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Peroxiredoxin-Mediated Redox Regulation of the Nuclear Localization of Yap1, a Transcription Factor in Budding Yeast

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ABSTRACT

A redox reaction involving cysteine thiol–disulfide exchange is crucial for the intracellular monitoring of oxidation status. The yeast transcription factor Yap1 is activated by formation of a disulfide bond, which inhibits nuclear export in response to peroxide stress, with resultant enhancement of the nuclear localization of Yap1. A glutathione peroxidase-like protein, Gpx3, which has peroxiredoxin activity, is required for formation of the disulfide bond in Yap1. We show here that the requirement for Gpx3 in the regulation of Yap1 is strain-specific. Thus, Tsa1, a ubiquitous thioredoxin peroxidase, is required for the activation of Yap1 in yeast strain Y700, which is derived from W303. The strain-specific utilization of different peroxiredoxins appears to be determined by Ybp1, a Yap1-binding protein. The Ybp1 of Y700 has a nonsense mutation, and a wild-type *YBP1* gene can restore the Gpx3-dependent activation of Yap1. These results suggest that Tsa1, a ubiquitous peroxiredoxin, has the potential for transducing redox signals to a particular sensor protein. *Antioxid. Redox Signal.* 7, 327–334.

INTRODUCTION

During respiration, oxygen is converted to harmful reactive oxygen species (ROS), which include hydroperoxide and superoxide, that can damage cellular macromolecules. Organisms have acquired defense systems to protect themselves from the toxicity of ROS and to maintain a reducing environment in the cytoplasm and in the nucleoplasm. Superoxide generated by mitochondrial respiration and by various NADPH oxidases can be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (12). H_2O_2 is relatively stable, and its amphiphilic properties allow it to permeate membranes. Recent reports indicate that ROS can also act as cellular signals in responses to growth factors, cytokines, and various types of stress (30). Cells control levels of ROS by exploiting glutathione peroxidase (Gpx) coupled with the glutathione reduction–oxidation (redox) cycle and peroxiredoxin (Prx) coupled with the thioredoxin (Trx) system, in which a proton is accepted from NADPH and then reduces H_2O_2 to H_2O. Thus, these systems play an essential role in maintenance of the redox status of cells and, possibly, in the sensing of intracellular levels of ROS (4).

Studies of mechanisms for sensing H_2O_2 in eukaryote have revealed the involvement in budding yeast of the eukaryotic transcription factor Yap1 (7, 8, 10, 17–19; for review, see 23). Upon exposure of yeast cells to H_2O_2, Yap1 is activated and induces the expression of various proteins that are involved in antioxidant systems, such as Trx (Trx2; 16, 20) and thioredoxin reductase (Trr) (Trr1; 5, 21). The localization of Yap1 is determined by constitutive nuclear import and nuclear export (Fig. 1; 14, 17, 18, 30). Thus, under nonstressed conditions, Yap1 is localized predominantly in the cytoplasm. However, it seems likely that, in response to oxidative stress, the conformation of the nuclear export signal (NES) of Yap1, which is embedded in the carboxy-terminal cysteine-rich domain (c-CRD; see Fig. 1) of Yap1, is altered, with resultant inhibition of binding of the nuclear export receptor Crm1 and subsequent accumulation of Yap1 in the nucleus (18, 31). Formation of a disulfide bond in the c-CRD is induced by the thiol-oxidant diamide (17, 18), or possible direct cross-link-

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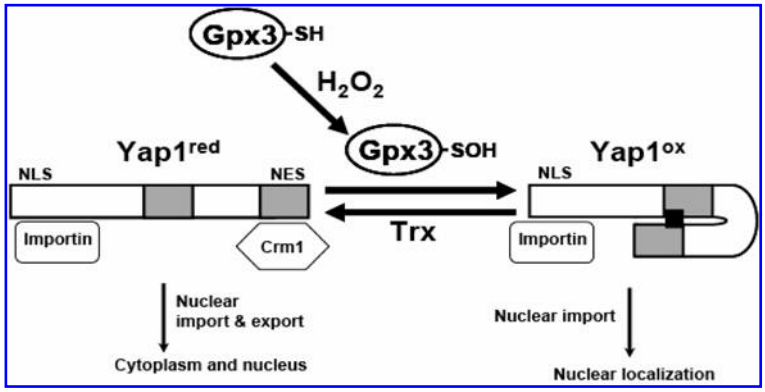

