Msn2p/Msn4p Act as a Key Transcriptional Activator of Yeast Cytoplasmic Thiol Peroxidase II*

We observed that the transcription of Saccharomyces cerevisiae cytoplasmic thiol peroxidase type II (cTPx II) (YDR453C) is regulated in response to various stresses (e.g., oxidative stress, carbon starvation, and heat-shock). It has been suggested that both transcription-activating proteins, Yap1p and Skn7p, regulate the transcription of cTPx II upon exposure to oxidative stress. However, a dramatic loss of transcriptional response to various stresses in yeast mutant strains lacking both Msn2p and Msn4p suggests that the transcription factors act as a principal transcriptional activator. In addition to two Yap1p response elements (YREs), TTAC-TAA and TTAGTAA, the presence of two stress response elements (STREs) (CCCTT) in the upstream sequence of cTPx II also suggests that Msn2p/Msn4p could control stress-induced expression of cTPx II. Analysis of the transcriptional activity of site-directed mutagenesis of the putative STREs (STRE1 and STRE2) and YREs (TRE1 and YRE2) in terms of the activity of a lacZ reporter gene under control of the cTPx II promoter indicates that STRE2 acts as a principal binding element essential for transactivation of the cTPx II promoter. The transcriptional activity of the cTPx II promoter was exponentially increased after postdiauxic growth. The transcriptional activity of the cTPx II promoter is greatly increased by rapamycin. Deletion of Tor1, Tor2, Ras1, and Ras2 resulted in a considerable induction when compared with their parent strains, suggesting that the transcription of cTPx II is under negative control of the Ras/cAMP and target of rapamycin signaling pathways. Taken together, these results suggest that cTPx II is a target of Msn2p/Msn4p transcription factors under negative control of the Ras-protein kinase A and target of rapamycin signaling pathways. Furthermore, the accumulation of cTPx II upon exposure to oxidative stress and during the postdiauxic shift suggests an important antioxidant role in stationary phase yeast cells.

Aerobically growing cells are continuously challenged by reactive oxygen species. Reactive oxygen species are potent oxidants capable of damaging all cellular components including DNA, protein, and membrane lipid. To protect against the toxicity of reactive oxygen species, aerobic organisms are equipped with an array of defense mechanisms (1). Among these, a new type of peroxidase, named thiol peroxidase, thioredoxin peroxidase (TPx),

*This work was supported by Grant 2001-1-20900-005-1 from the Basic Research Program of the Korea Science and Engineering Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Biochemistry, Paichai University, 439-6 Dowa-Dong Seo-Gu, Taejon 302-735, Republic of Korea. Tel.: 82-42-520-5379; Fax: 82-42-520-5594; E-mail: ihkim@mail.pcu.ac.kr.

Received for publication, November 28, 2001, and in revised form, January 22, 2002
Published, JBC Papers in Press, January 30, 2002, DOI 10.1074/jbc.M111341200

Seung-Keun Hong, Mee-Kyung Cha, Yong-Soo Choi, Won-Chiel Kim, and Il-Han Kim
 From the Department of Biochemistry, Paichai University, Taejon 302-735, Republic of Korea

