Previously, we reported that the yeast cytoplasmic thiol peroxidase type II isoform (cTPx II), a member of the TSA/AhpC family, showed a very low peroxidase activity when compared with other cytoplasmic yeast isoforms, and that cTPx II mutant (cTPx IIΔ) showed a severe growth retardation compared with that of the wild-type cells. To reveal the physiological function of cTPx II in yeast cell growth, we searched for proteins which react with cTPx II. In this study, we identified a novel interaction between cTPx II and CSR1p using the yeast two-hybrid system. CSR1p (SFH2p) has been known to be one member of Sec14 homologous (SFH) proteins. SFH2p exhibits phosphatidylinositol transfer protein activity. Interestingly, we found that cTPx II selectively bound to SFH2p among the five types of SFH proteins and Sec14p. The interaction required the dimerization of cTPx II. In addition, SFH2p also specifically bound to cTPx II among the yeast thiol peroxidase isoforms. The selective interaction of the dimer form of cTPx II (the oxidized form) with SFH2p was also confirmed by glutathione S-transferase pull-down and immunoprecipitation assays. The growth retardation, clearly reflected by the length of the lag phase, of cTPx IIΔ was rescued by deleting SFH2p in the cTPx IIΔ strain. The SFH2Δ strain did not show any growth retardation. In addition, the double mutant showed a higher susceptibility to oxidative stress. This finding provides the first in vitro demonstration of the specific interaction of cTPx II with SFH2p in an oxidative stress-sensitive manner and a novel physiological function of the complex of cTPx II and SFH2p.

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 (TPx),1 thiol peroxidase protector protein, thiol-specific antioxidant protein (TSA), or peroxiredoxin, has been known to eliminate H₂O₂ and alkyl hydroperoxides using a thiol-reducing equivalent (2–7). The new type of peroxidase with cysteine as the primary site of catalysis has been discovered from prokaryotes to eukaryotes (2–22). The TPx family, also referred to as the TSA/alkyl hydroperoxide reductase family or peroxiredoxin family, is a large family of a new type of antioxidant. In mammalian tissue, at least six types of TPx isoenzymes have been identified. Recently, in addition to two types of TPx isoenzymes (TSA I, YML028W (Ref. 2); TSA II/alkyl hydroperoxide reductase 1, YLR109W (Refs. 15 and 16)) described previously as yeast members of the TSA/alkyl hydroperoxide reductase family, we have characterized three TPx homologues (YDR453C, YBL064C, and YIL010W) as a new member of the yeast TPx family (22). Evidence from our recent work indicates that different TPx isoenzymes are localized in distinct cellular organelles, where they are likely to serve diverse functions in yeast cells (22). TSA I and TSA II (Ahp1) were described as a general hydroperoxide peroxidase to remove H₂O₂ and alkyl hydroperoxide (2, 15, 16, 22). Three novel isoforms showed a thiol peroxidase activity supported by thioredoxin and appeared to be distinctively localized in the cytoplasm, mitochondria, and nucleus. Each isoform was named after its subcellular localization such as cytoplasmic TPx I (cTPx I or TSA I), cTPx II (YDR453C), cTPx III (TSA II/alkyl hydroperoxide reductase 1), mitochondrial TPx (YBL064C), and nuclear TPx (YIL010W) (22).

Recently, we have reported that, unlike other TPx null mutants, cTPx II null mutant showed a slow growth phenotype and accumulation of G₁-phased cells during the log phase (23). The growth defect appeared to be caused by the accumulation of G₁-phased cells, even in the exponentially growing condition (22). We have demonstrated that Msn2p/4p-mediated transcription of the cTPx II gene under negative control of Ras–TOR signaling pathway is turned on at diauxic shift (23). Thus, on the basis of previous observations, we proposed that cTPx II might be one of the candidates for signaling mediators to maintain the aerobic life of stationary-phased yeast, although the function is not clearly understood (22, 23).