FIG. 1. Redox regulation of the nuclear localization of Yap1. Yap1 has a nuclear localization signal (NLS; 14), which can bind to the import receptor (Importin), and a nuclear export signal (NES; 18), which is recognized by the export receptor (Crm1). Under nonstress conditions, Yap1 is transported in and out of the nucleus, and Yap1 is localized predominantly in the cytoplasm. However, when Yap1 is oxidized in response to H_2O_2 stress, Crm1 no longer recognizes the NES of Yap1. As a result, Yap1 accumulates in the nucleus and expression of its target genes is activated. Formation of a disulfide bond between two cysteine-rich domains, namely, n-CRD and c-CRD (indicated by gray boxes), occurs in the presence of Gpx3, which has Prx activity (10).

ing of the electrophilic compound diethyl maleate (DEM) to the three cysteine residues in the c-CRD is responsible for the inhibition of interaction between Crm1 and the NES of Yap1 (c-CRD) (1). In addition to the c-CRD, three cysteine residues in the amino-terminal region (n-CRD) are also required for nuclear localization of Yap1 in the specific response to H_2O_2 (7). Our data suggest that the cysteine residues in the n-CRD are required for duration of nuclear localization of Yap1 (19). It has been suggested that formation of an intramolecular disulfide bridge between the first cysteine of n-CRD (Cys303) and the first cysteine of c-CRD (Cys598) might be required for the inhibition of binding of Crm1 to the NES of Yap1 that occurs in response to hydroperoxides such as H_2O_2 (8).

The recent finding that Gpx3, which has Prx (Trx peroxidase) activity, is an effecter of Yap1 in the sensing of H_2O_2 (10) suggests that the rapid response to H_2O_2 by Yap1 might involve the sensitivity of Gpx3 to H_2O_2. The *YBP1* (yap1-binding protein 1) gene has been identified as a high-copy-number suppressor of the sensitivity to peroxide of the *tsa1*Δ strain of cells that is derived from W303–1a cells (24). The disruption of *YBP1* (*ybp1*Δ) increased the sensitivity of those cells to H_2O_2. This phenotype was similar to that of cells with disruption of *GPX3* (*gpx3*Δ) and of the double-disruption mutant (*gpx3*Δ *ybp1*Δ), suggesting that Ybp1 might play an essential role in the Gpx3-catalyzed activation of Yap1.

Members of the Prx family of proteins are conserved from bacteria to human. The common catalytic property of proteins in the Prx family appears to be the ability to reduce hydroperoxides at the expense of thiols that are coupled with the Trx/Trr/NADPH redox cycle. An active cysteine in Prx is sensitive to hydroperoxide (for review, see 13). It is oxidized to sulfenic acid (Cys-SOH) or further oxidized to sulfinic acid (Cys-SO_2H). As Prx modulates intracellular levels of ROS, it has been postulated Prx might function in H_2O_2-mediated signal transduction (29).

In this study, we found that a Prx, Tsa1, catalyzed the H_2O_2-induced activation of Yap1 in a strain-dependent manner. Our results suggest that Prx can serve as a general transducer of the H_2O_2 signal to sensor protein.

MATERIALS AND METHODS

Yeast strains and media

Yeast cells were grown in synthetic dextrose (SD) medium supplemented with amino acids (SD dropout; 16) or in YPAD medium (1% peptone, 0.5% yeast extract, 2% glucose, 0.08 mg/ml adenine sulfate). The following strains of *Saccharomyces cerevisiae* were used in this study: Y17202 (*MAT*α *his3*Δ*1 leu2*Δ*0 lys2* Δ*0 ura3*Δ*0 trp1::kanMX4*), the *trp1*Δ cells from a knockout library constructed using BY4742 (EUROSCARF) derived from S288C, the strain used for genome sequencing; BY4742 yap1Δ [*MAT*α *his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0 trp1::kanMX4 yap1::URA3*); Y700 (*MAT*α *his3 can1–100 ade2 leu2 trp1 ura3*) [formerly designated W303B (16)]; and TW (the same as Y700 but *yap1*::*URA3 ura3*::*TRX2*p-lacZ) (19). The *TSA1* and *GPX3* genes were disrupted as follows. The PCR-amplified *TSA1* gene was cloned into pBlueScript SK (Stratagene, La Jolla, CA, U.S.A.) to yield pTSA1. The coding region of *TSA1* was replaced by the *HIS3* gene, and the resultant fragment was used to transfect to TW cells. *GPX3*::kanMX, in which the coding region of *GPX3* had been replaced by a kanamycin-resistance gene (26), was amplified by PCR and used to transfect TW cells. Transformants were selected on histidine-dropout SD medium and in YPAD that contained G418 (150 μg/ml; Nakarai Tesque Co., Kyoto, Japan), respectively, and genome replacement was confirmed by PCR. BY4742 *yap1*Δ was constructed in this study from Y17202, as described by Kuge and Jones (16).