The abbreviations used are: TPx, thiol peroxidase; YRE, Yap1 response element; STRE, stress response element; cTPx, cytoplasmic thiol peroxidase; TOR, target of rapamycin; PKA, protein kinase A; TSA, thiol-specific antioxidant protein; RT-PCR, reverse transcription-PCR.
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doxin reductase (25), GPx2 (glutathione peroxidase) (26), GLR1 (glutathione reductase) (27), TSA1 (cTPx I), cTPx II (27, 28), and AHP1 (cTPx III) (16). The transcriptional factor Yap1 is a bZIP DNA binding protein of the AP1 family (29) that binds the sequence TGT/GACTAA, named the Yap1 response element (YRE) (24, 30, 31). Yap1-mediated transcription can be activated by oxidative stress (24), and the activation is attributed to oxidative-stress-induced nuclear localization of Yap1 involving the nuclear export receptor Crm1 (Xpo 1) (32, 33). Another important transcriptional factor in S. cerevisiae is Msn2p and the partially redundant factor Msn4p are key regulators of oxidative-stress-responsive genes expression (34). In addition to their involvement in the oxidative stress response, they are also implicated in control of the multiple stress responses to carbon source starvation, osmotic stress, and heat stress (34). Msn2p and Msn4p, Cys2His2 zinc finger proteins, recognize and bind to STRE (CCCTT) (35, 36). The activation is attributed to the accumulation of both factors in the nucleus under these stress conditions. The nuclear localization of Msn2p/Msn4p is down-regulated by protein kinase A (PKA) activity or increasing levels of cAMP and up-regulated by stress (37). PKA, which has been implicated in the coordination of several essential events such as cell growth (38), entry into cell division (inhibition of G1 cyclin activity) (39, 40), and reprogramming of transcription at diauxic transition during yeast growth (41), acts as a potent repressor of STRE-mediated transcription.

In addition to two YREs, the promoter of the cTPx II gene contains two potential STREs, which suggests that Msn2p/Msn4p control expression of cTPx II. It has been suggested that both stress-related transcription-activating proteins, Yap1p and Skn7p, regulate the transcription of cTPx II in response to oxidative stress (28). In the present study, we have demonstrated for the first time that cTPx II is a transcriptional target of Msn2p/Msn4p. Furthermore, we have shown that transcriptional expression of the cTPx II promoter by Msn2p/Msn4p is attributed to stress-responsive down-regulation of the Ras/cAMP and TOR signaling pathways.

EXPERIMENTAL PROCEDURES

Strains and Media—S. cerevisiae strains used in this study were grown in rich medium (YPD; 1% yeast extract, 2% Bactopepton, and 2% glucose; YPG; 1% yeast extract, 2% Bactopepton, 3% glycerol, and 1% ethanol) or in synthetic minimal medium (SD) supplemented with the appropriate nutrients. The tor1 mutant strain NH349-3d (MATa, leu2–3,112, ura3–52, mtl1, trp1, his4, GAL1, HMLa, leu2Δ, tor1–ADE2–3/YCplac111:tor2–21ts) and their parent strain JK350-18a (MATa, leu2–3,112, ura3–52, rme1, trp1, his4, GAL1, HMLa, ade2Δ, tor2–ADE2–3/YCplac111:tor2–21ts) were kindly provided by Dr. Michael Jacquet (Universite Paris-Sud). The skn7 mutant strain (MATa, leu2–3,112, ura3–52, rme1, trp1, his4, GAL1, his3Δ200, lys2–801, ACT1::HisG, SKN7Δ) and its parent strain, W303-1a, were kindly donated by Dr. Michael N. Hall (Farber Cancer Institute, Harvard Medical School).

