oxidative stress response. We assessed the sensitivity to oxidative stress of the *hsf1^{ts}*, *skn7Δ*, and *skn7Δ hsf1^{ts}* strains by means of a standard plate assay (see MATERIALS AND METHODS). The *hsf1^{ts}* mutation alone caused a modest increase in hydrogen peroxide sensitivity relative to the wild-type strain MYY385 (Figure 6), and a more pronounced effect was observed with the *skn7Δ* mutation compared with its wild-type parent, W303-1a. However, the *skn7Δ hsf1^{ts}* strain showed an enhanced hypersensitivity to hydrogen peroxide stress relative to either the *skn7Δ* or the *hsf1^{ts}* strain alone (Figure 6). Introduction of *SKN7* on a CEN plasmid reversed this increased sensitivity, restoring it to that of the *hsf1^{ts}* strain, indicating that the phenotype was not due to genetic background effects. Confirming this result, an isogenic strain containing the *skn7Δ* deletion in an *hsf1^{ts}* background was also found to be hypersensitive to oxidative stress relative to either single mutant alone (A.L. Johnson, unpublished results). The additive effect of these mutations on stress sensitivity suggests that Skn7 and Hsf1 might in some way cooperate in the response to free radical stress.

In a complementary approach, we found that the W303-1a *skn7Δ* cells containing a high-copy plasmid with multiple HSE inserts exhibited significantly enhanced hydrogen peroxide sensitivity (D.C. Raitt, unpublished results). By presenting the cell with multiple copies of the binding site for Hsf1, the protein may have been competed away from its native binding sites, thereby decreasing its overall activity in the cell. Thus, when Hsf1 activity is depleted, either through competition for binding sites or by mutation, the survival of *skn7Δ* cells under stress is further compromised. This observation further supports the proposal that both Skn7 and Hsf1 contribute to cell survival during oxidative stress, perhaps through a related or shared pathway.

Previous data concerning the function of Skn7 indicated that phosphorylation of the conserved aspartate (D427)

within the receiver domain was not required for survival under conditions of oxidative stress (Morgan *et al.*, 1997). Significantly, an episomal D427N version of *SKN7* was also found to reverse the hypersensitivity of DR20-2b to hydrogen peroxide (Figure 6). Furthermore, the Skn7^{D427N} mutant also restored HSE2-*LacZ* expression in *skn7Δ* cells (our unpublished results), suggesting that phosphorylation of D427 is not required for Skn7p function through binding HSEs. In contrast, Skn7^{D427N} fails to activate *CLN1* and *CLN2* expression in a *swi4^{ts} swi6Δ* background (Morgan *et al.*, 1995) or to rescue the cell wall assembly defect of the *kre9Δ* mutant (Brown *et al.*, 1994). Hence, phosphorylation of the receiver domain, presumably by the SLN1-YPD1 phosphohistidine relay (Li *et al.*, 1998; Posas *et al.*, 1996), can direct the activation of Skn7 function to different target genes.

Skn7 and Hsf1 Interact Physically

To extend these data suggesting a genetic interaction between Skn7 and Hsf1, we undertook copurification analysis to determine if these proteins interact physically. Thus, the *SKN7* gene was placed under the control of the *GAL1* promoter in plasmid YCpIF16 (Foreman and Davis, 1994) and fused in frame to the hemagglutinin (HA) epitope, and the *HSF1* gene was fused in frame to the GST epitope in the high-copy expression plasmid p426-GAG. Cell extracts were prepared from galactose-induced cultures and incubated with GST-Sepharose beads (see MATERIALS AND METHODS). Immunoblot analysis indicated that Skn7-HA copurified with GST-Hsf1 on the GST-Sepharose beads (Figure 7A, lane 4). When the extracts were prepared from a strain containing the empty vector, p426-GAG, Skn7-HA was not detected in association with the beads (Figure 7A, lane 2). To confirm this result, we used extracts prepared from the same strain containing p426-GAG-*HSF1* grown on glucose to repress synthesis of Skn7-HA from the *GAL* promoter. Skn7 was found not to associate with the GST-Sepharose beads