Construction of plasmids

YBP1 genes (−425 to +2,689 of the initiation codon of Ybp1) were amplified by PCR from genomic DNA that had been isolated from BY4742 or Y700 using Takara Ex taq™ polymerase (Takara Co., Kyoto, Japan) and primers 5′-CAGAAATGTCACTCGCCAAA-3′ and 5′-TCCAAAATCCCTGAACGACA-3′.

The amplified fragments containing *YBP1* from BY4742 and Y700 were cloned between the *Xba*I (blunt-ended) and the *Hind*III (blunt-ended) sites of a multicopy-number vector, pRS425, and a low-copy-number vector, pRS315, and the resulting plasmids were designated pRS425-YBP1 (*YBP1* from BY4742), pRS425-ybp1–2 (*ybp1–2* from Y700), pRS315-YBP1, and pRS315-ybp1–2, respectively, after the sequence of the insert had been confirmed by sequencing.

Assay of β*-galactosidase activity and fluorescence microscopy*

The assay of β-galactosidase activity was performed as described by Kuge *et al*. (19). Yeast cells, grown in SD dropout medium (1.5 ml) until the absorbance at 600 nm had reached 0.5, were recovered and resuspended in prewarmed SD dropout medium (1.5 ml). After exposure to H_2O_2 at 0.5 m*M* or to 2 m*M* DEM for 1 h at 30°C, cells were recovered by centrifugation and resuspended in 50 µl of Tris-Triton (100 m*M* Tris-HCl, pH 7.5, 0.05% Triton X-100). Then after cells had been frozen at −80°C and thawed at room temperature, they were mixed with 250 µl of a mixture of 0.8 m*M* *o*-nitrophenyl β-D-galactopyranoside, 48 m*M* Na_2HPO_4, 32 m*M* NaH_2PO_4, 8 m*M* KCl, 0.8 m*M* $MgSO_4$, and incubated for a few minutes at 30°C. After the reaction had been stopped by addition of 125 µl of 1 *M* sodium carbonate, absorbance at 600 nm was determined to estimate the density of cells. Cells were pelleted by centrifugation, and absorbance of the supernatant at 420 nm was determined. The absorbance at 420 nm was normalized by reference to the absorbance at 600 nm and the reaction time (in minutes) for estimation of β-galactosidase activity. At least three samples from the individual respective yeast colony were analyzed, and results are given as means ± SD.

Fluorescence from green fluorescent protein (GFP) in yeast cells that harbored pRS cp GFP HA YAP1 (17) was recorded with a fluorescence microscope (Dmire 2; Leica Co., Wetzlar, Germany) equipped with a GFP:S filter block and a CoolSnap™ HQ CCD camera (Roper Scientific Co., Tucson, AZ, U.S.A.), and the images were analyzed with the MetaMorph™ program (Universal Imaging Co., Marlow, Buckinghamshire, U.K.).

Analysis of Yap1 in vivo *by western blotting*

Lysates of yeast cells that harbored pRS cp HA-YAP1 (17) were prepared essentially as described by Delaunay *et al*. (8) with slight modifications. Yeast cells, grown in SD dropout medium to an absorbance at 600 nm of 0.5, were recovered and resuspended in prewarmed medium. After exposure to H_2O_2 at 0.5 m*M*, cells were recovered at the indicated times. Cells were fixed in 20% TCA, and then frozen in 12.5% trichloroacetic acid (TCA). Lysates were prepared from the frozen cells by mixing vigorously with glass beads (G-8772; Sigma Chemical Co., St. Louis, MO, U.S.A.), precipitated in the TCA solution by centrifugation, and washed with acetone. The precipitates were dissolved in 50 m*M* iodoacetamide, 1% sodium dodecyl sulfate (SDS), 1 *M* Tris-HCl, pH 8.0, 1 m*M* EDTA plus a complete protease inhibitor cocktail (catalog no. 1836170; Roche Diagnostics, F. Hoffmann–La Roche Ltd., Basel, Switzerland), and incubated 37°C for 60 min. The reaction mixture was then dialyzed, treated with calf intestinal alkaline phosphatase (Roche), and subjected to nonreducing SDS-polyacrylamide gel electrophoresis (PAGE) as described above. Western blotting and detection of hemagglutinin (HA)-tagged Yap1 protein were performed as described elsewhere (17).

