Molecular Biology of the Cell
Vol. 11, 2631–2642, August 2000

Thioredoxin Peroxidase Is Required for the Transcriptional Response to Oxidative Stress in Budding Yeast

Sarah J. Ross, Victoria J. Findlay, Panagiota Malakasi, and Brian A. Morgan*

School of Biochemistry and Genetics, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, United Kingdom

Submitted December 6, 1999; Revised April 27, 2000; Accepted June 1, 2000
Monitoring Editor: Trisha N. Davis

A genetic screen was performed in *Saccharomyces cerevisiae* to identify mechanisms important for the transcriptional activation of genes encoding antioxidant proteins. Thioredoxin peroxidase, Tsa1p, of the thioredoxin system, was found to be essential for the transcriptional induction of other components of the thioredoxin system, *TRX2* (thioredoxin) and *TRR1* (thioredoxin reductase), in response to H_2O_2. The expression of *TRX2* and *TRR1* is known to be regulated by the transcription factors Yap1p and Skn7p in response to H_2O_2, and the Tsa1p-dependent regulation of *TRX2* requires the Yap1p/Skn7p pathway. The data suggest that expression of components of the thioredoxin system is dependent on the activity of Tsa1p in response to H_2O_2 in a Yap1p/Skn7p-dependent pathway.

INTRODUCTION

Oxidative stress (OS) is an unavoidable consequence of oxygen metabolism and therefore occurs in the cells of all aerobic organisms. OS is a state within the cell in which the level of reactive oxygen species, such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\cdot$), exceeds the available antioxidant defenses that scavenge and inactivate the reactive oxygen species. It is important that aerobic cells respond to OS, because reactive oxygen species are chemically very reactive and consequently damage intracellular components. Such damage has been implicated in aging, all stages of cancer, and numerous other human diseases (for a review, see Halliwell, 1987). Thus, oxygen-utilizing cells have evolved defense mechanisms to protect against the damage caused by OS called the oxidative stress response (OSR). However, despite the importance of the OSR in maintaining homeostasis, little is known about how this response is regulated.

The yeast *Saccharomyces cerevisiae* is an important model organism for the study of the eukaryotic OSR (Jamieson, 1998). The discovery of the OSR in *S. cerevisiae* followed observations that pretreatment of cells with low doses of an oxidizing agent led to an increase in resistance to subsequent treatment with a higher dose (Jamieson, 1992). The tolerant state is achieved by increasing the production of antioxidant defense proteins through an increase in gene expression induced by exposure to low doses of oxidants (Jamieson *et al.*, 1994).

* Corresponding author. E-mail address: b.a.morgan@ncl.ac.uk.

The thioredoxin system is an important conserved system for protection against OS by reducing peroxides such as H_2O_2 to harmless products. The system is composed of three proteins, thioredoxin peroxidase (Tsa1p), thioredoxin (Trx2p), and thioredoxin reductase (Trr1p) (Chae *et al.*, 1994; Netto *et al.*, 1996) (Figure 1A). The expression of genes encoding the components of the thioredoxin system in *S. cerevisiae*, *TSA1*, *TRX2*, and *TRR1*, is induced in response to H_2O_2 (Kuge and Jones, 1994; Morgan *et al.*, 1997; Godon *et al.*, 1998; Lee *et al.*, 1999).

Two transcription factors, Yap1p and Skn7p, are involved in the H_2O_2-induced expression of *TSA1*, *TRX2*, and *TRR1* in *S. cerevisiae* (Kuge and Jones, 1994; Morgan *et al.*, 1997; Lee *et al.*, 1999). Yap1p is a member of the c-Jun family of proteins, containing a basic leucine zipper domain characteristic of all Ap-1–like proteins. An important method of regulating Yap1p activity by the OSR involves regulation of the nuclear localization of Yap1p mediated by its cysteine-rich C-terminal region (Kuge *et al.*, 1997; Wemmie *et al.*, 1997). However, other regions of Yap1p have also been suggested to play a role in the OS regulation of Yap1p (Wemmie *et al.*, 1997). Although Skn7p has been shown to bind to the *TSA1* and *TRX2* promoters (Morgan *et al.*, 1997; Lee *et al.*, 1999), the mechanism by which OS regulates Skn7p activity is not understood but may involve regulation by the Ras/PKA pathway (Charizanis *et al.*, 1999). Skn7p is a member of the response regulator protein family (Brown *et al.*, 1993, 1994; Morgan *et al.*, 1995; Krems *et al.*, 1996), the members of which are usually transcription factors that regulate gene transcription through a two-component signal transduction system (for a review, see Stock *et al.*, 1989). However, results suggest that the two-component mechanism is

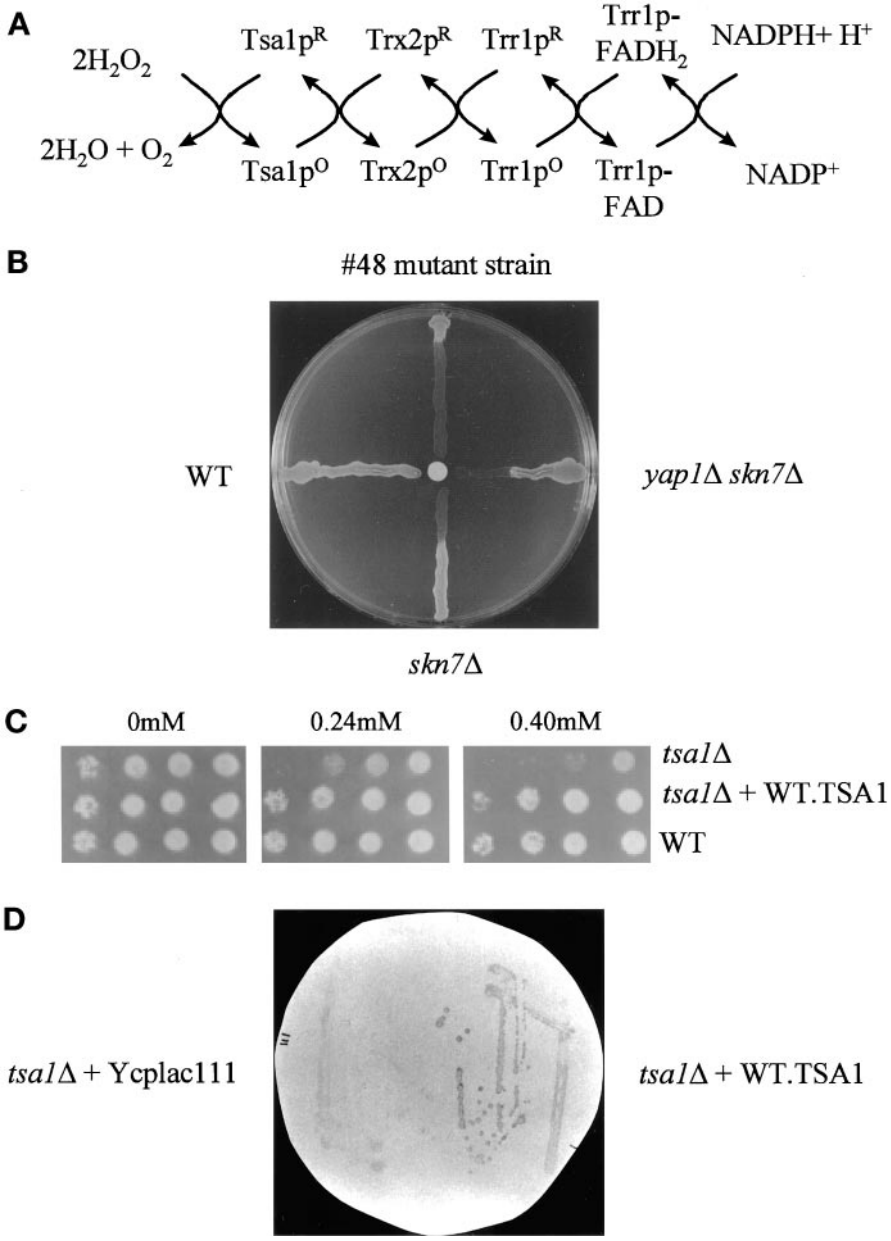

Figure 1. Genetic analysis of the H_2O_2-induced expression of the *TRX2* gene. (A) The likely mechanism by which the thioredoxin system reduces peroxides. The transfer of electrons from NADPH to peroxide in the thioredoxin system is thought to occur in sequence. The electrons flow from NADPH to two tightly bound FAD molecules and from FAD to a redox-sensitive disulfide in thioredoxin (Trx2p), which can then reduce the disulfide linkages within thioredoxin peroxidase (Tsa1p). Tsa1p then passes the electrons onto the peroxide, reducing it to a harmless product and completing the electron transfer. Adapted from Chae *et al.*, 1994. R and O indicate the reduced and oxidized forms of the respective proteins. (B) The sensitivity of W303-1a, *skn7Δ*, *yap1Δskn7Δ*, and the #48 mutant strains to H_2O_2. (C) Spot tests showing the sensitivity of the *tsa1Δ* strain to *t*BOOH. Strains were *tsa1Δ*, *tsa1Δ* containing WT.TSA1, and W303-1a. (D) H_2O_2-induced *lacZ* assays of the *tsa1Δ* strain containing either the Ycplac111 vector or the WT.TSA1 plasmid.

probably not important for the regulation of Skn7p in response to H_2O_2 (Morgan *et al.*, 1997). Thus, although Skn7p and Yap1p regulate gene expression in response to OS, the mechanisms by which regulation takes place are not well characterized. Furthermore, Skn7p and Yap1p may not be the only transcriptional mechanisms that are used for the regulation of *TRX2* expression in response to H_2O_2, because a *skn7Δyap1Δ* strain still shows some residual *TRX2* induction (Morgan *et al.*, 1997).