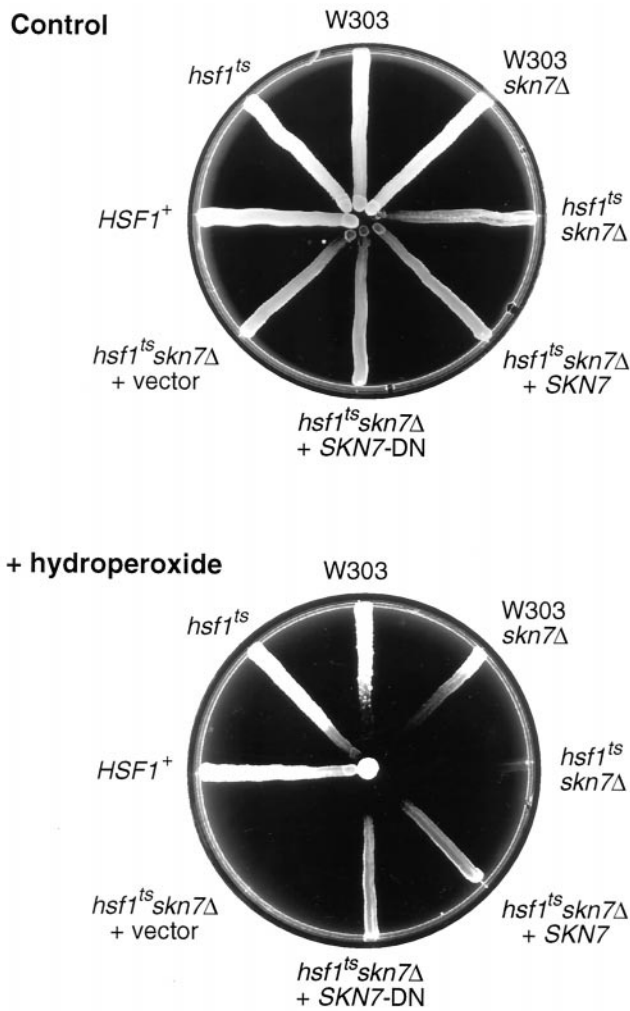


Figure 6. The double mutant *hsf1^{ts} skn7Δ* is hypersensitive to hydrogen peroxide. Sensitivity to hydrogen peroxide was assayed for W303-1a and isogenic *skn7Δ*, for MYY290 (*HSF1⁺*) and the *hsf1^{ts}* derivative MYY385, and for an *hsf1^{ts} skn7Δ* spore clone (DR20-2b) derived from a cross between *skn7Δ* and MYY385. Cell suspensions were streaked on a YPD plate (Control) and a YPD plate onto which 1 μ l of 7.7 M *t*-butyl hydrogen peroxide was spotted on a disk of Whatman 3MM paper positioned in the center of the plate (+ hydroperoxide). Suppression of hypersensitivity was judged by the ability to grow in the presence of hydrogen peroxide of a YCplac111 (Gietz and Sugino, 1988) plasmid expressing the *SKN7* gene (+*SKN7*) or an *SKN7^{D427N}* mutant (+ *SKN7*-DN) in which the conserved aspartate residue (D427) was mutated to asparagine. The *hsf1^{ts} skn7Δ* strain was also transformed with the YCplac111 vector alone (+ vector); plates were incubated for 2 d at 30°C.

(Figure 7B, lane 3; compare with lane 4, in which galactose-grown cells were used).

To determine whether Hsf1 also copurifies with Skn7, the complementary experiment was undertaken with an Ni²⁺-NTA affinity matrix to purify 6His-tagged Skn7p from a galactose-induced cell extract. Subsequent Western analysis of the proteins associated with the nickel-bound Skn7 indicated that Hsf1 copurified with the 6His-tagged Skn7 pro-

tein (Figure 7C, lane 3). No Hsf1 was pulled down in an extract without 6His-Skn7 (Figure 7C, lane 2). These results are consistent with the copurification of Skn7 with Hsf1 and strongly suggest that they interact physically in vivo.