Construction of Wild-type and Mutant cTPx II Promoter-β-Galactosidase (lacZ) Fusion—A cTPx II promoter-lacZ fusion plasmid was constructed using a PCR-amplified DNA fragment. A putative promoter sequence of cTPx II was identified by the analysis with the SGD program (Stanford University). The promoter sequence (position –601 to –1) was amplified using genomic DNA from a yeast strain (J7D-7C) and oligonucleotides TF (5′-CCGGGTAACCTGTACCCCTATA GACATTACC) (forward primer) and TR (5′-CCGGAATTCCATGTTTATTAGTACCGAG) (reverse primer). These primers introduce a Kpn1 (forward) and BamH1 (reverse) site (underlined) for in-frame directional cloning into plasmid digested with Kpn1 and BamH1. To introduce β-galactosidase-fused promoter sequences, the lacZ gene was amplified by primers 5′-CCGGGATCCATGACCATGATTACGGATTCACT (forward primer) and 5′-GGTGAAGCTTATATTATTTTTGACACCAGACC (reverse primer), digested with BamH1 and HindIII, and cloned into YEG6·HHR525 digested with the same enzymes to produce pHLa. The lacZ promoter of the cTPx II promoter were digested with Kpn1 and BamH1 and cloned into pHLa digested with Kpn1 and BamH1 to give pYcTPxII·lacZ fusion vector. DNA sequencing confirmed that no mutation had been introduced in the promoter during PCR amplification. Site-directed mutagenesis was carried out using an overlap extension PCR method. The T′ and TR primers for amplification of the cTPx II promoter were used as external primers. Four internal primers (5′-CCGGGTACCTGGTACCCCTATAATGACAAAAGGATGTYGATG, TRE1 forward; 5′-GCCAGGATACATCTTT TTCATTGCTA, YRE1 reverse; 5′-GTTTCTTTTGGAAAAAGCGCTACGC, YRE2 forward; and 5′-GTCCGATGGCCCTTCCTCACAAGAAATAAC, YRE2 reverse) were designed to introduce substitutions (TAACTACTAA, STRE1 site to AAAGCGCTACGC, STRE2 forward, and 5′-GAATTCATGATGATTACGGATTCACT (forward primer) and 5′-GGTGAAGCTTATATTATTTTTGACACCAGACC (reverse primer), digested with BamH1 and HindIII, and cloned into pYlac digested with Kpn1 and BamH1 to give pYcTPxII–Mutant-LacZ fusion vector. DNA sequencing confirmed that mutation had been introduced in the corresponding STRE and YRE sites in the TPx II promoter. All kinds of stress and YRE mutants (double, triple, and quadruple mutants) were made using the appropriate pYcTPxII·Mutant-LacZ vector and the combination of internal and external primers for the respective location of the respective YREs and STREs with reference to the cTPx II start codon is shown in Fig. 1.

Assay for β-Galactosidase Activity—Cells were harvested and disrupted by vortexing with glass beads, and β-galactosidase activity was assayed using O-nitrophenyl-β-d-galactoside essentially as described previously (42). The β-galactosidase activity is expressed as unit (increase in A123 nm from O-nitrophenyl-β-d-galactoside/10 min/μg β-galactosidase/100 μg protein).

RESULTS

The Transcription of cTPx II Is Induced in Response to Oxidative Stress—Previously, we reported that exposure of yeast cells harboring the cTPx II·lacZ gene fusion vector to oxidative stress increases the β-galactosidase gene activity (42). To investigate the cTPx II transcriptional response to oxidative stress, exponentially growing cells were exposed to varying concentrations of H2O2 (0.1, 0.2, and 0.5 mM) and diamide (0.5, 1, and 1.5 mM) for 30 min, and the intracellular levels of the cTPx II protein and mRNA were analyzed. The immunoblot analysis for cTPx II in late log-phase cells grown in a rich media (YPD;
yeast growth. To investigate the expression level of cTPx II as a function of yeast growth, each of the total soluble proteins obtained from yeast cells grown in a rich media (YPD) to 400 μg/ml (lane 2), 1000 μg/ml (lane 3), 1000 μg/ml (lane 4), and 1500 μg/ml (lane 5) was used for the Western blot (Growth). Lane 1 indicates the Western blot for 200 ng of cTPx II as standard. In YPD media, the yeast cells can grow to about 200 μg/ml cell density, A600 nm = 20. To investigate the induction of cTPx II in response to oxidative stress, increasing concentrations of H2O2 (from the left, 0.1, 0.2, and 0.5 mM) and diamide (from the left, 0.5, 1.0, and 1.5 mM) were exposed to exponentially growing cell (A). Exponentially growing yeast cells (cell density, A600 nm = 20) indicates that the protein level is very low (about 0.02% of total soluble protein) compared with that of cTPx I (Fig. 1A). There are very similar types of cytoplasmic TPxs in yeasts (i.e. cTPx I and cTPx II) (i.e. 96% identities, 96% positives) (22). Cytoplasmic TPx I (TSA1) is an abundant protein even under noninduced conditions (0.7% of total soluble protein), and its synthesis rate was constant throughout yeast life but increased upon exposure to oxidative stress (3, 22). Cytoplasmic TPx II is also an inducible protein in response to oxidative stress, but in contrast to cTPx I, cTPx II was elevated as a function of yeast growth (Fig. 1B) (22). A gradual increase of band intensity as a function of cell growth was consistent with the previous observation of growth-dependent increase of transcriptional activity in terms of the β-galactosidase activity under control of the cTPx II promoter (22). Comparative Western blot (Fig. 1B) and RT-PCR (Fig. 1C) analyses showed that the protein and mRNA levels in early log-phase cells (cell density, A600 nm = 5) were very low but that the expression of cTPx II was dramatically induced in response to exposure of yeast cells to H2O2 and diamide. Taken together, these results demonstrate that expression of cTPx II is increased as a function of yeast growth and that cTPx II is an inducible protein in response to oxidative stress.