Proteins are frequently engaged in multiple interactions, and that governance of protein interaction specificity is a primary means of regulating biological systems. One member of mammalian TPx isoenzymes (i.e. PAG, for proliferation-associated gene product), which is thought to be a mammalian counterpart of Saccharomyces cerevisiae cTPx II, is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity (24). To gain more insight into the physiological function of cTPx II in maintaining the aerobic life of stationary-phased yeast, we explored a possible protein interaction with cTPx II using the yeast two-hybrid system. We first identified a novel protein-protein interaction between cTPx II and CSR1p.
(SFH2p). SFH2p has been known to be one member of Sec14 homologous (SFH) proteins (25). SFH2p exhibits phosphatidylinositol transfer protein (PTP) activity, which is essential for Golgi function and cell viability (26, 27). In this study, we find the oxidative stress-mediated protein-protein interaction between the dimeric form (oxidized form) of cTPx II and SFH2p and the rescue of the growth defect in cTPx IIΔ strain by the deletion of SFH2p. These results indicate that cTPx II functions in the regulation of yeast cell proliferation driven by oxidative stress. This work may help to elucidate a reason for the slow growth phenotype and furthermore, a physiological function of cTPx II during post-diauxic growth in S. cerevisiae.

**EXPERIMENTAL PROCEDURES**

Construction of Bait and Prey Plasmid for the Two-hybrid System—pEG202, which was used as a vector to express the LexA-cTPx II fusion protein, contains the his3 selectable marker, yeast 2-μm origin, E. coli origin (pBR origin, and LexA DNA-binding domain. The cTPx II DNA fragment encoding cTPx II (192 amino acids) was amplified by PCR and cloned into EcoRI-XhoI sites of pEG202. The resulting plasmid, named pLexA-cTPx II, was used as the bait. The yeast strain EGY48 possesses the p80-LacZ reporter plasmid (Clontech). Plasmid pJJG4-5-contains the TRP1-selectable marker, yeast 2-μm origin, and E. coli pUC origin. Expression of the fusion protein in this plasmid is under the control of GAL1, a galactose-inducible promoter. Approximately 2 x 10^6 yeast transformants were screened according to the instruction from the manufacturer. The yeast strain EGY48/pLexA-cTPx II was transformed with the yeast genomic DNA library by the lithium acetate method. Transformants were selected for the B42 primer (5'-GAA TTC ATG GTA GCA GAA GTT CAA AAA CAA G-3'). cTPx II reverse, 5'-CTC CAG TTA ATT ATT GCC ATT TTA GAA ATA C-3'; C48S forward, 5'-CAT TTC TTG TCT CCA CTG AG-3'; C48S reverse, 5'-CTC AGT TGG AGA GAC AAA TG-3'; C171S forward primer; cTPx II reverse primer; C171S forward, 5'-ATG TCT GCC ACC CCA TGG AGC-3'; C171S forward, 5'-ATG TCT GCC ACC CCA TGG AGC-3'. EcoRI and XhoI restriction enzyme sites for cloning are underlined, and codon for each serine is represented as boldface characters, respectively.

**β-Galactosidase Assay**—The relative strength of the protein interaction between bait and prey proteins was determined by measuring the expression level of lacZ reporter gene. Cells were suspended in Z buffer (60 mM Na2HPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, pH 7.0) containing 2-mercaptoethanol and disrupted by vortexing with glass beads, and the β-galactosidase activity was assayed using o-nitrophenyl-β-D-galactoside (ONPG) as a artificial substrate according to the method described previously (23, 29). The β-galactosidase activity was expressed as unit (increase of OD at 420 nm that resulted from ONPG hydrolyzed by β-galactosidase per 10 min per mg of protein). Protein concentration was determined using Bradford protein assay kit (Bio-Rad). Yeast transformation, DNA, protein extraction from yeast, and other methods not mentioned were carried out according to supplier manual or a standard protocol described elsewhere (28).