RESULTS

Tsa1 is responsible for sensing of H_2O_2 *by Yap1*

Members of the Prx family of proteins are conserved from yeast to human. Deletion of the gene (*TSA1*) for a thiol-specific antioxidant that is a member of the Prx family in

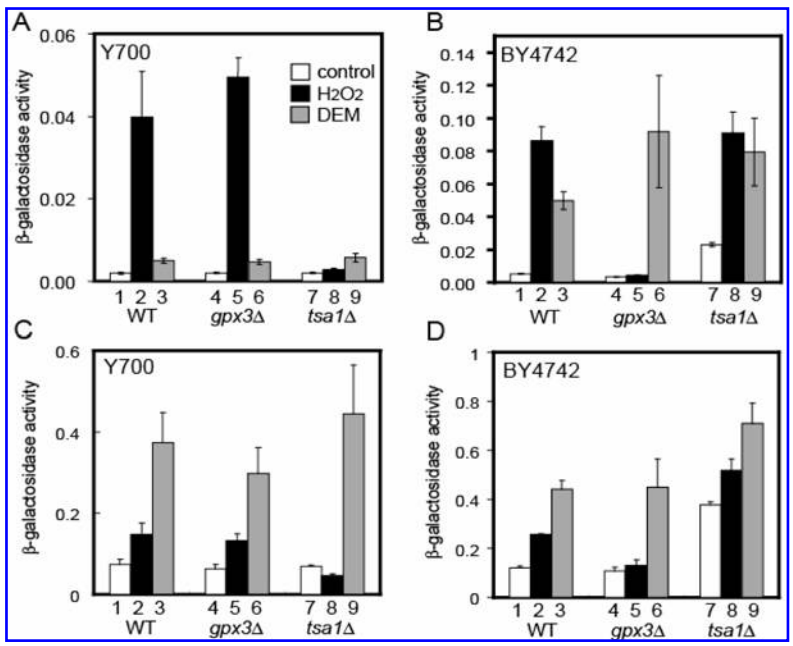

FIG. 2. Strain-specific dependence on proteins in the Prx family (Tsa1 and Gpx3) that participate in the activation of target genes by Yap1. The *TSA1* or the *GPX3* gene was disrupted in Y700 (**A** and **C**) and in BY4742 cells (**B** and **D**). The reporter genes for Yap1 cloned into low-copy number vectors, TRX2-LACZ (16; A and B) or SV40AP-1-LacZ (16; C and D), were introduced in these yeast strains, and β-galactosidase activity was assayed after each culture had been exposed to 0.4 m*M* H_2O_2 (black bars), to 2 m*M* DEM (gray bars), or to no treatment (open bars) for 1 h. See text for further details.

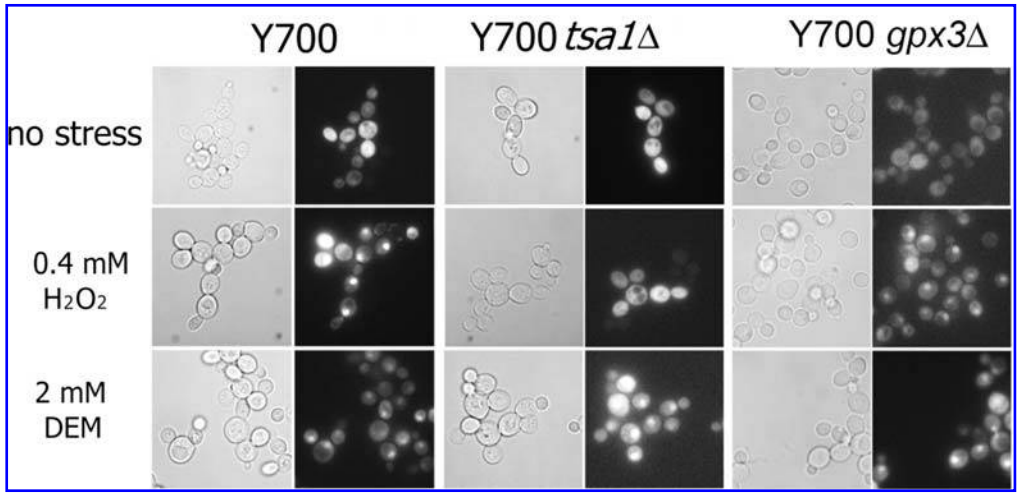

FIG. 3. Localization of GFP-Yap1 in Y700 *tsa1*Δ and in Y700 *gpx3*Δ. The GFP fluorescence was monitored in Y700 (TSA1), Y700 *tsa1*Δ, and Y700 *gpx3*Δ expressing GFP-fused Yap1, as described in the text, after treatment of the indicated cells with or without the indicated oxidants for 15 min. Bright-field images (**left panels**) and GFP fluorescence images (**right panels**) are shown.

yeast results in defects in Yap1-dependent transcription in response to oxidative stress (21). In this study, we examined first the effects of disruption of *TSA1* and *GPX3* on the Yap1-dependent activation of a reporter gene, driven by the TRX2 promoter, in response to H_2O_2 and to DEM in two different isogenic strains, namely, Y700, which is derived from W303. As shown in Fig. 2A, there was no difference in terms of the response to the electrophilic reagent DEM between the isogenic wild-type (*TSA1*) and *tsa1*Δ strains of Y700. However, no response to H_2O_2 was detected in Y700 *tsa1*Δ.