Skn7 Can Interact with Itself In Vivo

Because Hsf1 is known to form homotrimers through its leucine zipper, we determined whether Skn7 could also interact with itself, given that Skn7 contains a similar coiled-coil region (Figure 1). To address this question, coimmunoprecipitations were carried out with extracts from cells containing integrated 6Myc-tagged Skn7 under control of its own promoter and with high-copy HA-tagged *SKN7* under galactose-inducible expression. Western analysis with 12CA5 antibody revealed that precipitation with monoclonal 9E10 anti-myc antibody specifically coprecipitates HA-Skn7 (Figure 7D, lane 4) from a galactose-induced extract containing 6Myc-Skn7. In the absence of a Myc-tagged *SKN7* gene, or with the use of a cell extract derived from glucose-grown cells expressing GAL-HA-*SKN7*, no such coprecipitation was apparent (Figure 7D, lanes 2 and 3). These data suggest that Skn7 can oligomerize in vivo and that this may be central to its function, because disruption of the leucine zipper motif in Skn7 significantly compromises its function (Alberts *et al.*, 1998).

Skn7p Is Localized to the Nucleus

To determine whether regulated nuclear import and export is an important mechanism by which the activity of the Skn7 response regulator may be controlled, we constructed an *SKN7*-GFP fusion protein in the CEN plasmid pRS416-sGFP (a gift from P. Silver) and examined the localization of Skn7. Figure 8 (C) shows that Skn7-GFP colocalizes with the DAPI-stained nucleus (B) under nonstressed growth conditions. This localization was unaffected by the addition of *t*-butyl hydrogen peroxide (our unpublished results). The nuclear localization of Skn7 is consistent with its role as a transcriptional regulator.

DISCUSSION

Both Skn7 and Hsf1 have previously been shown to play important roles in the cellular response to oxidative stress. In the case of the response regulator Skn7, it has been shown to cooperate with the yeast AP-1 homologue Yap1 at the *TRX2* promoter and to specifically activate transcription of the thioredoxin and thioredoxin reductase genes in the presence of hydrogen peroxide (Morgan *et al.*, 1997). Similarly, Hsf1-dependent activation of the *CUP1* metallothionein gene was observed in yeast cells treated with the superoxide generator menadione (Liu and Thiele, 1996). Here, we provide evidence that Skn7 is required for efficient heat shock gene activation in response to hydrogen peroxide. We also show that Skn7 and Hsf1 interact physically and genetically and have identified target genes known to be regulated by Hsf1 that are also regulated by Skn7 in response to free radical stress.

Physical and Genetic Interactions between Hsf1 and Skn7

A genetic interaction between Hsf1 and Skn7 is indicated by several observations. First, the restrictive temperature of an