**In Vitro Protein Binding Assay Using Glutathione S-Transferase (GST) Pull-down and Immunoprecipitation Methods**—The complete coding region of SFH2 was inserted into pGEX-4T-1 (Amersham Biosciences). The resulting plasmid GST-SFH2 was transformed into E. coli BL21 (DE3). GST-SFH2 protein was induced by addition of 0.5 mM IPTG at 20 °C for 8 h and purified on glutathione (GSH)-Sepharose 4FF (Pharmacia). For the pull-down assay, 2 μg of GST-SFH2, 100 μl of buffer (50 mM Tris-Cl, pH 8.0, 150 mM NaCl) were mixed and incubated in the presence or absence of 0.1 mM DTT for 45 min at 4 °C. After addition of GST beads, the mixture was incubated at 4 °C for an additional 30 min, and the beads were separated by a brief centrifugation, washed five times with phosphate-buffered saline, and suspended in 25 μl of SDS sample loading buffer (50 mM Tris-Cl, pH 6.8, 100 mM DTT, 2% b-mercaptoethanol, 1% glycerol). The bound protein was analyzed by Western blot using GST and cTPx II antibodies. For the immunoprecipitation assay, 20 μg of cTPx II and 20 μg of GST-SFH2 were incubated and mixed in the presence or absence of 0.1 mM DTT for 45 min at 4 °C. For the addition of cTPx II, the mixture was incubated at 4 °C for another 30 min, and protein A-agarose beads (Amersham Biosciences) were added to the mixture. After a 30-min incubation at 4 °C, the beads were separated by a brief centrifugation and washed five times with the buffer. The beads were washed extensively with phosphate-buffered saline and then boiled in SDS-loading buffer for Western blot.

**Gene Disruption by Fusion PCR**—Null mutant of cTPx II was a laboratory stock used in previous work (22). The SFH2 gene was disrupted by a short flanking homology method. The 5' and 3' ends of SFH2 gene were amplified with a pair of primers, which were designed for amplification of the 0.41-kb DNA fragments from the ends. The TRP1 gene was used as select marker for the SFH2 strain. TRP1 gene was amplified by PCR with primers (5'-ATG TCT CTT ATT TTT TTT TTT TTT GAG TAC GGA GAA GTT CAA AAA CAA G-3' for TRP1 forward, and 5'-CTC CAG TTA ATT ATT GCC ATT TTA GAA ATA C-3' for TRP1 reverse). This marker was used for selecting a DNA fragment where the marker is flanked by the two 0.41-kb end regions of SFH2 gene by a fusion PCR. The first fusion PCR was performed by melting out the TRP1 gene, which was amplified with primer pair (5'-ATG TCT CTT ATT TTT TTT TTT TTT TTT GAG TAC GGA GAA GTT CAA AAA CAA G-3' for TRP1 forward, and 5'-CTC CAG TTA ATT ATT GCC ATT TTA GAA ATA C-3' for TRP1 reverse). This marker was used for selecting a DNA fragment where the marker is flanked by the two 0.41-kb end regions of SFH2 gene by a fusion PCR. The first fusion PCR was performed by melting out the TRP1 gene, which was amplified with primer pair (5'-ATG TCT CTT ATT TTT TTT TTT TTT TTT GAG TAC GGA GAA GTT CAA AAA CAA G-3' for TRP1 forward, and 5'-CTC CAG TTA ATT ATT GCC ATT TTA GAA ATA C-3' for TRP1 reverse).
primer are represented as TRPCR. The final PCR product was gel-purified and used for the disruption of SFH2 gene. The cTPx IIΔSFH2Δ strain was derived from cTPx IIΔ strain, and the SFH2Δ strain was derived from cTPx II wild strain, W303-1A. Correct replacement of the target open reading frames was confirmed by PCR directly on yeast cells.

**Determination of Thioredoxin-linked Peroxidase Activity of cTPx II**—Peroxidase reaction was performed in 350 μl of reaction mixture containing 50 mM Hepes-NaOH, pH 7.0, 0.8 μM thioredoxin, 0.3 μM thioredoxin reductase, 0.26 mM NADPH, 2.5 mM cTPx II, varying concentrations of SFH2p, and 300 μM t-butyldihydroperoxide at room temperature. The peroxidase activity of TPx linked to NADPH oxidation was traced for 3 min as the decrease of A_{412}. cTPx II and SFH2p were purified from BL21 strain carrying corresponding expression vector. For SFH2p, the E. coli strain was cultured for 12 h at 20 °C after addition of IPTG.