Next, we examined defects in *GPX3* on the genetic background of Y700. Deletion of *GPX3* did not affect the H_2O_2-induced activation of Yap1 in this context (Fig. 2A). These observations suggested that there might be some strain specificity in the requirement for specific proteins in the Prx family with respect to the response of Yap1 to H_2O_2. Therefore, we examined other isogenic strains, *gpx3*Δ and *tsa1*Δ, derived from BY4742 (BY4742 has been used for the EUROFAN II knockout project and is derived from S288C; 3). As shown in Fig. 2B, the disruption of *TSA1* increased the uninduced level of Yap1-dependent transcription, whereas disruption of *GPX3* decreased the H_2O_2-induced rate of Yap1-dependent transcription. These observations are consistent with previously reported results (10).

Activation of the TRX2 promoter requires two different activators of transcription, namely, Yap1 and Skn7 (20). Therefore, we examined transcription of another lacZ reporter gene that was driven exclusively by Yap1-binding sites (SV40AP-1-lacZ; 16). As shown in Figs. 2C and D, *TSA1,* but not *GPX3,* was required for the H_2O_2-induced expression of SV40AP1-lacZ in Y700. These observations demonstrated that Tsa1 and Gpx3 can each affect the response of Yap1 to H_2O_2, acting in a strain-dependent manner.

Next, we examined the induction of the nuclear localization of Yap1 that had been fused to GFP in response to oxidants in Y700 *TSA1* cells, Y700 *tsa1*Δ cells, and Y700 *gpx3*Δ cells. There was no difference in terms of the DEM-induced nuclear localization of Yap1 between Y700 *TSA1*, Y700 *tsa1*Δ, and Y700 *Gpx3*Δ cells. However, when we examined the response to H_2O_2, we found that disruption of *TSA1,* but not of *GPX3,* resulted in defects in the nuclear localization of Yap1 on the Y700 genetic background (Fig. 3). Thus, Tsa1 was required for the H_2O_2-induced nuclear accumulation of Yap1 in Y700 cells.

Tsa1-dependent formation of oxidized Yap1 in response to H_2O_2

Formation of an intramolecular disulfide bond between cysteine residues in two domains of Yap1, namely, n-CRD and c-CRD, results in a mobility shift during nonreducing SDS-PAGE (8). To examine the effects of Tsa1 on conforma-

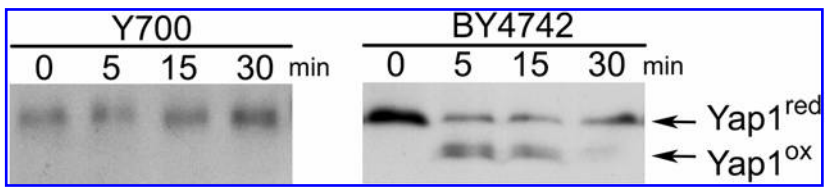

FIG. 4. Oxidation of Yap1 *in vivo*. Time-dependent oxidation of Yap1 in response to H_2O_2 was monitored. TW (for Y700 *yap1*Δ) cells and BY4742 *yap1*Δ cells expressing HA-Yap1 were treated with 0.5 m*M* H_2O_2 and lysed after fixation with TCA. Free Cys-SH residues in the lysate were allowed to react with iodoacetamide as described in the text. Yap1 was detected by western blotting with HA-specific antibodies.