To identify proteins involved in the signal transduction pathways responsible for sensing and regulating the H_2O_2-induced expression of the *TRX2* gene, a genetic screen was performed in *S. cerevisiae*. Mutants were isolated that affected the expression of a *TRX2* promoter *lacZ* fusion in response to H_2O_2. Cloning and characterization of one such mutant identified Tsa1p as a novel regulator of *TRX2* and *TRR1* expression in response to H_2O_2.

MATERIALS AND METHODS

Yeast Strains and Growth Conditions

The *Saccharomyces cerevisiae* strains used were W303-1a haploid (**a** *ade2-1 trp1-1 can1-100 leu2-3,112 his3-11 ura3*), W303-1a diploid (**a/α** *ade2-1 trp1-1 can1-100 leu2-3,112 his3-11 ura3*), *skn7Δ* (**a** *ade2-1 trp1-1 can1-100 leu2-3,112 his3-11 ura3 SKN7::HIS3*), and *yap1Δ* (**α** *ade2-1 trp1-1 can1-100 leu2-3,112 his3-11 ura3 YAP1::TRP1*).

S. cerevisiae strains were grown in either rich YPD medium for nonselective growth or minimal SD medium for selective growth (Sherman *et al.*, 1986). For sporulation, medium contained 1% po-

tassium acetate, 0.1% yeast extract, 0.05% dextrose, and 2% agar (Sherman *et al.*, 1986). All strains were grown at 30°C unless stated otherwise.

Thirty percent H_2O_2 and 70% tetra-butyl hydroperoxide (*t* BOOH) were both obtained from Sigma Chemical (St. Louis, MO) and used at the concentrations given below. Restriction enzymes and DNA polymerases were from Promega (Madison, WI).

Yeast Techniques

Yeast cells were transformed with the use of the lithium acetate method described by Schiestl and Gietz (1989). Plasmids were isolated from yeast cells as described by Robzyk and Kassir (1992). Genomic DNA was isolated from yeast cells as described by Hoffman and Winston (1987).

Genetic Screen

To identify mutants that affect the H_2O_2-induced expression of *TRX2* in *S. cerevisiae*, a reporter plasmid was used that contains the *Escherichia coli lacZ* gene fused to the *TRX2* promoter region (*TRX2lacZ*) (Kuge and Jones, 1994). When this plasmid is introduced into *S. cerevisiae*, the expression of the *lacZ* gene is induced in response to OS in a manner mimicking that of chromosomal *TRX2* (Kuge and Jones, 1994). Haploid wild-type *S. cerevisiae* cells containing the *TRX2lacZ* plasmid were mutagenized with UV light, and mutations were identified that showed reduced or abolished H_2O_2-induced expression of *lacZ*. As an additional means of selecting mutants affecting chromosomal *TRX2* expression, mutants that also displayed increased sensitivity to H_2O_2 were favored because a *trx2*Δ strain has increased sensitivity to H_2O_2 (Kuge and Jones, 1994).

LacZ Expression Assays

H_2O_2-induced *lacZ* expression studies were performed by β-galactosidase filter assays by the method of Guarente (1983). Filters of cells containing the *TRX2lacZ* plasmid (Kuge and Jones, 1994) were treated with 1 mM H_2O_2 for 90 min at 30°C, and then β-galactosidase filter assays were performed. The filters were incubated at 37°C and examined at 1-h intervals for β-galactosidase activity as determined by the development of blue color.

Deletion of the TSA1 Gene

The *tsa1*Δ strain was made by transformation of the wild-type W303-1a diploid strain with a 1.4-kilobase (kb) deletion fragment containing the *HIS3* gene flanked by approximately 70 bases of genomic sequence from upstream of the *TSA1* start codon and downstream of the *TSA1* stop codon. The deletion fragment was obtained by PCR from the YDp-H vector (Berben *et al.*, 1991) with the use of the oligonucleotide primers TSA1.3 and TSA1.4. Deletions were confirmed by PCR with the use of the primers TSA1.1 and TSA1.2. Haploid *tsa1*Δ cells were isolated by dissection of the heterozygous diploid strain. Attempts to obtain the *tsa1*Δ directly in a haploid strain were unreliable, because several different possible deletions were obtained that displayed peroxide sensitivities ranging from similar to that of the *skn7*Δ and *yap1*Δ strains to similar to that of the wild-type strain.

The *tsa1*Δ*skn7*Δ and *tsa1*Δ*yap1*Δ double mutants were obtained by dissection of heterozygous diploids constructed by mating a *tsa1*Δ strain with either the *skn7*Δ or the *yap1*Δ strain.

Oligonucleotide Primer Sequences

Lib.1 (5′CTGGTTGACTTGTGCATGAACACGAGC3′) and Lib.2 (5′ACCGAGGTGATACAATCTACC3′) were used to sequence the inserts in the suppressor library plasmids. TSA1.1 (5′CGTCAGATCAATGCCGAACCGTTC3′) and TSA1.2 (5′GAGCTAGTGTGAATAGCTTCTTAGACGG3′) were used to amplify and sequence the *TSA1* gene. TSA1.3 (5′CGGGCCTTCCCCTCGTTCAATTGCTCACAACCAACCACAACTACATACACATACATACACAATGGAATTCCCGGGGATCCGGTGATTG3′) and TSA1.4 (5′AAGTATAAACGTAAAGAGTGAATTTTAAATAAGTAGTCATTTAGACAACTCTGCAAGCGCTTTAAAGCTAGCTTGGCTGCAGGTCGACGC3′) were used to construct the *TSA1* disruption fragment. CTT1.1 (5′CCGTTGGTGGTGAAAGTGGTACAC3′) and CTT1.2 (5′GGACACTGTTCGGCAGTGTATTGG3′) were used for amplification of the catalase probe. TSA1.M1 (5′GCAATGCGATGTGGCCACGTTATATAATGC3′), TSA1.M2 (5′GAGTGGTTGGTGTCAGACAACAATGGAATG3′), TSA1.M3 (5′CTTCTTCGATCGTGACACCATAGTCTCTGG3′), and TSA1.M4 (5′CGGGCACCAGAAGGAATTCGCGGTG3′) were used to form the TSA^{AS}, TSA^{LT}, and $TSA^{AS/LT}$ constructs.

Plasmid Constructs

To form the WT.TSA1 construct, a Yep24 library plasmid, containing the wild-type *TSA1* gene, was digested with *Pst*I and a 2.25-kb fragment containing the *TSA1* gene and promoter region was isolated. The *TSA1*-containing fragment was then ligated into *Pst*I-digested Ycplac111 to generate WT.TSA1. Ycplac111 is a centromeric *LEU2* plasmid (Gietz and Sugino, 1988).

The high-copy *TSA1* plasmid (Yep24-TSA1) contains the *TSA1* gene and promoter region in the high-copy 2μ vector Yep24 and was isolated as a library plasmid that could suppress the #48 mutant strain phenotypes.

To form the construct $TSA1^{AS}$, a two-step PCR method was used. This method introduced a G/C-to-T/A base pair (bp) substitution at position 303 in the *TSA1* DNA sequence, resulting in an alanine-to-serine amino acid change. In the first step, a 0.576-kb *TSA1* DNA product was amplified by PCR from the WT.TSA1 plasmid with the use of the primers TSA1.M1 and TSA1.M2. TSA1.M2 contains the C-to-A base change at position 303 in *TSA1* and amplifies a PCR product that contains the G/C-to-T/A base mutation at bp 303. In the next PCR step, a 1.18-kb full-length *TSA1* product (also containing the G/C-to-T/A bp change) was amplified from the WT.TSA1 plasmid with the use of the 0.576-kb *TSA1* product amplified from the first PCR as one primer and TSA1.M4 as the other primer. The *TSA1* PCR product was then digested with *Eco*RI and ligated into Ycplac111 digested with *Eco*RI and *Sma*I to create $TSA1^{AS}$. The $TSA1^{LT}$ construct was generated with the use of the same two-step PCR method described to make $TSA1^{AS}$ except that a *TSA1* sequence containing the TT/AA-to-AC/TG bp changes at positions 342 and 343, encoding the leucine-to-threonine amino acid change, was amplified. The oligonucleotide primers used in the first stage PCR were TSA1.M1 and TSA1.M3 (containing the AA-to-TG base changes at 342 and 343), which produced a 0.616-kb product. The first-step PCR product was then used in the second-stage PCR with the oligonucleotide primer TSA1.M4 to amplify the full-length *TSA1* gene containing the TT/AA-to-AC/TG bp changes. The *TSA1* PCR product was then digested with *Eco*RI and ligated into Ycplac111 digested with *Eco*RI and *Sma*I to create $TSA1^{LT}$. The $TSA1^{AS/LT}$ construct, which encodes a Tsa1p containing both the alanine-to-serine and leucine-to-threonine amino acid changes, was generated essentially as described for the $TSA1^{LT}$ construct. However, instead of WT.TSA1, the $TSA1^{AS}$ construct was used as a DNA template for the first- and second-step PCR reactions.