**RESULTS**

**Screening of cTPx II-interacting Proteins in the Yeast Two-hybrid System**—The protein interaction with cTPx II was studied using a yeast two-hybrid system. Approximately 2 × 10^{6} independent transformants were pooled and re-suspended on the selection plates (Ura-, His-, Trp-, Leu-) containing galactose. Among approximately 2500 colonies selected on the plates, a total of 56 colonies showed galactose-dependent β-galactosidase activity. For determining the relative expression level of the lacZ reporter gene caused by the protein-protein interaction, 56 colonies were cultured in the presence of galactose, and the expressed β-galactosidase activities were measured. Among the 56 colonies tested, 2 colonies showed relatively stronger β-galactosidase activities (data not shown). The two colonies were finally selected for sequencing. Sizes of the DNA inserts appeared to be same (2.4 kbp) on agarose gel. Sequence analysis revealed that the clones encode the C-terminal fragment of SFH2p. The DNA fragment encoded SFH2p (the region from Ala-109 to the C-terminal end, Val-408). To investigate the interaction of cTPx II and complete SFH2p, the full SFH2 gene was amplified by PCR, and the PCR fragments were inserted into pJG4-5. The plasmids were co-transformed with pLexA-cTPx II. The induced lacZ reporter gene caused by the protein-protein interaction between cTPx II and SFH2p was ~20% higher than the SFH2p fragment-mediated expression level (data not shown), which indicates the protein-protein interaction between SFH2p and cTPx II.

**Cytoplasmic TPx II Protein Specifically Binds to SFH2p**—Yeast expresses six members of a family of PITPs including Sec14p dedicated to divergent sets of cellular functions. The sequences of the family share a high degree of sequence similarity with Sec14p (from 43 to 79%) (25). Four of these proteins (SFH2p, SFH3p, SFH4p, and SFH5p) exhibit phosphatidylinositol transfer activity (30). Thus, to investigate a possible protein-protein interaction between cTPx II and other members of PITP family, the full genes of PITP family (SFH2p, Sec13p, SFH1p, SFH3p, SFH4p, and SFH5p) were fused in frame to the B42-activating domain of the pJG4-5 vector and used as prey for cTPx II. Each vector was co-transformed with pLexA-cTPx II. The induced β-galactosidase activity of the transformed yeast cells by galactose was measured. As a control test, the activity of the yeast cells grown on medium containing glucose as a carbon source was also measured. The β-galactosidase activity was assayed. The β-galactosidase activity is expressed as increase of OD at 412 nm that resulted from ONPG hydrolyzed by β-galactosidase (OD increase per mg of protein per 10 min).

To examine the effect of the dimerization of cTPx II on the protein-protein interaction with SFH2p, pLexA-cTPx II mutants (C48S, C171S, and C48S/C171S) were replaced with serine as a bait, and pJG4-5-cTPxII for prey. To analyze in vitro dimerization of cTPx II, the bait plasmid (pLexA-cTPx II mutant) was co-transformed with the prey plasmid (pJG4-5-cTPx II). The relative β-galactosidase activities and the growth capability on the selection media show that the wild-type cTPx II (bait) binds to the cTPx II expressed from the prey plasmid, whereas any other cTPx II mutant proteins as a bait could not dimerize with the wild-type prey protein (Fig. 2), confirming the in vivo dimerization via the intermolecular disulfide linkage.

**Dimer Form of cTPx II Protein Selectively Binds to SFH2p**—Among the yeast members, four TPx isoforms (cTPx 1, cTPx II, cTPx III, and mTPx) (but not nTPx) were dimerized among each other via intermolecular disulfide bond (22). The alignment of amino acid sequences revealed one highly conserved cysteine within the TPx homologous proteins. The conserved cysteine (Cys-48 for cTPx II) was previously reported to serve as one part of a sulfhydryl group forming the intermolecular disulfide linkage (22). For cTPx II, Cys-171 acts as a counterpart of a sulfhydryl group to form intermolecular sulfide bond. Thus, it is interesting to investigate which form among the monomeric and dimeric forms is more favorable for protein interaction with SFH2p. To answer the question, first, for the purpose of confirming the dimerization of cTPx II in vivo using yeast two-hybrid system, we constructed pLexA-cTPx II mutant, in which conserved cysteine(s) (C71S, C171S, and C48S/C171S) is replaced with serine as a bait, and pJG4-5-cTPx II for prey. To analyze in vitro dimerization of cTPx II, the bait plasmid (pLexA-cTPx II mutant) was co-transformed with the prey plasmid (pJG4-5-cTPx II). The relative β-galactosidase activities and the growth capability on the selection media show that the wild-type cTPx II (bait) binds to the cTPx II expressed from the prey plasmid, whereas any other cTPx II mutant proteins as a bait could not dimerize with the wild-type prey protein (Fig. 2), confirming the in vivo dimerization via the intermolecular disulfide linkage.
II. To see the dimerization effect on the protein-protein interaction, the expressed wild type of cTPx II. Each vector was co-transformed with pJG4-5-SFH2 (the plasmid for prey). The expressed β-galactosidase activity in the resulting yeast cells in the presence of galactose was measured. The growth defect on plate deleting Ura, His, Trp, and Leu was shown in inset. The β-galactosidase activity is expressed as increase of OD at 412 nm that resulted from ONPG hydrolyzed by β-galactosidase (OD increase per mg of protein per 10 min).