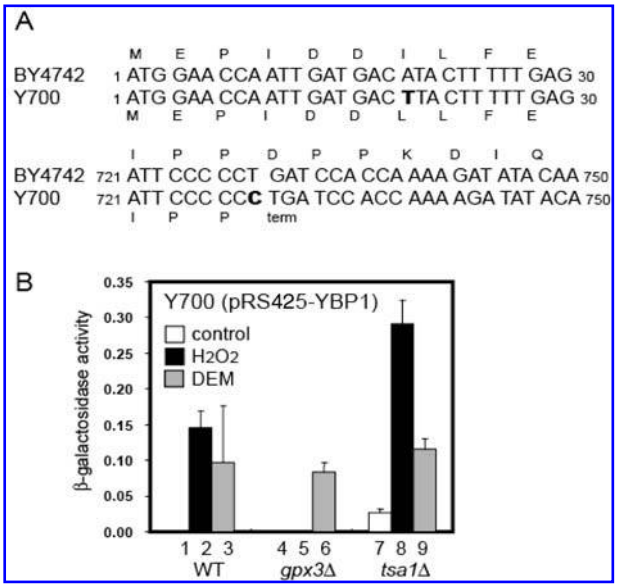

FIG. 5. ***YBP1*** **and** ***ybp1–2*** **are responsible for the strain-dependent utilization of Tsa1 or Gpx3.** (**A**) Direct sequencing of *YBP1* alleles in yeast genomic DNA after amplification by PCR. To prevent any impact of errors during PCR, products of at least three amplifications by PCR were subjected to sequencing reactions. Bold face indicates mutations found in *ybp1–2*. Insertion of "C" at position 729 generated a termination codon at Asp (243) in Ybp1 of Y700. (**B**) Y700 cells expressing the wild-type (BY4742) allele of *YBP1* (pRS425-YBP1), as monitored as described in the text, after each culture was exposed to 0.4 m*M* H_2O_2 (black bars), to 2 m*M* DEM (gray bars), or to no treatment (open bars) for 1 h.

tional changes in Yap1 in Y700 cells, we treated cells that expressed HA-tagged Yap1 (HA-Yap1) with H_2O_2. Free thiols were blocked by iodoacetamide to prevent artificial oxidation during preparation of cell lysates, as well as during nondenaturing SDS-PAGE. Consistent with the data reported previously (8), a higher-mobility form of HA-Yap1 was detected 5–15 min after the start of treatment of BY4742 cells with H_2O_2 (Fig. 4). By contrast, no such shift in the mobility of HA-Yap1 was observed in Y700 (TW) cells in response to H_2O_2 treatment (Fig. 4).

Mutation of YBP1 *of Y700 cells*

W303–1a cells have a mutant allele of *YBP1* (*ybp1–1*) and exhibit enhanced sensitivity to hydroperoxide (24). As Y700 cells are derived from W303 cells, we determined the sequence of the *YBP1* gene in genomic DNA from BY4742 cells and Y700 cells, respectively. There were no differences between the sequences of the *YBP1* gene from BY4742 and the sequence in the yeast genome database (data not shown). However, we found that the *YBP1* gene from Y700 has an insertion of deoxycytidine at nucleotide position 729 of the coding region of *YBP1*, which resulted in the introduction of a nonsense mutation at codon 244 of Ybp1 (Fig. 5A). In addition, all the mutations found in *ybp1–1* (I7L, F328V, K343E, and N471D; 24) were present in *YBP1* of Y700 (data not shown; Fig. 5A). We refer to the gene for *YBP1* of strain Y700 as *ybp1–2*.

To examine the possible involvement of *ybp1–2* in the response of Yap1 to H_2O_2, we introduced the *YBP1* gene, which was isolated from BY4742 cells, into Y700 cells. As shown in

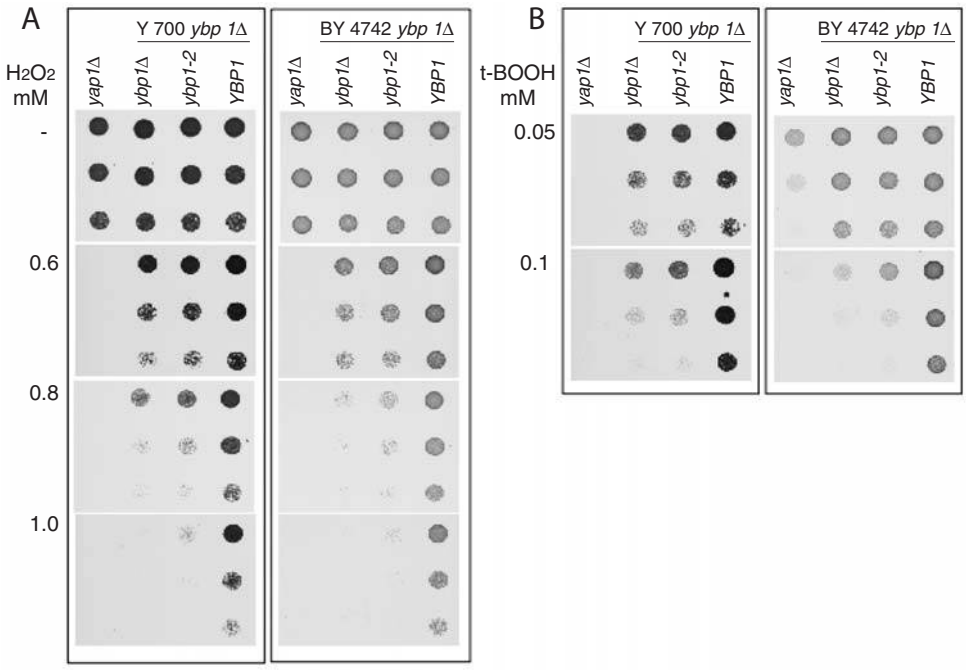

FIG. 6. Hydroperoxide resistance of cells carrying ***YBP1*** **and** ***ybp1–2*****.** Series of dilution (4.3×10^2, 1.4×10^2, and 4.8×10^2 cells/spot) of Y700 *ybp1*Δ cells carrying pRS315-ybp1–2 (*ybp1–2*) or pRS315-YBP1 (*YBP1*), and Y700 *yap1*Δ were spotted on SD agar plate (supplemented with all required nutrients) containing the indicated concentrations of H_2O_2 (**A**) or t-BOOH (**B**). For BY4742 *ybp1*Δ cells carrying pRS315-YBP1 and pRS315-ybp1–2, 4×10^4, 1.3×10^4, and 4.3×10^3 cells/spot were used for the assay. The spotted plates were incubated at 30°C for 20 h.