DNA Sequencing

All DNA sequencing was performed by the Molecular Biology Sequencing Center (University of Newcastle upon Tyne) with the use of the appropriate oligonucleotide primers.

Sensitivity Tests

To test the sensitivity of the isolated mutants to H_2O_2, cells were resuspended in 20 μl of water and streaked onto SD medium. A

3-mm filter paper circle (Whatman [Clifton, NJ] microfiber paper) was soaked in 15 μl of 10% H_2O_2 and placed in the center of the plate. Plates were incubated for 2 d at 30°C, and the zone of inhibited growth was measured.

For spot tests, strains were grown to midlog phase ($\sim 1 \times 10^7$ cells/ml) and serial 10-fold dilutions were made. Five microliters of undiluted strain and each of the dilutions were spotted onto medium containing various concentrations of *t*BOOH. Plates were incubated at 25°C for 2–3 d, and sensitivity was examined.

RNA Analysis

RNA was extracted, with the use of a method described previously by Aves *et al.* (1985), from cells of midlog-phase strains growing in SD medium. Northern blotting was performed as described previously (Morgan *et al.*, 1995), and the blots were probed with various gene-specific probes. The *TRX2* and *TRR1* probes were obtained by PCR with the use of gene-specific oligonucleotides (Morgan *et al.*, 1997). The *CTT1* probe was obtained by PCR with the use of the gene-specific oligonucleotides CTT1.1 and CTT1.2. The *ACT1* probe was used as a loading control (Morgan *et al.*, 1997). Probed membranes were autoradiographed with Fuji Medical (Tokyo, Japan) x-ray film (Super RX) for the desired time and then developed. Alternatively, membranes were exposed to a Phosphorimager plate and analyzed with the use of a Phosphorimager (Bio-imaging analyzer Fuji film Bas-1500). The data obtained were quantitated with the use of Tina 2.0 software (Raytest, Straubenhardt, Germany).

RESULTS

Genetic Screen for Mutations That Regulate the OSR

A genetic screen was performed to isolate mutations in genes important for the H_2O_2-induced regulation of the *TRX2* gene (see MATERIALS AND METHODS). One mutant isolated from the screen, mutant #48, was of particular interest because of several phenotypes. First, H_2O_2-induced expression of *lacZ* from a *TRX2lacZ* reporter plasmid was reduced. Second, it was extremely sensitive to peroxides, showing a much greater sensitivity than the isogenic wild-type, *skn7*Δ, and *yap1*Δ*skn7*Δ strains (Figure 1B). To further characterize the mutation(s) in the #48 mutant, the strain was mated with a wild-type haploid and the heterozygous diploid was sporulated. Analysis of tetrads suggested that the abolished H_2O_2-induced expression of *lacZ* from the *TRX2lacZ* plasmid and the increased sensitivity to peroxides (H_2O_2 and *t*BOOH) were the result of a single mutation or very closely linked gene mutations. In addition, characterization of the heterozygous diploid indicated that both of the phenotypes exhibited by the #48 mutant were only partially rescued by the presence of the wild-type locus.

Identification of the Mutated Gene

The ability of the heterozygous diploid to grow on medium containing 0.16 mM *t*BOOH, a concentration lethal to the haploid #48 mutant, was used to clone the wild-type allele. A high-copy *S. cerevisiae* genomic library, ligated into the Yep24 vector (kindly provided by L.H. Johnston, NIMR, London), was introduced into the haploid #48 mutant and transformants were identified that could grow on medium containing 0.16 mM *t*BOOH. Approximately 30,000 transformants were screened, and 10 plasmids that enabled the mutant to grow on 0.16 mM *t*BOOH were isolated and analyzed. Sequencing of the inserts from the suppressor plasmids with the use of the oligonucleotide primers Lib.1 and Lib.2 revealed that 8 of the 10 suppressor plasmids contained genomic inserts, which shared a region of chromosome XIII spanning two hypothetical ORFs and *TSA1*, a gene that had previously been shown to protect against OS.

To determine whether the *TSA1* gene was responsible for suppression of the phenotypes observed in the #48 mutant, the wild-type *TSA1* gene and promoter region were ligated into the centromeric vector, Ycplac111 (WT.TSA1) (Gietz and Sugino, 1988). The WT.TSA1 plasmid or the Ycplac111 vector was then introduced into the #48 mutant haploid strain for complementation studies. The Ycplac111 vector was unable to complement any of the phenotypes observed in the #48 mutant (our unpublished results). However, WT.TSA1 partially complemented the #48 mutant phenotypes of increased peroxide sensitivity and reduced H_2O_2-induced expression of *lacZ* from the *TRX2lacZ* plasmid, behaving very similarly to the #48/wild-type heterozygous diploid (our unpublished results). This is the expected result if the #48 mutant phenotypes are due to mutation(s) of the *TSA1* gene, because the #48 mutant phenotypes are only partially rescued in a heterozygous diploid.

To confirm that *TSA1* was indeed mutated in the #48 mutant strain, the *TSA1* gene was amplified independently several times by PCR with the use of the oligonucleotide primers TSA1.1 and TSA1.2 from the genome of the #48 mutant and DNA sequences were obtained. Analysis of the DNA sequences revealed that *TSA1* from the #48 mutant encoded two amino acid changes, an alanine-to-serine substitution at position 102 and a leucine-to-threonine substitution at position 114.

Phenotype of the tsa1Δ Strain

To examine the role of Tsa1p in the regulation of gene expression, a *tsa1*Δ strain was constructed. The *tsa1*Δ haploids were examined for sensitivity to H_2O_2 and *t*BOOH and also to H_2O_2-induced expression of the *lacZ* gene from the *TRX2lacZ* plasmid. Similar to mutant #48, the *tsa1*Δ strain was more sensitive than the isogenic wild-type strain to both H_2O_2 (our unpublished results) and *t*BOOH (Figure 1C) and also showed a reduction in H_2O_2-induced expression of *lacZ* (Figure 1D). Introduction of WT.TSA1 into the deletion strain rescued both the peroxide sensitivity and the reduced H_2O_2-induced expression of the reporter construct, confirming that both of these phenotypes were the result of losing Tsa1p function (Figure 1, C and D). The similar phenotypes of the *tsa1*Δ strain and mutant #48 strongly suggest that the phenotypes of the #48 mutant are the result of the loss of Tsa1p function. However, the #48 mutant phenotypes are only partially rescued in a heterozygous diploid, suggesting that the mutant *tsa1* is semidominant but that *tsa1*Δ is recessive. Hence, the mutant Tsa1p in the #48/wild-type heterozygous diploid may also be interfering with the activity of the wild-type Tsa1p.

Loss of Tsa1p Reduces the H_2O_2-induced Expression of Native Chromosomal TRX2

To determine whether the reduced H_2O_2-induced expression of *lacZ* from the *TRX2lacZ* plasmid represented a reduction in induction of the normal *TRX2* promoter in response to H_2O_2, Northern blot analysis was performed on