Msn2p/Msn4p Act as Key Transcription Factors to Regulate Expression of cTPx II—Recently, it has been reported that both transcription factors Yap1p and Skn7p regulate cTPx I and cTPx II expression (28). However, the difference in expression pattern between cTPx II and cTPx I during yeast life suggests that another transcription factor could be involved in cTPx II expression. To test this possibility, the mRNA level of cTPx II was investigated in various mutant strains lacking oxidative-stress-related transcription factors such as Yap1p, Skn7p, and Msn2p/Msn4p (22). Comparative analyses of the cTPx II transcripts in yeast mutants lacking Yap1p, Skn7p, and Msn2p/Msn4p (i.e. yap1Δ, skn7Δ, and msn2/4Δ, respectively) were performed. To determine whether Msn2p/Msn4p are involved in the induction of cTPx II in response to H2O2 and diamide, reverse transcription was carried out, followed by amplification. The fold induction in msn2/4Δ in response to H2O2 and diamide was very low compared with that of its parent strain, W303-1a (Fig. 2A). To compare the inducibility of the transcription factors in response to H2O2, the levels of the transcripts in yap1Δ, skn7Δ, and msn2/4Δ were analyzed using RT-PCR (Fig. 2B) and Northern blots (Fig. 2C) after treatment of the exponentially growing cells (A600 nm = 5) with 0.5 mM H2O2. In wild-type cells, the cTPx II mRNA level was dramatically induced upon H2O2 treatment. Only in msn2/4Δ was a significant induction of the cTPx II mRNA level not observed. In skn7Δ, the induced level was significantly higher compared with that in msn2/4Δ, although the level is significantly lower.
lacZ fusion vector. cTPx II was used to construct a cTPx II promoter-lacZ fusion vector.

The cTPx II Promoter Contains Functional STREs—Comput- er-assisted analysis of the cTPx II promoter identified two potential YREs located at positions −582 (YRE1; 5'-TTACTAA) and −472 (YRE2; 5'-TTAGTAA) relative to the ATG translation initiation codon (Fig. 3). In addition, the cTPx II promoter also contains two putative STREs located at positions −316 (STRE1; 5'-CCCCCT) and −267 (STRE2: 5'-CCCCCT) (Fig. 3). Either or both of the two YRE sites (YRE1 and YRE2) were replaced with AAAGAAA. Also, either or both of the STRE sites were replaced with AACAT (Fig. 3). The wild-type and mutated cTPx II promoters were fused to the Escherichia coli lacZ gene in plasmid pHylac to give cTPxII-lacZ fusion vectors. The lacZ expression vectors under control of wild-type and mutated cTPx II promoters were transformed to obtain WcTPxII carrying wild-type cTPx II promoter-lacZ fusion vector and strains carrying mutated cTPx II promoter-lacZ fusion vectors (MutYRE1, MutYRE2, MutYRE1/2, MutSTRE1, MutSTRE2, and MutSTRE1/2; Fig. 3). The β-galactosidase activity was assayed for each of the transformants grown in a synthetic media. The mutations in each binding element are named as described in the figure. The 601-bp DNA fragment spanning the region extending from next to the stop codon of YDR545C to before the initiation codon of cTPx II (YDR545C) was used to construct a cTPx II promoter-lacZ fusion vector.