**FIG. 2. Verification of the dimerization of cTPx II using yeast two-hybrid system.** Each point-mutated cTPx II gene (wild (W), C48S (C1S), C171S (C2S), and C48S/C171S (C1/2S)) was fused in frame to the LexA-DNA binding domain of the pLexA vector and used as bait for the wild type of cTPx II. Each vector was co-transformed with pG4-5-cTPx II (plasmid for prey). The expressed β-galactosidase activity in the resulting yeast cells is shown in inset. The β-galactosidase activity is expressed as increase of OD at 412 nm that resulted from ONPG hydrolyzed by β-galactosidase (OD increase per mg of protein per 10 min).

**FIG. 3. CTPx II dimeric form-specific interaction of SFH2p.** Each point-mutated cTPx II gene (wild (W), C48S (C1S), C171S (C2S), and C48S/C171S (C1/2S)) was fused in frame to the LexA-DNA binding domain of the pLexA vector and used as bait for SFH2p. Each vector was co-transformed with pG4-5-SFH2 (the plasmid for prey). The expressed β-galactosidase activity in the resulting yeast cells in the presence of galactose was measured. The β-galactosidase activity is expressed as increase of OD at 412 nm that resulted from ONPG hydrolyzed by β-galactosidase (OD increase per mg of protein per 10 min).

**FIG. 4. CTPx II-dimeric form-specific interaction of SFH2p.** Each full gene of yeast TPx isoform (cTPx I, cTPx II, cTPx III, mTPx, and nTPx) was fused in frame to the LexA-DNA binding domain of the pLexA vector and used as bait for SFH2p. Each vector was co-transformed with pG4-5-SFH2 (the plasmid for prey). The expressed β-galactosidase activity in the resulting yeast cells in the presence of galactose was measured. The β-galactosidase activity is expressed as increase of OD at 412 nm that resulted from ONPG hydrolyzed by β-galactosidase (OD increase per mg of protein per 10 min).

action, the same experiment as that of cTPx II was performed using cTPx I mutant, in which the corresponding cysteine was replaced with serine. In contrast to the case of cTPx II, the β-galactosidase activities induced by the protein-protein interaction were regardless of presence or absence of the conserved cysteine (data not shown). This result suggests that the protein-protein interaction between cTPx I and SFH2p is different from that between cTPx II and SFH2p. Taken together, these results suggest that the protein-protein interaction between cTPx II and SFH2p is very specific, and that SFH2p selectively binds to the dimeric form of cTPx II.

Biochemical Verification of Two-hybrid Interaction of Dimeric Form of cTPx II with SFH2p—Interaction between the dimeric form of cTPx II and SFH2p was verified by both methods of immunoprecipitation and GST pull-down. For immunoprecipitation assay, after purified GST-SFH2 fusion protein and native cTPx II protein were incubated in the presence or absence of DTT, cTPx II antibody was employed to pull down GST-SFH2 fusion protein in vitro. GST protein was used as a control to eliminate a possible binding of GST itself to cTPx II protein. Immunoblot analysis of the protein complex with GST antibody demonstrates that GST-SFH2 fusion protein interacts to the dimeric (oxidized) form of cTPx II, but not to the monomeric (reduced by DTT) form of cTPx II (Fig. 5A). GST protein itself did not bind to cTPx II protein (data not shown).

For GST pull-down assay, after purified GST-SFH2 fusion protein and native cTPx II protein were incubated in the presence or absence of DTT, SFH2-GST fusion protein was employed to pull down cTPx II protein in vitro. GST protein was used as a control to test the specificity of the binding. Immunoblot analysis of the bound proteins with GST and cTPx II antibodies was performed. Fig. 5B shows that the dimeric form of cTPx II protein, which was incubated without DTT, binds to immobilized GST-SFH2 protein, whereas the monomeric form of cTPx II, which was incubated in the presence of DTT, does not bind to the GST-SFH2 fusion. Cyttoplasmic TPx II did not bind to immobilized GST protein alone (data not shown).