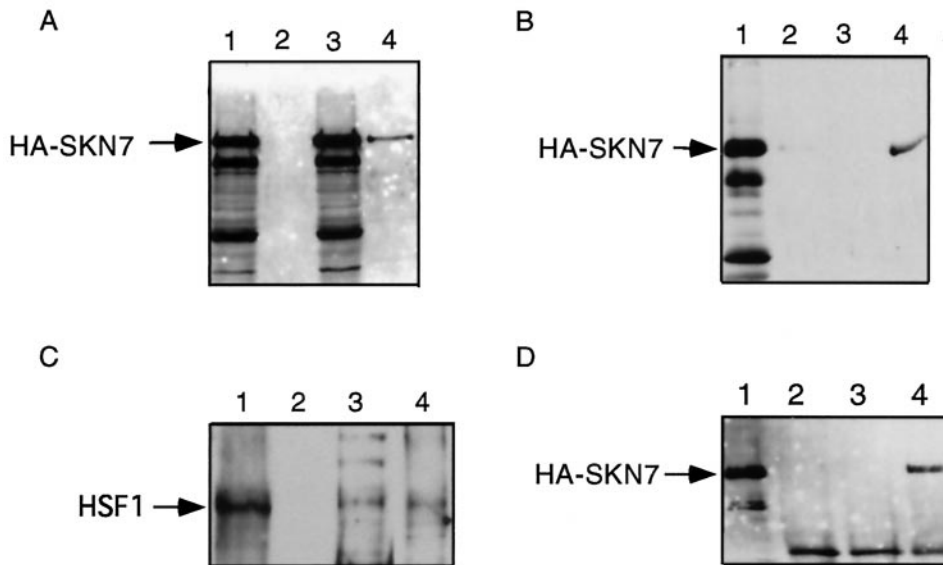


Figure 7. Hsf1 and Skn7 copurify. (A) Western blot analysis with 12CA5 mAb reveals that pGAL-Skn7-HA copurifies with GST-Hsf1. Lane 1, crude extract containing GST vector alone and pGAL-Skn7-HA; lane 2, GST pull-down from extract containing pGAL-Skn7-HA and GST vector alone; lane 3, crude extract containing pGAL-Skn7-HA and GST-Hsf1; lane 4, GST pull-down from cells containing pGAL-Skn7-HA and GST-Hsf1. (B) Lane 1, 20 μ g of input protein (HA-Skn7); lane 2, GST pull-down of extract from cells containing empty GST vector and pGAL-Skn7-HA; lane 3, GST pull-down of extract from cells containing GST-Hsf1 vector and pGAL-Skn7-HA and grown in glucose; lane 4, GST pull-down of extract from galactose-grown cells containing pGAL-Skn7-HA and pGST-Hsf1. (C) Nickel-affinity copurification of Hsf1 with 6His-tagged Skn7. Lane 1, immunoprecipitate with anti-Hsf1 antibody from 1 mg of whole cell extract of galactose-grown cells expressing pGAL-SKN7-6His; lane 2, Ni^{2+} -NTA agarose beads plus 1 mg of cell extract that does not contain the 6His-Skn7 protein; lane 3, Ni^{2+} -NTA agarose beads plus 1 mg of galactose-induced extract from cells expressing pGAL-SKN7-6His; lane 4, 20 μ g of input galactose-induced extract. (D) Skn7p can interact with itself. Coimmunoprecipitations from cell extracts containing galactose-induced pGAL-Skn7-HA and 6Myc-Skn7 were performed with the use of 9E10 mAb followed by 12CA5 Western blot analysis. HA-Skn7p expression was under the control of the GAL promoter, and integrated 6Myc-Skn7 was under the control of its own promoter. Immunoprecipitations with 1.5 μ g of 9E10 were as follows: lane 1, 20 μ g of extract from galactose-grown cells; lane 2, immunoprecipitation of extract from glucose-grown cells; lane 3, immunoprecipitation of extract from galactose-grown cells that did not contain the Myc-tagged SKN7; lane 4; immunoprecipitation of extract from galactose-grown cells containing 6Myc-Skn7 and pGAL-Skn7-HA. Western blot analysis was carried out with 12CA5 mAb.

hsf1^{ts} skn7 Δ strain is decreased relative to that of the parental *hsf1^{ts}* strain. Second, the growth defect of the *hsf1^{ts}* strain is partially suppressed by high-copy expression of the SKN7 gene. That this suppression is partial indicates that Skn7 can fulfill some but not all of the functions of Hsf1 in the cell. This observation is not surprising given the pleiotropic nature of the *hsf1-m3* mutation (Smith and Yaffe, 1991). Third, by reducing the activity of Hsf1 in *skn7 Δ* cells through construction of the double mutant strain *hsf1^{ts} skn7 Δ* , the sensitivity of *skn7 Δ* cells to hydrogen peroxide is greatly

enhanced. This hypersensitivity could be suppressed by ectopic expression of either SKN7 or SKN7^{D427N}. These observations indicate that Skn7 and Hsf1 have overlapping functions in the stress response, presumably in the activation of particular stress-responsive genes.

Consistent with these observations, we have obtained several lines of evidence suggesting that Skn7 and Hsf1 interact physically. First, in extracts prepared from strains coexpressing Skn7-HA and GST-Hsf1, HA-tagged Skn7 copurified with GST-Hsf1 on glutathione-Sepharose beads. Physical