The transactivation of the cTPx II promoter, the transcriptional activity of the cTPx II promoter was maximized by exposure to H2O2. In Fig. 4B, comparison of responses to H2O2 treatment, which was carried out under the same conditions, showed that the mutation in YRE1 (MutYRE1), YRE2 (MutYRE2), and STRE2 (MutSTRE2) resulted in a remarkable decrease in the level of transactivation of the cTPx II promoter induced by H2O2, with a reduction in β-galactosidase activity of 52%, 20%, and 46%, respectively, but that the mutation in STRE1 (MutSTRE1) resulted in no decrease in the transactivation level when compared with with the wild-type cTPx II promoter (Wild-type). Exposure of the cells to elevated concentrations of H2O2 (Fig. 4C) induced transcriptional activity of the cTPx II promoter (curve 1) and showed that STRE and YRE sites were required for full transcriptional activity upon oxidative stress (curves 2 and 3, respectively). Simultaneous mutation of all binding elements (MutYRE1/2 and STRE1/2) resulted in a near complete loss of β-galactosidase activity even in the presence of 0.5 mM H2O2 (Fig. 4D). Fig. 4B also shows that a change of the carbon source in the media from glucose (SD) to glycerol and ethanol (SGE) resulted in a significant elevation of the transcriptional activities in MutSTRE1, MutYRE1, and MutYRE2. In contrast, MutSTRE2 did not respond to such a carbon source change from a fermentable sugar to a nonfermentable sugar, suggesting the possibility that transactivation of the cTPx II promoter was suppressed in yeast growth using a fermentable sugar as a carbon source.

Taken together, these results demonstrate that (i) all of the YREs and STREs are potentially functional; (ii) however, under non-oxidative-stress growth conditions (i.e. physiological conditions), STRE2 located closest to the initiation codon is a key binding element essential for transactivation of the cTPx II promoter; and (iii) YRE2 and STRE1 play an additive role in full transactivation of the cTPx II promoter under oxidative stress and physiological conditions, respectively.

As mentioned above, STREs and YREs in the cTPx II promoter could be functional. To test whether Msn2p/Msn4p and Yap1p participate in respective STRE- and YRE-mediated activation of cTPx II promoter, we introduced the wild-type cTPx II promoter-lacZ fusion plasmid into the Msn2p/Msn4p double mutant (msn2Δ/msn4Δ), the Yap1 mutant (yap1Δ), and their parent strains (W303-1A and SEY6210, respectively). The resulting β-galactosidase activity in msn2Δ/msn4Δ was almost completely abolished (Fig. 5A), even under the oxidative stress conditions caused by H2O2 and diamide (Fig. 5C). In contrast, the β-galactosidase activity in yap1Δ was rather increased when compared with with the parent type as seen in the Northern blot (Fig. 2D), although under the oxidative stress condition and in the stationary-phase cells, β-galactosidase activity in yap1Δ was slightly decreased when compared with with the parent type (Fig. 5,
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...activity of the expressed tional response of cTPx II were exposed to 0.5 mM H2O2. Values represent the average of five independent experiments. A...activity in yap1 and YRE sites (MutYRE1 and MutYRE2) as the promoter of lacZ structural gene were transformed in strain JD7-7C and grown in a minimal media containing 2% glucose (SD) or 3% glycerol plus 1% ethanol (SGE) as a carbon source at 30°C. The expressed β-galactosidase (β-Gal) activities were determined. A, late log-phase transactivation of the cTPx II promoter. The cells were cultured in SD medium and harvested to determine the activity of the expressed β-galactosidase at the indicated time. The growth was determined in the terms of the increase in absorbance at 600 nm. B, transactivation of the cTPx II promoter in response to H2O2 and during growth on a nonfermentable carbon source. The first and second bars [■] and [□] in each set of experiments indicate the expressed β-galactosidase activities in cells grown in SD and SGE to a cell density of A500 nm = 8, respectively. The third bar [□] shows that the transcriptional response of cTPx II promoters after a 40-min exposure to 0.5 mM H2O2 before harvest at A500 nm = 8. The cells were grown in SD media. Values represent the average of five independent experiments. C and D, induction of STRE- and YRE-mediated transactivation of the cTPx II promoter in response to H2O2. The experimental procedure was as described in B. Curves 1–3 in C show the changes of β-galactosidase activities in cells containing wild-type, MutYRE1/2, and MutSTRE1/2 in response to increasing concentrations of H2O2 for 40 min, respectively. To investigate the response of the promoter lacking both STRE and YRE sites (MutYRESTRE) to H2O2, exponentially growing cells were exposed to 0.5 mM H2O2 (D). The cells were grown in SD media. Values represent the average of five independent experiments.