The Growth Phenotype of cTPx IIΔSFH2 Strain—Recently, we have reported that, unlike other TPx null mutants, cTPx II null mutant showed a slow growth phenotype and cells were arrested at G1-phase during the log phase. The growth defect was not recovered by prolong the cell culture to the stationary phase (22). The transcription of cTPx II was activated by the diauxic shift, which is strictly negative-regulated by Ras-TOR signaling pathway (23). The previous data suggest that cTPx II
has a physiological role in stationary-phased growth. Therefore, it is worth investigating how cTPx II functions in the stationary-phased growth. In an attempt to reveal the physiological role of cTPx II interaction with SFH2p in stationary phase, we constructed the SFH2Δ strain from cTPx IIΔ and its parent strains (W303-1a) and monitored their effects on cell growth. Fig. 6 shows that the colony-forming units are significantly higher for the cTPx IIΔ cells relative to those of the wild type, SFH2Δ, and double mutant cells, with the latter three cases forming large colonies. SFH2Δ strain did not give any a slow growth phenotype compared with its wild-type strain. However, the deletion of SFH2 in cTPx IIΔ strain resulted in recovering a slow growth phenotype shown by cTPx II strain, which suggests the inhibitory action of a free form of SFH2p on a cTPx II-dependent growth. Therefore, the growth retardation of cTPx IIΔ strain could be interpreted as the result of an inhibitory action of a free form of SFH2p in the absence of cTPx II protein. Taken together, these results could provide a new function of the cTPx II-SFH2p complex. The growth recovery of the cTPx IIΔ strain by deletion of SFH2p supports the in vitro protein-protein interaction between cTPx II and SFH2p.

To investigate further the physiology of the double mutant, the growth was monitored throughout the yeast growth phases. Fig. 7A shows that the growth of the double mutant exhibits a shorter lag phase relative to that of the cTPx IIΔ strain, which is visualized in Fig. 6, but the growth is decreased to a lower level than that of cTPx IIΔ strains during stationary-phased growth. The growth of SFH2pΔ strain was nearly the same as that of its parent strain, which is consistent with the result shown by Fig. 6. To test the possibility that the severe growth retardation of the double mutant during the stationary phase might be caused by the high susceptibility of the double mutant to oxidative stress, oxidative stress was subjected to the mutant (Fig. 7B). Exponential- and stationary-growth-phased cells were plated on the YPD plate containing 0.1 mM t-butyl hydroperoxide, and the cell viability was measured in terms of the number of the survival colonies. For the viability test using exponential-phased cells, each single mutant did not show any significant change in cell viability against the oxidative stress. However, the cell viability of the double mutant was significantly lower than either of the single mutants and its wild-type strain. In case of the stationary-phased cells, except for the wild-type strain, the viability of all mutants (cTPx IIΔ, SFHΔ,
and cTPx II (Sfh2p) is significantly lower when compared with the viability derived from the corresponding exponential cells. For the wild-type strain (W303-1a), stationary-phased cells survived more than those plated with cells from the exponential growth phase, which is consistent with the fact that stationary-phased cells are more resistant to oxidative stress. Analysis of viability data indicated that deletion of SFH2p is more harmful for the stationary-phased yeast cells in the presence of oxidative stress. Collectively, these results demonstrate that the growth retardation of the aged double mutant is caused by the high susceptibility to oxidative stress, which is acquired by deletion of SFH2p rather than by cTPx II. Therefore, the inhibitory action of Sfh2p on yeast growth (cTPx II-dependent growth) before the diauxic shift (i.e. before the induction of cTPx II) (23) is necessary for maintaining yeast life against oxidative stress.