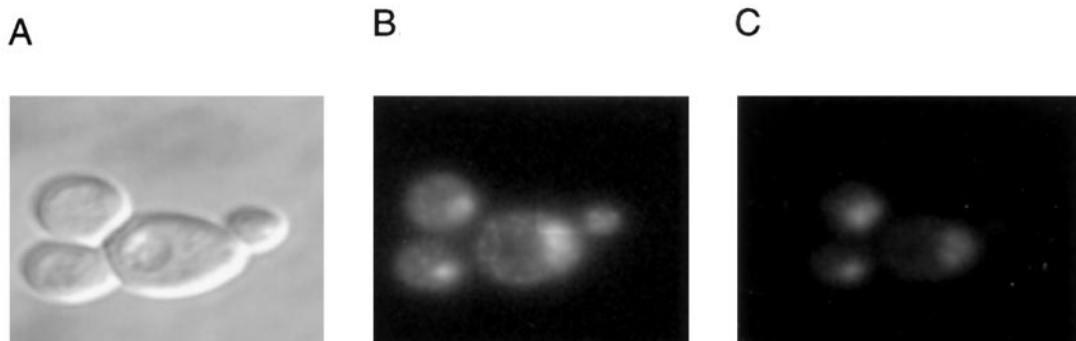


Figure 8. The Skn7 response regulator is localized in the nucleus. An SKN7-GFP fusion protein expressed from a CEN plasmid was visualized in cells from a midlog-phase culture (A; Nomarski image). The Skn7p-GFP fusion protein visualized by fluorescence (C) colocalizes with the DAPI signal of nuclear DNA (B).

interaction between these proteins was also shown with the use of a complementary pull-down assay; Hsf1 associated with 6His-tagged Skn7 on nickel-agarose beads. Previous work had suggested that Skn7 interacts with a number of proteins, including Yap1 (Morgan *et al.*, 1997), Rho1 (Alberts *et al.*, 1998), Sln1-Ypd1 (Li *et al.*, 1998), and Mbp1 (Bouquin *et al.*, 1999). In no case has coimmunoprecipitation been demonstrated with any of these proteins. Only a small proportion of total cellular Skn7 may associate with any of these proteins at one time, rendering coimmunoprecipitation studies difficult. Nonetheless, the copurification of Skn7 with Hsf1 described here argues strongly for a physical interaction.

Skn7 Is Required to Activate Heat Shock Gene Expression Specifically in Response to Hydrogen Peroxide

An implication of the interaction between Hsf1 and Skn7 is a role for Skn7 in regulating heat shock gene expression. Preliminary studies with the use of an *SSA1-LacZ* fusion construct indicated that Skn7 was required for HSE-mediated *LacZ* induction in response to hydrogen peroxide but was not required for the heat shock induction of the reporter (Table 1). Northern analysis of heat shock gene expression in wild-type and *skn7Δ* cells confirmed that *SKN7* was not required for induction of these genes in response to heat shock. For example, when cells are shifted from 25 to 39°C, the heat shock induction of *HSP12* in *skn7Δ* cells was identical to that in the isogenic wild-type strain. In contrast, the 5-fold induction of this gene by hydrogen peroxide in the wild type was largely abolished in the isogenic *skn7Δ* strain. The *skn7Δ* mutation also significantly reduced the 20-fold induction of *HSP26* and the 9-fold induction of *HSP104* by hydrogen peroxide without affecting their responses to heat shock activation (Figure 4). The residual induction of these genes in response to oxidative stress could be dependent on the other known activators of these genes, such as Hsf1 and the Msn2/Msn4 transcription factor. The latter can activate expression through upstream activation sequence elements unrelated to HSEs (Martínez-Pastor *et al.*, 1996; Schmitt and McEntee, 1996).

The requirement for Skn7 in hydrogen peroxide induction of *HSP12* is intriguing given that induction of this gene by a variety of other stresses has been shown to be mediated through STRE sequences by Msn2/Msn4 (Martínez-Pastor *et al.*, 1996) and, in response to osmotic shock, by the high osmolarity glycerol (HOG) MAPK pathway (Varela *et al.*, 1995). We note, however, that hydrogen peroxide induction of this and other STRE-regulated genes does not appear to be affected significantly by mutations in Msn2 or Msn4 (Schüller *et al.*, 1994). It appears likely, therefore, that regulation of *HSP12* (and perhaps other STRE-regulated genes such as *HSP26* and *HSP104*) in response to hydrogen peroxide-generated oxidative stress is mediated principally by Skn7 and Hsf1, whereas activation in response to other stress conditions is regulated through the STRE elements and downstream effectors of the HOG pathway. In this context, it is interesting that the HOG1 pathway is itself regulated by the *SLN1* histidine kinase, which also seems to regulate the activity of the Skn7 response regulator (Li *et al.*, 1998).