B and D). The slight but significant reduction of β-galactosidase activity in yap1Δ upon exposure to H2O2 (Fig. 5D) suggests that cTPx II is also a target of the Yap1 transcription factor, which is consistent with previous results (28). Based on these observations, a slight increase of the transcriptional activity in Yap1Δ under the non-oxidative stress condition (Fig. 5B) could be explained in terms of oxidative stress-induced Msns2p/Msn4p-mediated induction, which is caused by deletion of Yap1p. It is believed that deletion of Yap1p, acting as an important stress-responsive transcription factor in the cells, resulted in an increase of oxidative stress in the cell (34).

Collectively, these results demonstrate that, as reported previously (28), both Skn7p and Yap1p participate in part in the transactivation of the cTPx II promoter, but Msns2p/Msn4p act as key transcription factors to regulate expression of cTPx II. Msns2p/Msn4p-mediated Heat shock Response of cTPx II—We observed that the transcription of cTPx II is increased in response to heat-shock (Fig. 6). The β-galactosidase activity in yeast cells harboring the cTPx II-lacZ fusion was increased upon the heat-shock, which is consistent with the results of Northern blot analysis. To examine the possibility that Msns2p/Msn4p or Yap1p could be involved in the heat-shock-induced increase, we introduced wild-type cTPx II promoter-lacZ fusion plasmid into msn2/4Δ, yap1Δ, and their parent strains (W303-1A and SEY6210, respectively). The heat-shock re-
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Response was abolished only in the msn2/4Δ, suggesting Msn2p/Msn4p-mediated heat-shock response of cTPx II transcription (44). The Msn2p/Msn4p-dependent heat-shock response of cTPx II transcription could be taken as evidence supporting the observation that Msn2p/Msn4p act as key transcriptional activators of cTPx II.

Transcription of the cTPx II Gene Is Turned On at the Diauxic Shift—When the cells are cultured in a liquid rich medium in which the major carbon source is a fermentable carbohydrate (e.g. YPD), they exhibit two distinct growth phases, followed by a stationary phase in which cells cease to divide. During the first phase, cells meet their energy requirements primarily by fermentation. The second growth phase is initiated when cells exhaust most of the fermentable carbon source, undergo a major physiological change, and begin to grow at a much slower rate. The shift between these two phases is called the diauxic shift (a switch from fermentative to oxidative metabolism) (45). Several genes such as HSP26 are transcriptionally induced during the diauxic shift, in which dramatic changes in gene expression occur (46). Previously, we have shown transcriptional activation of the cTPx II gene upon changing the carbon source from a fermentable carbohydrate (i.e. glucose) to a nonfermentable carbohydrate (glycerol plus ethanol) (Fig. 4B). To exactly follow growth-dependent transcription of the cTPx II gene, we examined whether transcription of cTPx II is dependent on the yeast growth cycle from log phase to stationary phase. Comparison of growth curves with their corresponding transcriptional activities (Fig. 4A) indicates that the transcriptional activity of cTPx II is not fully activated in cells carrying a cTPx II-LacZ fusion vector until the yeast cells reach the late log phase. The transcriptional activity is almost completely abolished only in the case of msn2/4Δ (Fig. 5A). Taken together, these results suggest the possibility that cTPx II is transcriptionally induced during the diauxic shift.