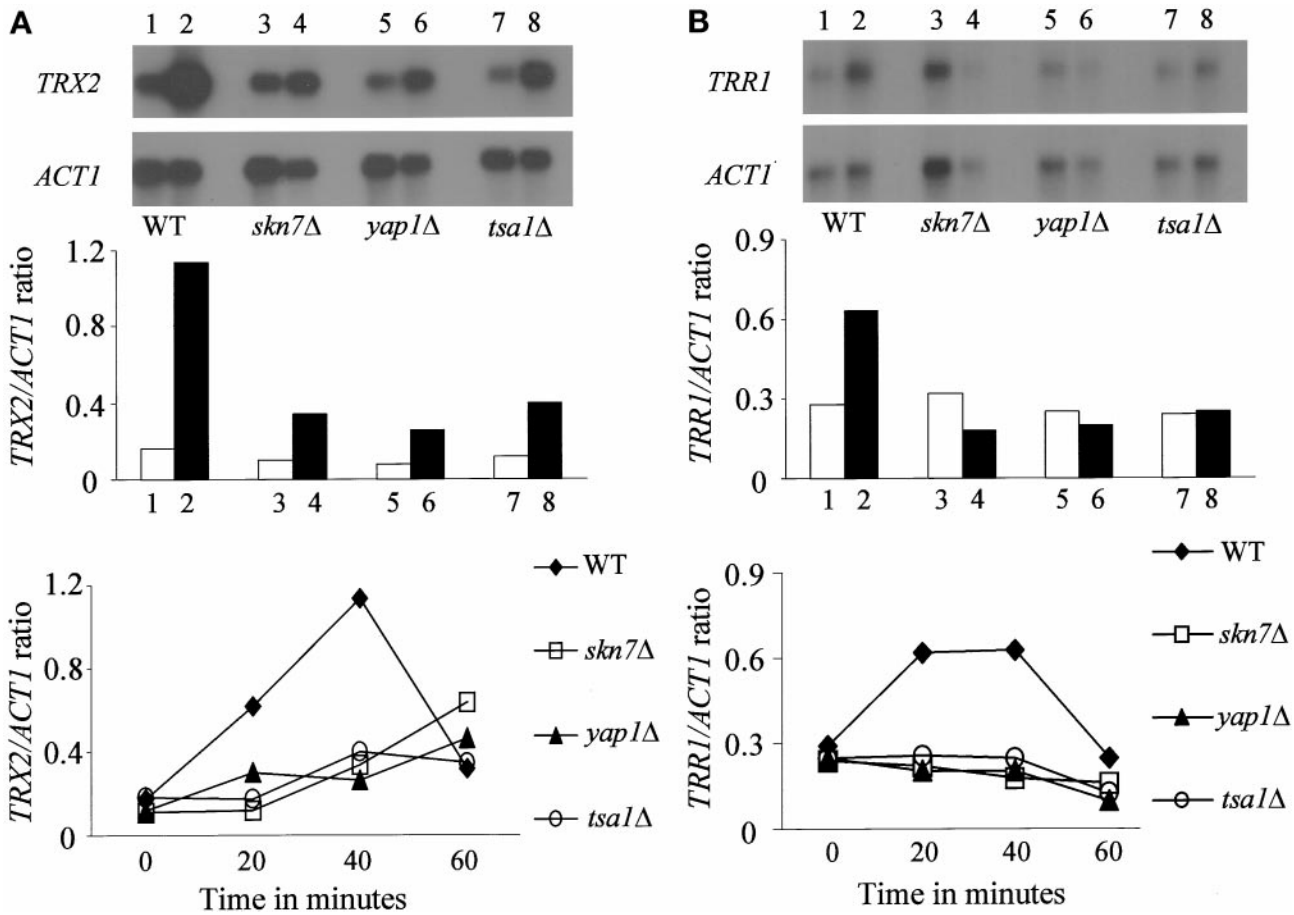

Figure 2. Tsa1p regulates the expression of *TRX2* and *TRR1* in response to H_2O_2. Northern blot analysis of RNA isolated from different midlog-phase growing cultures either untreated (lanes 1, 3, 5, and 7) or treated with 0.1 mM H_2O_2 (lanes 2, 4, 6, and 8) for 40 min with the use of probes specific for *TRX2* (A) or *TRR1* (B) and *ACT1*. Strains were W303-1a (lanes 1 and 2), *skn7*Δ (lanes 3 and 4), *yap1*Δ (lanes 5 and 6), and *tsa1*Δ (lanes 7 and 8). The panels below the Northern blots show quantitation of the *TRX2* and *TRR1* transcript levels relative to the *ACT1* transcript. The line graphs represent the kinetics of *TRX2* and *TRR1* induction in the W303-1a, *skn7*Δ, *yap1*Δ, and *tsa1*Δ strains during 60 min of incubation with 0.1 mM H_2O_2.

RNA isolated from *tsa1*Δ cells. Total cellular RNA was isolated from untreated cells and cells treated with 0.1 mM H_2O_2 and examined for both *TRX2* and *ACT1* RNA levels (Figure 2A). These results showed that deletion of the *TSA1* gene did not affect the basal expression of *TRX2*. However, the *tsa1*Δ strain showed reduced H_2O_2-induced expression of chromosomal *TRX2* compared with the isogenic wild-type strain, in agreement with the *lacZ* reporter analysis. In addition, the *tsa1*Δ strain behaved very similarly to the *skn7*Δ and *yap1*Δ strains in that a weak residual induction of *TRX2* was evident. Yet, although the *tsa1*Δ, *skn7*Δ, and *yap1*Δ strains show a weak residual induction of *TRX2*, the kinetics of *TRX2* induction is different from that observed in the wild-type strain (Figure 2A). The wild-type strain responds rapidly to treatment with 0.1 mM H_2O_2, with *TRX2* maximally expressed after 40 min. In contrast, the *yap1*Δ, *skn7*Δ, and *tsa1*Δ strains do not reach maximal H_2O_2-induced *TRX2* expression until at least 60 min of incubation.

Trr1p is also involved in the thioredoxin system (Figure 1A) and, like *TRX2*, *TRR1* expression is induced in response to H_2O_2 in a Skn7p- and Yap1p-dependent manner (Morgan *et al.*, 1997). Hence, it was possible that *TRR1* expression may also be affected by the loss of Tsa1p. Indeed, Northern blot analysis showed that, like *TRX2*, the H_2O_2-induced expression of *TRR1* is reduced in the *tsa1*Δ strain compared with the wild-type strain (Figure 2B). The pattern of H_2O_2-induced expression of *TRR1* in the *tsa1*Δ strain is very similar to that seen in the *skn7*Δ and *yap1*Δ strains, with no induction of *TRR1* expression apparent (Figure 2B).

To confirm that the reduction in H_2O_2-induced *TRX2* and *TRR1* expression was the result of the loss of Tsa1p, gene expression was examined in a *tsa1*Δ strain that contained either Ycplac111 or the WT.TSA1 plasmid. The results demonstrated that the *tsa1*Δ strain containing the Ycplac111 vector alone showed a reduction in H_2O_2-induced expression of the *TRX2* (Figure 3A) and *TRR1* (Figure 3B) genes, whereas the introduction of WT.TSA1 restored the H_2O_2-dependent induction of the *TRX2* (Figure 3A) and *TRR1* (Figure 3B) genes to wild-type levels.

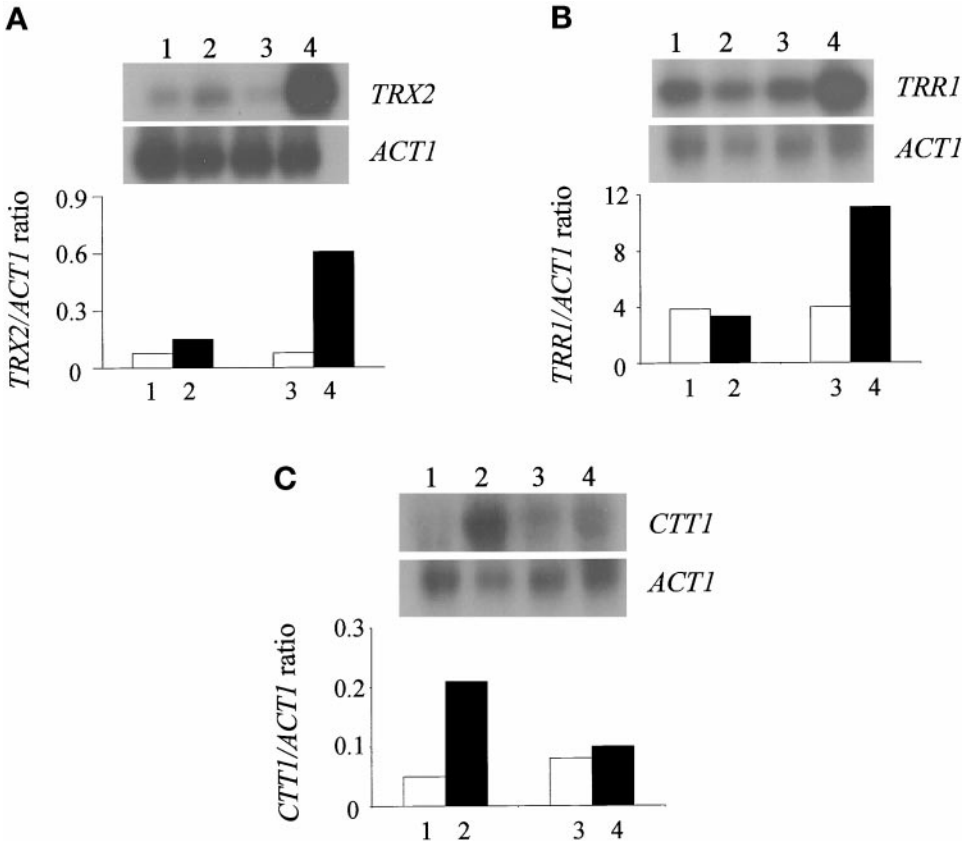

Figure 3. Introduction of the wild-type *TSA1* gene into a *tsa1*Δ strain restores the normal H_2O_2 induction of *TRX2*, *TRR1*, and *CTT1*. Northern blot analysis of RNA isolated from a midlog-phase culture of a *tsa1*Δ strain containing either Ycplac111 (lanes 1 and 2) or WT.TSA1 (lanes 3 and 4), which were either untreated (lanes 1 and 3) or treated with 0.1 mM H_2O_2 for 20 min (lanes 2 and 4) with the use of probes specific for *TRX2* (A), *TRR1* (B), or *CTT1* (C) and *ACT1*. The panels below the Northern blots show quantitation of the different transcripts relative to the *ACT1* transcript.