Fig. 5B, the pattern of the expression of the Yap1-dependent reporter gene was similar to that in BY4742 cells: the H_2O_2-induced expression of the reporter gene depended on *GPX3*, and the basal level of expression of *tsa1*Δ was enhanced.

To investigate further the role of *ybp1–2* in the oxidative stress response of Y700 cells, we examined the effect of *ybp1–2* on the resistance to H_2O_2 and *tert*-butyl hydroperoxide (t-BOOH). Consistent with previously reported results (24), *yap1*Δ cells showed more sensitivity to H_2O_2 and t-BOOH than *ybp1*Δ cells (Fig. 6). Furthermore, *ybp1–2* increased the resistance of *ybp1*Δ cells to H_2O_2 and t-BOOH, although the level of the activity of *ybp1–2* was lower than that of *YBP1* (Fig. 6), suggesting that a truncated mutant of Ybp1 protein encoded by the *ybp1–2* allele has the ability to activate Yap1 in response to hydroperoxide.

DISCUSSION

Prxs, which are ubiquitous from bacteria to mammals, reduce hydroperoxides. We showed, in this study, that the Prx Tsa1 can function as a transducer of oxidative stress to Yap1. A previous study indicated that Gpx3, which has Prx activity, can catalyze formation of a disulfide bond in Yap1 (10). Recently, *YBP1* was identified as a third factor in the hydroperoxide-induced activation of Yap1. In addition to the mutation found in *ybp1–1* of W303 (24), we found a nonsense mutation, which produce truncated Ybp1 ($Ybp1^{1–243}$), in *ybp1–2*. Thus, it appears that the truncated $Ybp1^{1–243}$ might somehow determine the utilization of Tsa1 in the activation of Yap1. It is possible that $Ybp1^{1–243}$ might prevent oxidation of Yap1 by Gpx3, allowing access by Tsa1 to Yap1 (Fig. 7). This hypothesis is supported by the observation that Ybp1 can bind to Yap1 (24).

We showed previously that Yap1 is activated in Y700 cells in response to hydroperoxide stress (17–19). The H_2O_2-induced oxidation of Yap1 in Y700 cells appeared to differ from that in BY4742 cells (Fig. 4). The faster migration of Yap1 during nondenaturing SDS-PAGE is the result of a disulfide bond between Cys303 and Cys598 (8). However, Yap1 did not include such a faster migrating component in Y700 cells (Fig. 4). We showed previously (19) that a disulfide bond is formed in the NES-containing regulatory domain c-CRD in response to H_2O_2, when the c-CRD was expressed in cells of Y700 background, and such disulfide bond formation might be sufficient to prevent binding of Crm1 to the NES of Yap1, suggesting that Yap1 in Y700 might form a disulfide bond in c-CRD in response to H_2O_2. However, the cysteine residues of n-CRD are required for the long-term duration of the H_2O_2-induced nuclear localization of Yap1 (19). Therefore, there might be an additional disulfide bond(s) or some other type of oxidation of residues in n–CRD that might stabilize the disulfide bond within the c-CRD. The slower mobility of Yap1 during nondenaturing SDS-PAGE 5 min after addition of H_2O_2 (Fig. 4) might be due to such multiple oxidation forms. It should be noted that an oxidation of Yap1 without the faster migrating band in SDS-PAGE was observed during nuclear localization of Yap1 in response to carbon stress induced by changes of carbon source in the medium (25).

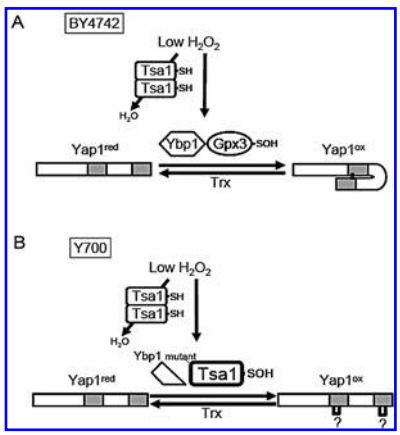

FIG. 7. Model of the participation of Tsa1 and Gpx3 in the activation of Yap1. At low concentrations of H_2O_2, Tsa1 serves as a Prx to reduce H_2O_2 and catalyzes the oxidation of the sensor protein Yap1 in the presence of the mutant Ybp1 (*ybp1–2*) (**B**). In the presence of wild-type Ybp1, Gpx3 may be recruited by Ybp1 for the oxidation of Yap1 (**A**).