Rapamycin forms a complex with FKBP12 that inhibits components of signal transduction pathways, named the TOR (target of rapamycin) pathway. The target of the complex was first identified in yeast as Tor1p and Tor2p (48). Loss of TOR function at the diauxic shift induces several other physiological changes characteristic of starved cells entering the stationary phase. Inhibition of the TOR signaling pathway by lack of fermentable carbon induces nuclear translocation of the carbon-sensitive transcription factor Man2p/Msn4p (49, 50).

To test the possibility that Msn2p/Msn4p mediated transactivation of the cTPx II promoter at the diauxic shift, we investigated transcription of the cTPx II gene after treatment with rapamycin. The levels of cTPx II mRNA shown in Fig. 7A and the inset of Fig. 7B show that rapamycin significantly increased the transcript in wild-type yeasts (W303-1a and SEY6210) and the yap1Δ strain. In contrast, rapamycin did not increase the transcript in the msn2/4Δ strain. Fig. 7B also shows that rapamycin increased the transcriptional activity of the wild-type cTPx II promoter about 3-fold. Mutation in YRE1/2 (MutYRE1/2) resulted in a still considerable increase in β-galactosidase activity with a ~2.3-fold induction in the presence of a sufficient amount of rapamycin. In contrast, mutation in STRE1/2 (MutSTRE1/2) resulted in no significant response to rapamycin. Taken together, these results suggest that Msn2p/Msn4p-mediated transcription of the cTPx II gene is induced in response to the inhibitory action of rapamycin on the TOR pathway.

Tor1p and Tor2p are key components of the TOR pathway. Cells lacking Tor1 exhibit only mild growth defects (51), but tor2Δ mutant does not survive because Tor2p is an essential protein that regulates cell growth (52). The dramatic increase of the cTPx II transcript upon exposure to rapamycin (Fig. 7A and B) suggests that cTPx II is under down-regulation of the TOR pathway. To demonstrate the negative control on transactivation of the cTPx II promoter by the TOR pathway, we tested the transcriptional activity of the cTPx II promoter in tor1Δ and tor2Δ mutants (ΔTor1 and ΔTor2). The Tor2 mutant is a temperature-sensitive mutant (permissive and nonpermissive temperatures are 30 °C and 37 °C, respectively) (53). Fig. 7C shows that disruption of Tor1 resulted in an increase in β-galactosidase activity with ~2-fold induction. Disruption of Tor2 at a nonpermissive temperature resulted in an increase in β-galactosidase activity with ~1.7-fold induction when compared with the Tor2 mutant grown at a permissive temperature (data not shown). Northern blot analysis (inset of Fig. 7C) also suggests that Tor1p repressed the transcriptional activity of the cTPx II promoter. Collectively, these results demonstrate that Tor1p and Tor2p suppress transactivation of the cTPx II promoter via activation of the TOR pathway.

In S. cerevisiae, Ras proteins, which encode GTP-binding proteins, are activated by both growth signals (e.g. glucose) (54) and stress signals (e.g. UV radiation and starvation) (47, 55, 56). The unregulated Ras/CAMP pathway suppresses the activity of the stress-responsive transcription factors Msn2p/Msn4p, which are responsible for activation of a large number of stress-related genes (47, 55, 56). Ras proteins activate the CAMP-de-
transcriptional activity as a function of yeast cell growth (Fig. 8, inset) of Fig. 8C). Taken together with previous reports that activation of Msn2p/Msn4p at the diauxic shift is under negative control of the TOR and Ras/cAMP pathways (37, 41, 49, 50), these results suggest that transactivation of the cTPx II promoter results from activation of Msn2p/Msn4p at the diauxic shift.