Loss of Tsa1p Induces Expression of the Catalase Gene

To determine whether the loss of Tsa1p reduced the transcription of another antioxidant-encoding gene, or whether it was specific for the thioredoxin system, the H_2O_2-induced expression of *CTT1* was examined in a *tsa1*Δ strain. The *CTT1* gene encodes the antioxidant protein catalase, which is involved in the detoxification of both H_2O_2 and superoxide radicals from the cell (Winkler *et al.*, 1988). Like that of *TSA1*, *TRX2*, and *TRR1*, the expression of *CTT1* is induced in response to H_2O_2. However, the level of *CTT1* induction is dose dependent, whereas *TRR1* levels appear to be maximally induced at low concentrations of H_2O_2 (Godon *et al.*, 1998). Northern blot analysis was performed on RNA isolated from *tsa1*Δ cells with the use of probes specific to *CTT1* and *ACT1*. Unlike the expression of *TRX2* and *TRR1*, the loss of Tsa1p was found to increase the H_2O_2-induced expression of *CTT1* (Figure 3C) compared with the wild-type strain. Introduction of WT.TSA1 into the *tsa1*Δ strain restored the wild-type expression of *CTT1* (Figure 3C). At the concentration of H_2O_2 used, only a small induction of *CTT1*, if any, was expected (Godon *et al.*, 1998; our unpublished results). In the *tsa1*Δ mutant, the effective H_2O_2 concentration is likely to be higher than that in the wild-type strain because one of the main pathways for H_2O_2 detoxification has been weakened considerably.

Analysis of the Effects of TSA1 Point Mutations on Tsa1p Function

To determine whether the amino acid alterations encoded by the mutant *TSA1* gene were important for the phenotypes that were observed in the #48 mutant, three constructs were made in the centromeric plasmid Ycplac111. These constructs contained the *TSA1* gene encoding the Ala^{102}-to-Ser^{102} amino acid substitution (construct $TSA1^{AS}$), the Leu^{115}-to-Thr^{115} amino acid substitution (construct $TSA1^{LT}$), or both of the amino acid substitutions together (construct $TSA1^{AS/LT}$). Ycplac111, WT.TSA1, and the mutant *TSA1* constructs were introduced into a *tsa1*Δ strain to determine whether they could complement the peroxide sensitivity and the effects on H_2O_2-induced expression of *TRX2* associated with the *tsa1*Δ strain.

The $TSA1^{AS}$ construct increased the resistance of the *tsa1*Δ strain to OS induced by *t*BOOH to almost wild-type levels. In contrast, the *tsa1*Δ strain containing either the $TSA1^{LT}$ or $TSA1^{AS/LT}$ construct showed similar sensitivity to the *tsa1*Δ strain containing the Ycplac111 vector (Figure 4A). Northern blot analysis revealed that the $TSA1^{AS}$ construct, but not the $TSA1^{LT}$ and $TSA1^{AS/LT}$ constructs, increased the H_2O_2-induced expression of the *TRX2* gene compared with the *tsa1*Δ strain containing the Ycplac111 vector (Figure 4B), although none of the constructs restored expression to the levels observed with WT.TSA1. Hence, these results suggest that the point mutations observed in *TSA1* from mutant #48, in par-

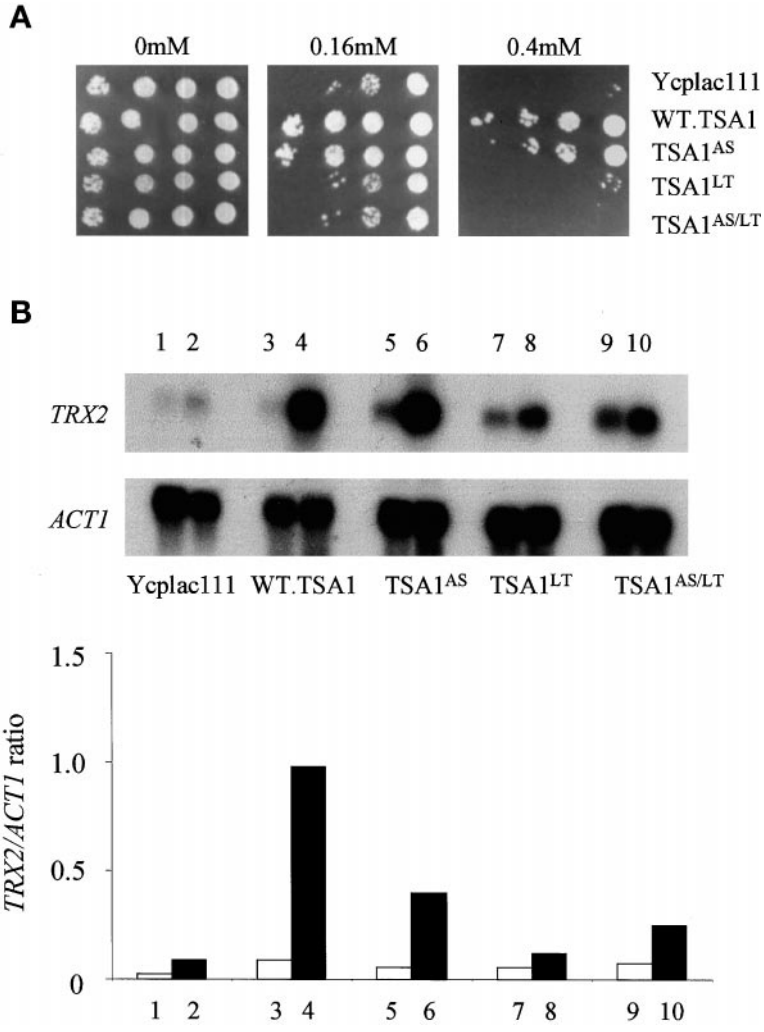

Figure 4. Analysis of the *TSA1* point mutations. (A) Sensitivity of the *TSA1* point mutations to *t*BOOH. Cultures of the *tsa1*Δ strain containing Ycplac111, WT.TSA1, $TSA1^{AS}$, $TSA1^{LT}$, or $TSA1^{AS/LT}$ were grown to midlog phase, and 10-fold serial dilutions were spotted onto SD medium containing various concentrations of *t*BOOH. (B) H_2O_2-induced expression of *TRX2*. Northern blot analysis of RNA isolated from midlog-phase cultures either untreated (lanes 1, 3, 5, 7, and 9) or treated with 0.1 mM H_2O_2 for 20 min (lanes 2, 4, 6, 8, and 10) with the use of probes specific for the *TRX2* and *ACT1* transcripts. The *tsa1*Δ strain contained Ycplac111 (lanes 1 and 2), WT.TSA1 (lanes 3 and 4), $TSA1^{AS}$ (lanes 5 and 6), $TSA1^{LT}$ (lanes 7 and 8), or $TSA1^{AS/LT}$ (lanes 9 and 10). The panel below the Northern blots shows quantitation of the *TRX2* transcript level relative to the *ACT1* transcript.

ticular the Leu^{115}-to-Thr^{115} substitution, affect peroxide sensitivity and H_2O_2-induced expression of *TRX2*.

Tsa1p-dependent Regulation of TRX2 Is through a Skn7p- and Yap1p-dependent Pathway

Skn7p and Yap1p are directly involved in the H_2O_2-induced expression of *TRX2* (Kuge and Jones, 1994; Morgan *et al.*, 1997). A *skn7*Δ*yap1*Δ strain shows the same level of residual H_2O_2-induced *TRX2* expression as either of the single-deletion strains alone, indicating a Skn7p/Yap1p-independent pathway that regulates *TRX2* expression in response to H_2O_2 (Morgan *et al.*, 1997). Hence, to investigate whether Tsa1p regulates the H_2O_2-induced expression of *TRX2* through this independent pathway, *tsa1*Δ*skn7*Δ and *tsa1*Δ*yap1*Δ double mutants were constructed and H_2O_2-induced expression of *TRX2* was examined. If Tsa1p functions in a Skn7p/Yap1p-independent pathway, then the *tsa1*Δ*skn7*Δ and *tsa1*Δ*yap1*Δ double mutants should show a greater reduction in H_2O_2-induced *TRX2* expression than the single deletions alone. Northern blot analysis revealed that both the *tsa1*Δ*skn7*Δ and *tsa1*Δ*yap1*Δ double mutants showed similar H_2O_2-induced *TRX2* expression as the single-deletion strains (Figure 5), suggesting that Tsa1p functions within the Skn7p/Yap1p pathway.