Tsa1 is an abundant protein in yeast cells (0.7% of total soluble protein of the cells; 5, 15) and is estimated at 91.4% (3.78×10^5 molecules per cells) among total molecules of five Prx family proteins, as well as Gpx3, whereas Gpx3 is estimated at 2% among these proteins (11). Thus, Tsa1 might be a major antioxidant in these cells. The abundance and reactivity to H_2O_2 of Tsa1 suggest that Tsa1 might activate Yap1 in the absence of Ybp1 and/or in the presence of $Ybp1^{1–243}$ by a system analogous to the system that involves Gpx3. A redox-active distal cysteine residue in Tsa1, Cys47, is first oxidized by H_2O_2 to sulfenic acid (Cys47-SOH), and then it reacts with a distal cysteine residue (Cys171) to form a dimer (13). We found that the $Tsa1^{C171T}$ mutant was cross-linked specifically with Yap1, but not with Trx or with Trr in the Trx/Trr/NADPH reduction system *in vitro* in response to H_2O_2 (Okazaki and Kuge, unpublished observations). Thus, the sulfenic acid form of Cys47 (Cys47-SOH) in Tsa1 might attack several cysteine residues to induce formation of disulfide bonds in Yap1.

At higher concentrations of H_2O_2, the unstable Prx with a Cys47-SOH moiety is further oxidized to Prx with a sulfinic acid (Prx Cys-SO_2H) moiety, but not a disulfide (Prx S-S Prx), and Prx Cys-SO_2H is reduced to Prx Cys-SH (27, 28). Thus, it has been suggested (27) that Prx might act as a floodgate for the H_2O_2 signal until the level of H_2O_2 exceeds the level that is protected by the formation of Prx Cys-SO_2H (floodgate model). Recent findings indicate that the constitutive reduction of Tsa1 Cys-SO_2H to Tsa1 Cys-SH is catalyzed by sulfiredoxin (Srx1) during the response to oxidative stress (2). We demonstrated here that the sensitive and appropriate response of Yap1 to the H_2O_2 signal involves member of the Prx family of proteins. Thus, in addition to the redox cycle of Prx by sulfiredoxin (Cys-SH/Cys-SO_2H), the redox cycle of Prx by Trx (Cys-SHs/Cys-S-S-Cys) might participate in transduction of the H_2O_2 signal via different oxidation states that depend on the intracellular level of H_2O_2 in the eukaryote.

Mammalian Prxs are also abundant proteins (0.1–0.8% of total soluble protein of the cells; 9). It is possible that an analogous redox-sensing system is exploiting Prxs in mam-

malian cells, in view of the recent finding that a mammalian transcription factor, Hic-5, is regulated at a nuclear export step by a possible redox reaction that involves a Yap1-like NES (22).

While the reviewing process of this article was in progress, Veal and Morgan perceived that there is a single nucleotide insertion of deoxycytidine at nucleotide position 729 of the coding region of *ybp1–1* that has not been indicated in the previous literature (14). This reveals that *ybp1–1* is identical to *ybp1–2*. A discrepancy of the observed characteristics between Y700 and W303–1a is transcriptional activation of LacZ reporter driven by SV40AP1 Yap1-binding sites (Figs. 1C and 2C in reference 24). This may be due to the difference of constructs of the reporter gene used in this study and the other study (24). We show here that Tsa1, an abundant Prx, can regulate nuclear localization of Yap1, and suggest that the utilization of Tsa1 on the activation of Yap1 is more efficient in cells carrying *ybp1–1* (*ybp1–2*) than in cells carrying *ybp1*Δ or *YBP1*.

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ABBREVIATIONS

c-CRD, C-terminal cysteine-rich domain of Yap1; DEM, diethyl maleate; GFP, green fluorescent protein; Gpx, glutathione peroxidase; HA, hemagglutinin; H_2O_2, hydrogen peroxide; n-CRD, N-terminal cysteine-rich domain of Yap1; NES, nuclear export signal; PAGE, polyacrylamide gel electrophoresis; Prx, peroxiredoxin; ROS, reactive oxygen species; SD, synthetic dextrose; SDS, sodium dodecyl sulfate; t-BOOH, *tert*-butyl hydroperoxide; TCA, trichloroacetic acid; Trr, thioredoxin reductase; Trx, thioredoxin; Ybp1, Yap1-binding protein 1.

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