**DISCUSSION**

In the present study, we have investigated Msn2p/Msn4p-mediated transactivation of the cTPx II promoter, and, based on the results of a series of the experiments (from Figs. 1–6), we have demonstrated that the expression of cTPx II in response to various stresses including oxidative stress, carbon starvation, and heat-shock is under the control of Msn2p/Msn4p transcription factors. Moreover, several lines of evidence as described below indicate the Msn2p/Msn4p-mediated transactivation of the cTPx II promoter under the negative control of the Ras/cAMP and TOR signaling pathways: (i) mutation of STRE2 abolished its transcriptional activity in response to the diauxic shift (Fig. 4A); (ii) major components of
signaling pathways (Ras1p, Ras2p, Tor1p, Tor1p, and Tor2p) (Figs. 7 and 8), which negatively regulate nuclear translocation of Msn2p and Msn4p as their targets (34, 37, 47, 54–56), derepressed the transcription of cTPx II (Figs. 7 and 8); and (iii) the addition of rapamycin induces transactivation of the cTPx II promoter. Rapamycin is known to induce nuclear import of Msn2p and Msn4p and induction of the stress-inducible STRE genes negatively regulated by the TOR pathway (57).

In terms of Man2p/Msn4p-mediated transactivation of the cTPx II promoter under negative control of the Ras/cAMP and TOR signaling pathways, we can explain a growth-dependent cTPx II transcription activation phenomenon as follows. Ras proteins activate the cAMP-dependent PKA activity, which in turn suppresses the activation of transcription factors Man2p and Msn4p by turning on the TOR pathway. At the phase of diauxic transition, glucose starvation begins to occur, which inhibits PKA activity, which leads to activation of the transcription factor Man2p/Msn4p by turning off the TOR pathway (i.e. inhibition of TOR kinase activity and activation of protein phosphatase (PPase) activity).

In the present study, we also have shown that STREs and YREs are functionally different. The YRE2 site appears to be involved in the transactivation of the cTPx II promoter in response to oxidative stress, but it does not function as the transactivation element without the stress (see Fig. 4). YRE1 and STRE1 sites play additive roles, which are necessary for the maximum transactivation in response to various stresses including carbon starvation and oxidative stress. The STRE2 site acts as a pivotal binding element for the transactivation. Without the involvement of the binding element, significant transactivation does not occur without an oxidative stress (Fig. 4), which is consistent with the in vivo analysis of the cTPx II mRNA level in the msn2Δ strain (Fig. 2). Based on a series of in vivo and in vitro studies on the transcriptional regulation of cTPx II, we have concluded that Msn2p and Msn4p are principal transcription factors for transactivation of the cTPx II promoter in response to various stresses including oxidative stress, limitation of carbon source, and heat-shock.

Yap1p and Skn7p are two yeast transcriptional regulators in the control of oxidative stress. A previous study (28) has shown that these regulators activate cTPx II in response to oxidative stress. In the present study, we also have shown that Yap1p and Skn7p are required for the induction of cTPx II in response to oxidative stress, but their functions as transcriptional regulators to activate cTPx II are not as pivotal as those of Msn2p/Msn4p (Figs. 2 and 5). It is worthwhile to investigate the physiological functions of Yap1p and Skn7p, although they seem to be required for full transactivation of the cTPx II promoter.

Reactive oxygen species are generated and removed by all aerobic organisms, leading to either physiological concentrations required for normal cell function or excessive quantities (the state called oxidative stress). A balance between oxidant and antioxidant intracellular systems by antioxidant enzymes is hence vital for cell function, regulation, and adaptation to diverse growth conditions (1). Recently, we reported that the cTPx II-null mutant shows a slow growth phenotype and a remarkable decrease in stationary-phase growth in contrast to its parent and the other cytoplasmic TPx isoenzyme mutants (cTPx IΔ and cTPx IIIΔ) (22). Therefore, taken together with the growth phenotype of the cTPx II mutant mentioned above, the accumulation of cTPx II during the postdiauxic shift suggests the possibility that cTPx II plays an important antioxi-
dant role, particularly in late log-phase and stationary-phase yeast cells.

Despite a clear involvement of the Ras/cAMP and TOR sig-

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