Overexpression of TSA1

Analysis of the *tsa1*Δ strain and the *TSA1* point mutations suggests that Tsa1p regulates the expression of components of the thioredoxin system. Such a result was completely unexpected, because it might have been predicted that a deletion of the *TSA1* gene would have increased *TRX2* and *TRR1* expression by increasing the effective OS of the cell. In this scenario, overexpression of the *TSA1* gene would be predicted to result in the reduction of *TRX2* and *TRR1* gene expression. Hence, the effects of overexpression of the *TSA1* gene on peroxide resistance and H_2O_2-induced expression of *TRX2* was examined (Figure 6). Overexpression of *TSA1* increased both the peroxide resistance and the H_2O_2-induced expression of the *TRX2* gene in the wild-type strain (Figure 6, A and B), although basal *TRX2* expression remained unaffected. Thus, overexpression of *TSA1* has the opposite affect than deletion of the *TSA1* gene on peroxide

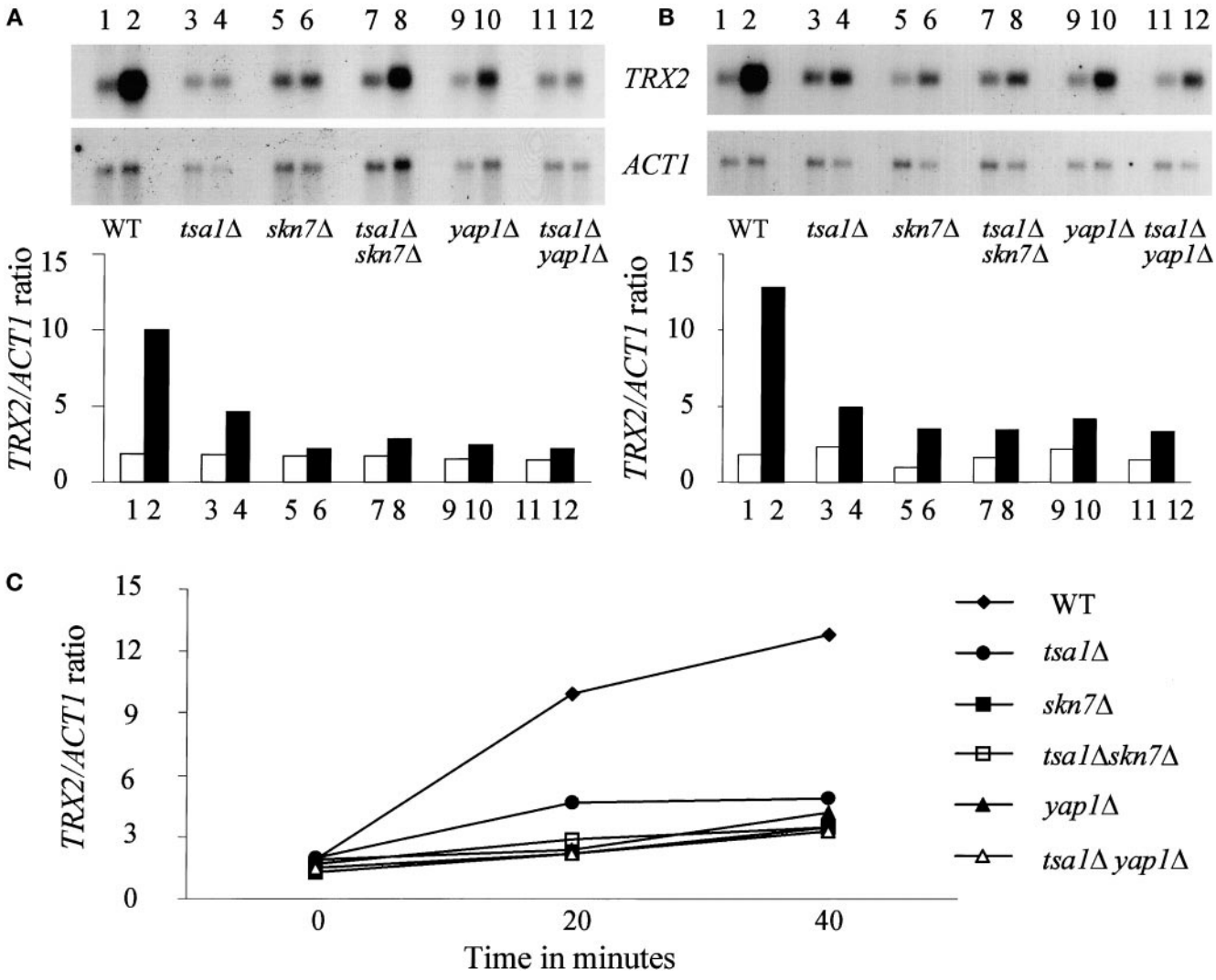

Figure 5. Northern blot analysis of RNA isolated from different midlog-phase cultures either untreated (lanes 1, 3, 5, 7, 9, and 11) or treated with 0.1 mM H_2O_2 (lanes 2, 4, 6, 8, 10, and 12) for 20 (A) and 40 (B) min with the use of probes specific for the *TRX2* and *ACT1* transcripts. The strains were W303-1a (lanes 1 and 2), *tsa1*Δ (lanes 3 and 4), *skn7*Δ (lanes 5 and 6), *tsa1*Δ*skn7*Δ (lanes 7 and 8), *yap1*Δ (lanes 9 and 10), and *tsa1*Δ*yap1*Δ (lanes 11 and 12). The panels below the Northern blots show quantitation of the different transcripts relative to the *ACT1* transcript. (C) Kinetics of *TRX2* induction. This graph shows the induction of *TRX2* expression in the W303-1a, *tsa1*Δ, *skn7*Δ, *tsa1*Δ*skn7*Δ, *yap1*Δ, and *tsa1*Δ*yap1*Δ strains during 40 min of incubation with 0.1 mM H_2O_2.

resistance and H_2O_2-induced *TRX2* expression. However, although the peroxide resistance of the *skn7*Δ and *yap1*Δ strains was increased on overexpression of *TSA1* (Figure 6A), the H_2O_2-induced expression of the *TRX2* gene remained unaffected (Figure 6B). Thus, the protective antioxidant function of Tsa1p is independent of Skn7p and Yap1p, whereas the H_2O_2-induced expression of *TRX2* via Tsa1p requires both transcription factors.

DISCUSSION

The mechanisms by which eukaryotic cells sense and respond to redox conditions are not well understood. In this study, we describe a genetic screen that was designed to isolate proteins involved in the regulation of *TRX2*, a key gene implicated in the OSR in *S. cerevisiae*. The results demonstrate that a loss-of-function mutation or a deletion of the *TSA1* gene, which encodes the antioxidant thioredoxin peroxidase, reduced the induction of expression of the chromosomal *TRX2* and *TRR1* genes in response to H_2O_2 without affecting basal-level transcription. These results were very unexpected, because loss of the antioxidant Tsa1p might be expected, if anything, to have the opposite affect on the transcription of the thioredoxin system genes. Indeed, deletion of other components of the thioredoxin system induces the expression of thioredoxin system genes even in the absence of H_2O_2 treatment (Izawa *et al.*, 1999; our unpublished results). Overexpression of the *TSA1* gene in a wild-type