**Peroxidase Activity of the Complex of cTPx II and SFH2p**—Previous data suggest that the protein-protein interaction between cTPx II and SFH2p could act as a signal event involved in yeast growth. The biochemical characterization of complex is the obvious key to understanding how the complex-forming process is involved in yeast growth. The physiology of the double mutant described previously suggests that the complex itself are necessary for maintaining yeast aerobic life. Now, it is interesting to examine the changes of antioxidant activity as forming the complex. The change of thioredoxin-linked peroxidase activity given by cTPx II was monitored as the concentration of SFH2p was increased to one equivalent to the concentration of cTPx II. After a 30-min incubation of the mixture consisting of cTPx II and SFH2p for forming the complex, the peroxidase activity in terms of NADPH decrease was spectrophotometrically traced at 340 nm (Fig. 8). The peroxidase activity was not significantly changed throughout the experiments, which shows that the activity of the free form of cTPx II is nearly same as that of the complex form. Therefore, we ruled out the possibility that some changes of peroxidase activity of cTPx II by forming the complex could directly participate in changing the phenotype of the double mutant (i.e. susceptibility against oxidative stress).

**DISCUSSION**

ROS do not have exclusively toxic effects. Low levels of ROS can act as signaling molecule under physiological condition (31). In mammals, ROS such as H2O2 produced in physiological condition can activate transcription factor, such as NFkappaB and AP-1 (32), and can function as signals in apoptosis that is induced by tumor necrosis factor-alpha (33). It is known that at least six types of TPx isoenzymes exist in mammalian. Evidence from our recent work indicates that different TPx isoenzymes are localized in distinct cellular organelles, where they are likely to serve diverse functions in yeast cells (22). We reported that five types of TPx isoforms exist in yeast. The amino acid sequence similarity among yeast TPx homologous proteins is 11.6%. Two cytoplasmic isoforms (cTPx I and cTPx II) share the highest degree of amino acid sequence similarity (86% identity, 96% positives) among yeast TPx isoforms, but thioredoxin-dependent thiol peroxidase activity of cTPx II is much lower than that of cTPx I (22). In addition, we also found that cTPx II null mutant showed a slow growth phenotype when compared with its parent and the other TPx mutants (22). Furthermore, the protein expression of cTPx II, which is very low compared with other TPx isoforms, is highly sensitive to intracellular redox state (22, 23). In the present study, we have provided a line of evidence that cTPx II may act as a candidate for signaling mediator upon oxidative stress. In our search for interacting molecules with cTPx II, we have isolated SFH2p using the yeast two-hybrid system, and demonstrated that cTPx II protein specifically binds the oxidative form (dimeric form) of SFH2p. Taken together with our observations, our results suggest the possibility that cTPx II might be a potential candidate for the oxidative-stress-mediated signaling mediator. Similarly, a putative mammalian counterpart for yeast cTPx II, PAG (for proliferation-associated gene product), gene expression of which occurs in two cellular events (oxidative stress and proliferation), is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity (24).

Previously we reported that transcription of cTPx II is turned on at diauxic shift, which is under negative control of Ras/cAMP-TOR signaling pathway (23). This result implies that cTPx II works at the stationary phase of yeast life cycle. In addition to the selective interaction of SFH2p with the dimeric (oxidative) form of cTPx II protein, we have found a line of evidence supporting the in vivo physiological function of the CTPx II-SFH2p complex. The rescue of the growth defect in cTPx IIΔ strain by deletion of SFH2p supports a role for cTPx
II in triggering a cTPx II-dependent growth, especially stationary-phased growth through the action of the cTPx II and SFH2p complex. We suggest that the process for formation of the complex is necessary for yeast cell viability against oxidative stress, thus, especially, for maintaining the aerobic life of stationary-phased yeast cells. It is generally believed that some of the TSA/AhpC family, including cTPx II, undergo a reversible conversion between the monomeric and dimeric forms upon redox state of cells. Thus, the protein-protein interaction between cTPx II and SFH2p regulated by intracellular redox state could act as a signal event to trigger oxidative stress-mediated cell signaling.

We demonstrate that the changes of the antioxidant activity given by cTPx II-SFH2p complex do not directly participate in the function of the complex. It remains to be solved how SFH2p inhibits cTPx II-dependent growth (i.e. stationary-phased growth) and how the cTPx II-SFH2p complex works. Although more characterization of the complex consisting of cTPx II protein and SFH2p is the obvious key to understanding of the function of the complex in oxidative stress-mediated cell signaling, overall, the results reported here suggest that the intracellular redox state-dependent protein interaction between cTPx II and SFH2p is a physiological process to mediate growth signaling involved in cTPx II-dependent growth, probably stationary-phased growth.

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