Structural Homology between Skn7 and Hsf1

With regard to the Skn7-Hsf1 interaction in the oxidative stress response, the structural similarities of the two proteins are of particular interest. We have shown through EMSA that Skn7 purified from yeast or *E. coli* can bind to a 26-base pair probe derived from the HSE2 regulatory region of the *SSA1* promoter. This binding is of a similar specificity to that of Hsf1, insofar as mutation of the GAAnnTTC sequence to AAAnnTTT ablates binding of both Hsf1 and Skn7. Previously, we identified a regulatory site within the TRX2 promoter through which Skn7 can act (Morgan *et al.*, 1997). The site contains the sequence CCGAAA in which mutation of the CG nucleotides to AT was found to reduce the binding of Skn7 by 20-fold. The common motif between this regulatory sequence and HSEs is the GAA triplet, three inverted repeats of which in the sequence AGAAAn constitute a consensus HSE. Although the exact consensus binding site for Skn7 has not been established, the triplet GAA evidently represents a potential core recognition sequence.

In terms of sequence specificity of Skn7 recognition of HSEs versus that of Hsf1, there is one notable divergence between their otherwise highly conserved DNA-binding domains. This occurs at the last residue of the $\alpha 3$ sequence recognition helix of Hsf1 (Harrison *et al.*, 1994), where the invariant M58 and G60 residues are replaced by K58 and D60, respectively, in the Skn7 protein (with residues numbered according to Figure 1). These substitutions may have a significant effect on DNA-binding specificity or the stability of Skn7 relative to Hsf1 because G60 of Hsf1 has been proposed to contact the DNA (Damberger *et al.*, 1994). All other residues proposed to contact the DNA, however, are conserved between Skn7 and Hsf1.

The other region of structural homology between these two proteins lies between residues 222 and 303 of the Skn7 protein. This stretch contains five heptad repeats, with hydrophobic residues at positions 1 and 4 and polar residues elsewhere in the repeat units, characteristic of regions that form coiled-coil structures (Lupas, 1996). Hsf1 contains six heptad repeats that have been shown to mediate trimerization of the protein through the formation of triple-stranded α -helical coiled coils (Sorger and Nelson, 1989; Peteranderl and Nelson, 1992; Rabindran *et al.*, 1993). These coiled coils are also known to mediate heterodimerization and homodimerization, e.g., of the yeast GCN4 member of the bZIP transcription factor family (Harbury *et al.*, 1993). We have demonstrated that Skn7, in common with Hsf1, can interact with itself *in vivo* and that these proteins can interact with each other. We have also shown that the Skn7 response regulator is constitutively localized to the nucleus and can bind to HSEs with a similar specificity to that of Hsf1. We are currently exploring the possibility that it is through the formation of heterodimers and/or heterotrimers that Skn7 and Hsf1 can mediate the activation of heat shock genes, and possibly other sets of genes, in response to oxidative stress. The markedly increased sensitivity to oxidative stress of an *hsf1^{ts} skn7Δ* strain relative to either single mutant (Figure 6) supports such a cooperative association.

In summary, we have shown that Skn7 interacts with Hsf1 and can bind to the same consensus sequence as Hsf1. We have further shown that *SKN7* is required for the induction of heat shock genes in response to *t*-butyl hydrogen peroxide, although it is not required for their

heat shock induction. In addition, we have demonstrated for the first time the nuclear localization of a response regulator in yeast, which was found to be independent of the presence or absence of oxidative stress. In the light of these data, we propose a model whereby Skn7 becomes activated in response to hydrogen peroxide stress and either by dimerization or through the formation of a heterotrimeric Skn7–Hsf1 complex binds to the promoters of stress-responsive genes. The actual mechanism by which either Skn7 or Hsf1 activates gene expression in response to stress remains unclear, although recent evidence suggests that Hsf1 can interact with TATA-binding protein (Mason and Lis, 1997) and with a phosphatase that may modulate the transcriptional activity of a subset of promoters, including CUP1, through interaction with the repressor region of Hsf1 (Lin and Lis, 1999). Hsf1 can also antagonize nucleosomal repression (Erkine *et al.*, 1996). It is possible, therefore, that the transcription activation function of Hsf1 is enhanced by its interaction with Skn7 or that Skn7 itself potentiates the interactions with components of the basal transcription apparatus at heat shock core promoters in response to oxidative stress.

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