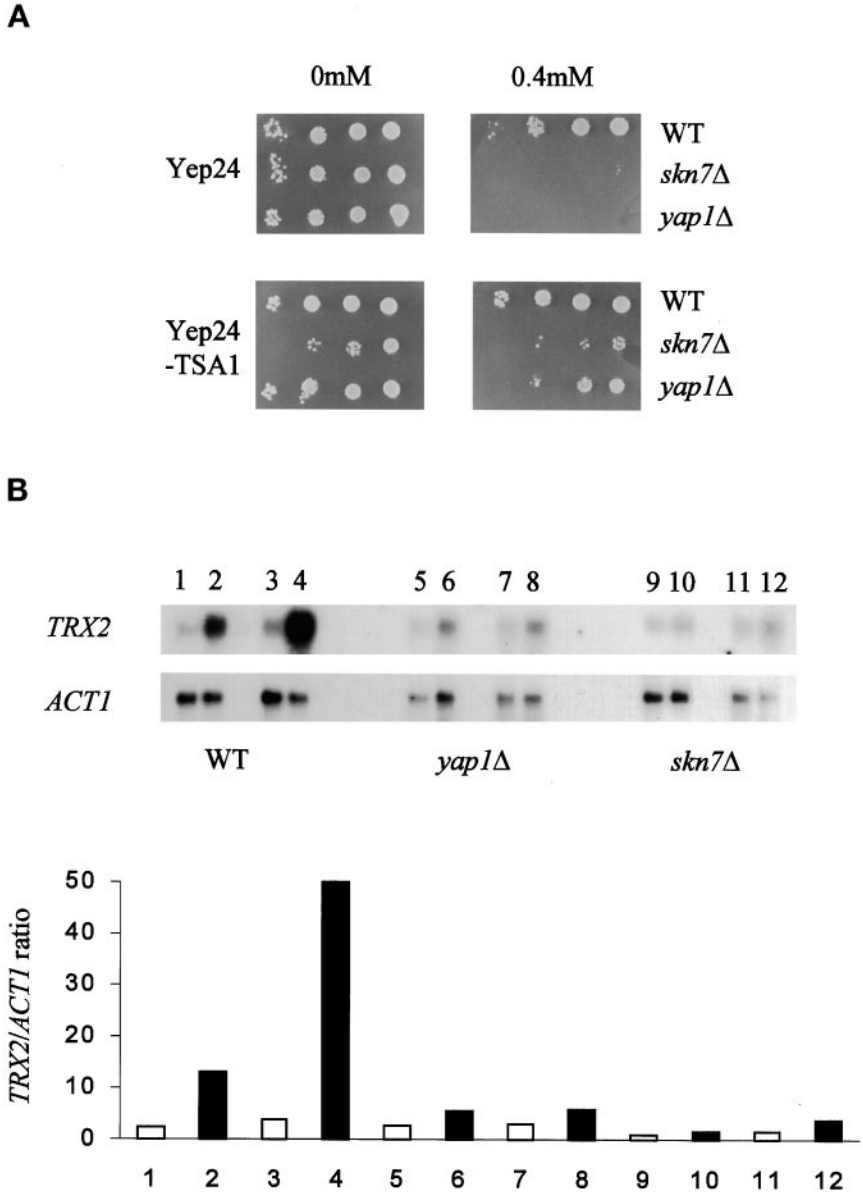

Figure 6. Overexpression of *TSA1*. (A) Spot tests showing the sensitivity to *t*BOOH after overexpression of *TSA1*. Strains were W303-1a, *skn7*Δ, and *yap1*Δ containing either the Yep24 vector or the Yep24-TSA1 plasmid. (B) Northern blot analysis of RNA isolated from different midlog-phase cultures either untreated (lanes 1, 3, 5, 7, 9, and 11) or treated with 0.1 mM H_2O_2 for 20 min (lanes 2, 4, 6, 8, 10, and 12) with the use of probes specific for *TRX2* and *ACT1*. Strains were W303-1a containing Yep24 (lanes 1 and 2), W303-1a containing Yep24-TSA1 (lanes 3 and 4), *yap1*Δ containing Yep24 (lanes 5 and 6), *yap1*Δ containing Yep24-TSA1 (lanes 7 and 8), *skn7*Δ containing Yep24 (lanes 9 and 10), and *skn7*Δ containing Yep24-TSA1 (lanes 11 and 12). The panel below the Northern blots shows quantitation of the *TRX2* transcript relative to the *ACT1* transcript.

strain has the opposite affect than the gene deletion, resulting in higher and/or faster induced expression of *TRX2* without affecting the basal level. Together, these results demonstrate that Tsa1p, but not Trx1p, Trx2p, or Trr1p, is required for the induction of gene expression after treatment with low concentrations of H_2O_2.

It is possible that Tsa1p is required for the induction of all H_2O_2-induced genes. Hence, the expression of the *CTT1* gene, encoding catalase, was investigated. Analysis of Ctt1p levels after H_2O_2 treatment has revealed that the amount of Ctt1p present is dependent on the concentration of oxidizing agent used (Godon *et al.*, 1998). At the concentration of H_2O_2 used here, a relatively low induction of *CTT1* expression was expected in the wild-type control. However, it was suspected that the loss of induction of the genes involved in the thioredoxin system might increase the induction of *CTT1*, and this was observed; in a *tsa1*Δ strain, *CTT1* expression was induced approximately threefold higher than in the wild-type after H_2O_2 treatment. Hence, only a subset of genes show a loss of H_2O_2 induction in a *tsa1*Δ strain, indicating that Tsa1p is not a global regulator of OS–induced genes.

Previous studies have shown that *S. cerevisiae* cells demonstrate an OSR at low concentrations of oxidizing agents, which results in increased resistance to higher doses of the agent (Jamieson, 1992; Jamieson *et al.*, 1994). The results presented here demonstrate that Tsa1p is involved in the transcriptional response to low doses of H_2O_2. Indeed, treatment of *tsa1*Δ cells with much higher, damaging doses of H_2O_2 results in the induction of *TRX2* expression even in the absence of the *TSA1* gene (our unpublished results). It may be relevant to this point that Tsa1p is susceptible to substrate

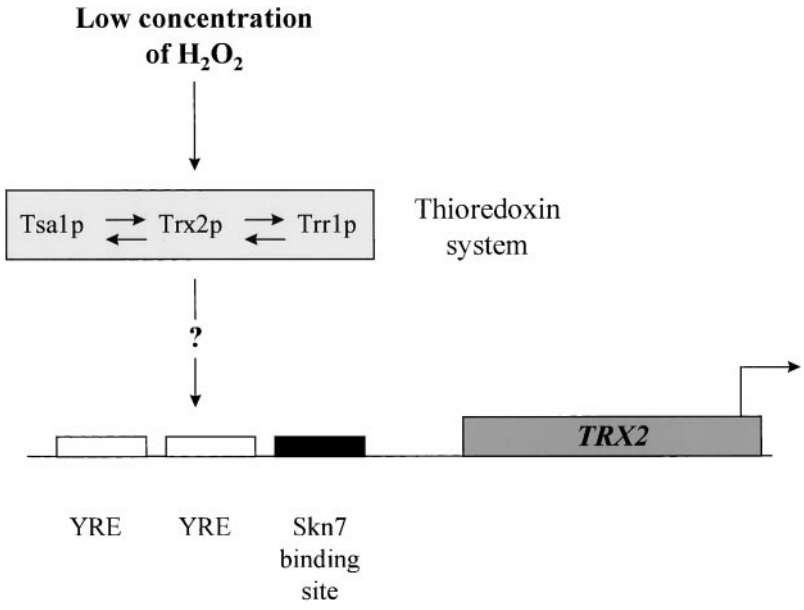

Figure 7. Model for the regulation of *TRX2* expression by Tsa1p. The data suggest that thioredoxin peroxidase may regulate the expression of thioredoxin through the activity of the Skn7p and Yap1p transcription factors. This regulation by Tsa1p could involve the direct regulation of the redox status of Skn7p and/or Yap1p. Alternatively, the redox status of an interacting regulatory protein or the phosphorylation state of Skn7p and/or Yap1p could be regulated. It is possible that the affect of Tsa1p is indirect, i.e., that it alters the redox status of one or more members of the thioredoxin pathway and that this protein is the regulatory element for OS–induced expression of *TRX2*. The arrows between the proteins of the thioredoxin system represent changes in the redox status of the proteins, and YRE represents the Yap1p-binding sites.

inhibition at high concentrations of H_2O_2 (Netto *et al.*, 1996). These data indicate that the regulation of *TRX2* gene expression is different at low and high doses of H_2O_2. The differences observed in *TRX2* expression are thus likely to be related to the increased cellular damage that occurs at the higher concentrations of H_2O_2. Our results also demonstrate that Tsa1p does not affect the basal levels of *TRX2* and *TRR1* gene expression. However, the effect on *TRR1* expression is in contrast to the results observed by Inoue *et al.* (1999), which showed an increased basal expression of *TRR1* in a *tsa1*Δ strain, although induced expression was not examined. The basis of this observed difference in basal *TRR1* expression is unclear.

Thioredoxin peroxidase proteins are highly conserved throughout evolution, and in higher eukaryotes these proteins have been found to have regulatory functions in addition to their antioxidant properties. For example, a human thioredoxin peroxidase, AEO372, has been shown to negatively regulate the activity of the transcription factor NF-κB through an unknown mechanism that modulates the phosphorylation state of IκB-α (Jin *et al.*, 1997). In addition, the human cytokine TRANK (thioredoxin peroxidase–related activator of NF-κB and c-Jun N-terminal kinase), which is highly homologous to thioredoxin peroxidase, activates both NF-κB and JNK (Haridas *et al.*, 1998). In *S. cerevisiae*, the transcription factors Skn7p and Yap1p are involved in the regulation of *TSA1*, *TRX2*, and *TRR1* expression in response to H_2O_2, suggesting that Tsa1p may regulate the activity of these proteins (Kuge and Jones, 1994; Morgan *et al.*, 1997; Lee *et al.*, 1999). Indeed, analysis of the *tsa1*Δ*skn7*Δ and *tsa1*Δ*yap1*Δ strains suggests that Tsa1p functions in the Skn7p/Yap1p pathway to regulate *TRX2* expression (Figure 5). Furthermore, although overexpression of Tsa1p increases the peroxide resistance of the wild-type, *skn7*Δ, and *yap1*Δ strains, only the wild-type strain shows an increase in the H_2O_2-induced expression of *TRX2* (Figure 6), strongly suggesting that Tsa1p-dependent expression of *TRX2* requires Skn7p and Yap1p. The regulation of Skn7p and Yap1p by the OSR is only partially understood. Previous studies have suggested that the DNA-binding ability of these transcription factors to the *TRX2* promoter is largely unaffected by H_2O_2 (Kuge and Jones, 1994; Morgan *et al.*, 1997). However, in response to various oxidizing agents, including H_2O_2, Yap1p localizes to the nucleus (Kuge *et al.*, 1997). A cysteine rich domain (CRD) at the C terminus of Yap1p regulates the OS localization (Kuge *et al.*, 1997), and it is possible that the regulation of the CRD region by OS is through the redox status of these cysteine residues. Hence, the localization of Yap1p could have been affected in a *tsa1*Δ strain after H_2O_2 treatment. However, Yap1p localizes to the nucleus normally in the *tsa1*Δ strain after H_2O_2 treatment (our unpublished results). Thus, the basis of the Tsa1p effect on *TRX2* and *TRR1* expression is not due to inhibition of Yap1p nuclear localization in oxidizing conditions.

The oxidation of other cysteine residues in Skn7p and/or Yap1p may be sensitive to the activity of the thioredoxin pathway. Indeed, deletion analyses have identified regions of Yap1p, in addition to the CRD region, that are important for activity in response to different oxidizing agents (Wemmie *et al.*, 1997). The basis of the regulation of Yap1p by these different oxidizing agents is not understood but may be related to the regulation of the protein by Tsa1p. The regulation of Skn7p by the OSR is also poorly understood but is likely to involve the function of a coiled-coil domain in the protein and repression of Skn7p activity by the PKA pathway (Alberts *et al.*, 1998; Charizanis *et al.*, 1999). Hence, it is possible that Tsa1p may affect the regulation of Skn7p through these two pathways.

The ability of Tsa1p to interact directly with Skn7p or Yap1p was tested by two hybrid studies. However, in the presence or absence of OS, no interaction was observed (our unpublished results). In addition, the high- and low-dose responses of *TRX2* expression to H_2O_2 are dependent on both Yap1p and Skn7p, whereas only the low-dose response is dependent on Tsa1p. Hence, Yap1p and Skn7p are able to respond to higher concentrations of H_2O_2 in a Tsa1p-inde-

pendent manner. Thus, the basis of the regulation of *TRX2* and *TRR1* expression by Tsa1p in the OSR is unclear. It is possible that Tsa1p does not directly regulate *TRX2* and *TRR1* expression but rather the redox status of other proteins in the thioredoxin pathway regulates their expression (Figure 7).

The identification of thioredoxin peroxidase, a conserved abundant protein that reduces reactive oxygen species, as a specific inducer of thioredoxin and thioredoxin reductase gene expression in response to OS in *S. cerevisiae* suggests that this protein is part of an important conserved sensing mechanism for redox conditions in eukaryotes. Thioredoxin peroxidase is one of the main cellular enzymes for the detoxification of H_2O_2 through the thioredoxin system, and the observation that Tsa1p is required for the induction of the other components in the thioredoxin system suggests the presence of a positive feedback loop in which at low levels of OS the redox state of Tsa1p regulates the expression of the other components of the pathway. Further experiments to understand the basis of this regulation in *S. cerevisiae* should provide insight into the detection processes and cellular responses to OS.

ACKNOWLEDGMENTS

We thank Janet Quinn, Mark Toone, Simon Whitehall, and Elizabeth Veal for their valuable advice and comments and Lee Johnston and Shusuke Kuge for the kind gift of plasmids. This work was funded partly by The Royal Society, grant 18793, and partly by the Medical Research Council (MRC), grant G9802083. S.J.R. was funded by an MRC studentship, V.J.F. was funded by a Biotechnology and Biological Science Research Council studentship, and P.M. was funded partly by the MRC grant listed above.

REFERENCES

Alberts, A.S., Bouquin, N., Johnston, L.H., and Treisman, R. (1998). Analysis of RhoA-binding proteins reveals an interaction domain conserved in heterotrimeric G protein β subunits and the yeast response regulator Skn7. J. Biol. Chem. *273*, 8616–8622.

Aves, S.J., Durkacz, B.W., Carr, A., and Nurse, P. (1985). Cloning, sequencing and transcriptional control of the *Schizosaccharomyces pombe cdc10* 'start' gene. EMBO J. *4*, 457–463.

Berben, G., Dumont, J., Gilliquet, V., Bolle, P.A., and Hilger, F. (1991). The YDp plasmids: a uniform set of vectors bearing versatile gene disruption cassettes for *Saccharomyces cerevisiae*. Yeast *7*, 475–477.

Brown, J.L., Bussey, H., and Stewart, R.C. (1994). Yeast Skn7p functions in a eukaryotic two-component regulatory pathway. EMBO J. *13*, 5186–5194.

Brown, J.L., North, S., and Bussey, H. (1993). *SKN7*, a yeast multicopy suppressor of a mutation affecting cell wall β-glucan assembly, encodes a product with domains homologous to prokaryotic two-component regulators and to heat shock transcription factors. J. Bacteriol. *175*, 6908–6915.

Chae, H.Z., Chung, S.J., and Rhee, S.G. (1994). Thioredoxin-dependent peroxide reductase from yeast. J. Biol. Chem. *269*, 27670–27678.

Charizanis, C., Juhnke, H., Krems, B., and Entian, K.-D. (1999). The oxidative stress response mediated via Pos9/Skn7 is negatively regulated by the Ras/PKA pathway in *Saccharomyces cerevisiae*. Mol. Gen. Genet. *261*, 740–752.

Gietz, R.D., and Sugino, A. (1988). New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene *74*, 527–534.

Godon, C., Langniel, G., Lee, J., Buhler, J.M., Kieffer, S., Perrot, M., Boucherie, H., Tolendano, M.B., and Labarre, J. (1998). The H_2O_2 stimulon in *Saccharomyces cerevisiae*. J. Biol. Chem. *273*, 22480–22489.

Guarente, L. (1983). Yeast promoters and *lacZ* fusions designed to study expression of cloned genes in yeast. Methods Enzymol. *101*, 181–191.

Halliwell, B. (1987). Oxidants and disease: some new concepts. FASEB J. *1*, 358–364.

Haridas, V., *et al.* (1998). Trank, a novel cytokine that activates NF-κB and c-Jun N-terminal kinase. J. Immunol. *161*, 1–6.

Hoffman, C.S., and Winston, F. (1987). A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene *57*, 267–272.

Inoue, Y., Matsuda, T., Sugiyama, K.-I., Izawa, S., and Kimura, A. (1999). Genetic analysis of glutathione peroxidase in oxidative stress response of *Saccharomyces cerevisiae*. J. Biol. Chem. *274*, 27002–27009.

Izawa, S., Maeda, K., Sugiyama, K.-I., Mano, J., Inoue, Y., and Kimura, A. (1999). Thioredoxin deficiency causes the constitutive activation of Yap1, an AP-1-like transcription factor in *Saccharomyces cerevisiae*. J. Biol. Chem. *274*, 28459–28465.

Jamieson, D.J. (1992). *Saccharomyces cerevisiae* has distinct adaptive responses to both hydrogen peroxide and menadione. J. Bacteriol. *174*, 6678–6681.

Jamieson, D.J. (1998). Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. Yeast *14*, 1511–1527.

Jamieson, D.J., River, S.L., and Stephen, D.W.S. (1994). Analysis of *Saccharomyces cerevisiae* proteins induced by peroxide and superoxide stress. Microbiology *140*, 3277–3283.

Jin, D.J., Chae, H.Z., Rhee, S.G., and Jeang, K.T. (1997). Regulatory role for a novel human thioredoxin peroxidase in NF-κB activation. J. Biol. Chem. *272*, 30952–30961.

Krems, B., Charizanis, C., and Entian, K.-D. (1996). The response regulator-like protein Pos9/Skn7 of *Saccharomyces cerevisiae* is involved in oxidative stress resistance. Curr. Genet. *29*, 327–334.

Kuge, S., and Jones, N. (1994). *YAP1* dependent activation of *TRX2* is essential for the response of *Saccharomyces cerevisiae* to oxidative stress by hydroperoxides. EMBO J. *13*, 655–664.

Kuge, S., Jones, N., and Nomoto, A. (1997). Regulation of yAP-1 nuclear localization in response to oxidative stress. EMBO J. *16*, 1710–1720.

Lee, J., Godon, C., Lagniel, G., Spector, D., Garin, J., Labarre, J., and Toledano, M.B. (1999). Yap1 and Skn7 control two specialized oxidative stress response regulons in yeast. J. Biol. Chem. *274*, 16040–16046.

Morgan, B.A., Banks, G.R., Toone, W.M., Raitt, D., Kuge, S., and Johnston, L.H. (1997). The Skn7 response regulator controls gene expression in the oxidative stress response of the budding yeast *Saccharomyces cerevisiae*. EMBO J. *16*, 1035–1044.

Morgan, B.A., Bouquin, N., Merrill, G.F., and Johnston, L.H. (1995). A yeast transcription factor bypassing the requirement for SBF and DSC1/MBF in budding yeast has homology to bacterial signal transduction proteins. EMBO J. *14*, 5679–5689.

Netto, L.E.S., Chae, H.Z., Kang, S.W., Rhee, S.G., and Stadtman, E.R. (1996). Removal of hydrogen peroxide by thiol-specific antioxidant enzyme (TSA) is involved with its antioxidant properties. TSA possesses thiol peroxidase activity. J. Biol. Chem. *271*, 15315–15321.

Robzyk, K., and Kassir, Y. (1992). A simple and highly efficient procedure for rescuing autonomous plasmids from yeast. Nucleic Acids Res. *20*, 3790.

Schiestl, R.H., and Gietz, R.D. (1989). High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Curr. Genet. *16*, 339–346.

Sherman, F., Fink, G.R., and Hicks, J.B. (1986). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Stock, J.B., Ninfa, A.J., and Stock, A.M. (1989). Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. *53*, 450–490.

Wemmie, J.A., Steggerda, S.M., and Moye-Rowley, W.S. (1997). The *Saccharomyces cerevisiae* AP-1 protein discriminates between oxidative stress elicited by the oxidants H_2O_2 and diamide. J. Biol. Chem. *272*, 7908–7914.

Winkler, H., Adam, G., Mattes, E., Schanz, M., Hartig, A., and Ruis, H. (1988). Co-ordinate control of synthesis of mitochondrial and non-mitochondrial hemoproteins: a binding site for the Hap1 (Cyp1) protein in the UAS region of the yeast catalase T gene (*CTT1*). EMBO J. *7*, 1799–1804.