Ybp1 Is Required for the Hydrogen Peroxide-induced Oxidation of the Yap1 Transcription Factor*

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We describe the characterization of Ybp1, a novel protein, in Saccharomyces cerevisiae, that is required for the oxidative stress response to peroxides. Ybp1 is required for H2O2-induced expression of the antioxidant encoding gene TRX2. Our data indicate that the effects of Ybp1 are mediated through the Yap1 transcription factor. Indeed, Ybp1 forms a stress-induced complex with Yap1 in vivo and stimulates the nuclear accumulation of Yap1 in response to H2O2 but not in response to the thiol-oxidizing agent diamide. The H2O2-induced nuclear accumulation of Yap1 is regulated by the oxidation of specific cysteine residues and is dependent on the thiol peroxidase Gpx3. Our data suggest that Ybp1 is required for the H2O2-induced oxidation of Yap1 and acts in the same pathway as Gpx3. Consequently, Ybp1 represents a novel class of stress regulator of Yap1. These data have important implications for the regulation of protein oxidation and stress responses in eukaryotes.

The cells of all aerobic organisms are exposed to reactive oxygen species (ROS) that include the superoxide (O2•−) and hydroxyl (OH•) radicals and H2O2. ROS are highly reactive and can damage many cellular components. Indeed, cells of the immune system make use of the damaging effects of ROS to eliminate invading pathogens such as fungi (for a review see Ref. 1). Organisms have an array of proteins that protect against ROS-induced damage. For example, antioxidant proteins scavenge and inactivate ROS. However, if the level of ROS exceeds the antioxidant defenses then oxidative stress occurs, triggering a mechanism, called the oxidative stress response (OSR), that increases the production of antioxidant proteins to restore homeostasis. ROS have been linked with a wide range of diseases and hence the characterization of the mechanisms of the OSR has important implications for understanding disease processes in higher eukaryotes.

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1 The abbreviations used are: ROS, reactive oxygen species; OSR, oxidative stress response; CRD, cysteine-rich domain; ORF, open reading frame; BOOH, butyl hydroperoxide; GFP, green fluorescent protein; DAPI, 4’6-diamidino-2-phenylindole.

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The budding yeast Saccharomyces cerevisiae is an important model organism in studies of the regulation of the OSR (2). The Yap1 and Skn7 transcription factors are required for the OSR to regulate the H2O2-induced expression of many antioxidant encoding genes. These include the TRX2, TTR1, and TSA1 genes (3–5), encoding thioredoxin, thioredoxin reductase, and thioredoxin peroxidase, respectively, which together make up the thioredoxin system, a conserved pathway that reduces peroxides such as H2O2 to harmless products (6, 7). Yap1 and Skn7 act in the same pathway to regulate TRX2 and TTR1 gene expression (3, 4), although the mechanism of co-operation has not been elucidated.

In eukaryotes, AP-1-like transcription factors, such as mammalian c-Jun, are involved in stress responses (see Ref. 8 for a review). AP-1-like proteins contain a bZIP structural motif consisting of a leucine-rich zipper region and an adjacent basic region that are important for dimerization and DNA binding, respectively. Yap1 is a member of a subfamily of AP-1-like transcription factors that also includes Cap1 from Candida albicans and Pap1 from Schizosaccharomyces pombe (8–10). Oxidative stress induces the accumulation of Yap1 in the nucleus (11). This is linked to changes in the redox status of two cysteine-rich domains (CRDs) in Yap1, the n-CRD and c-CRD, that prevent the interaction of Yap1 with the nuclear export factor Crm1 (11–16). In contrast, the nuclear import of Yap1 has been found to be unaffected by the redox status of Yap1 (17). During non-stressed conditions Yap1 and Crm1 interact, resulting in export of Yap1 from the nucleus, whereas oxidative stress treatment weakens this interaction resulting in the nuclear accumulation of Yap1 (12, 13). Interestingly, different oxidative stress agents appear to regulate Yap1 by different mechanisms. In particular, H2O2 and diamide (a free thiol oxidizing agent) cause oxidation of different cysteine residues in the CRDs of Yap1 (15, 16). However, both H2O2 and diamide treatment result in the nuclear accumulation and activation of Yap1. Although the mechanism of regulation of Yap1 by diamide is unclear, Delaunay et al. (18) demonstrated that a thiol peroxidase, Gpx3, forms a complex with Yap1 and is important for the H2O2-induced oxidation of the protein.

Here, we have identified an open reading frame (ORF), YBR216c, which we have named YBP1 (Yap1-binding protein), that is important for the OSR in S. cerevisiae. Moreover, we demonstrate that Ybp1 forms a stress-induced complex with Yap1 in vivo and influences the nuclear localization of Yap1 in response to H2O2 but not diamide. Yap1 oxidation is induced by H2O2 and is important for the regulation of the protein. Our data reveal that Ybp1 is required for the H2O2-induced oxidation of Yap1 and acts in the same pathway as Gpx3. Thus, Ybp1 represents a new class of redox regulator protein.
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β-Galactosidase Assays—β-Galactosidase assays were performed (33) on cells grown to mid-log phase in SD media.

Protein Interaction Studies—Crude protein extracts were prepared from ~2 × 10^9 cells by lysis cells in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM imidazole, 0.5 g/liter 100 ml Nonidet P-40, with 2 μg/ml pepstatin A, 2 μg/ml leupeptin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM/liter 100 ml aprotinin). For purification of His₆FLAG-tagged Yap1, crude extract was added to 30 μl of nickel-nitrotriacetic acid agarose (Qiagen) pre-equilibrated in the lysis buffer. After incubation at 4°C for 60 min, the beads and any coupled proteins were pelleted, washed with lysis buffer, and then resuspended in SDS-PAGE loading buffer. The ability of Myc-tagged Yap1 protein to co-purify with His₆FLAG-tagged Ybp1 was analyzed by SDS-PAGE and Western blotting using monoclonal anti-His antibodies (9E10, Sigma) to detect the oxidized and reduced forms of Myc-tagged Yap1.

Preparation of Cell Extracts for Monitoring Yap1 Oxidation—Cell extracts were prepared as described by Delaunay et al. (15). Iodoacetic-acid-treated proteins were treated with alkaline phosphatase and then separated by non-reducing SDS-PAGE. Gels were analyzed by Western blotting using anti-Myc monoclonal antibodies (9E10, Sigma) to detect the oxidized and reduced forms of Myc-tagged Yap1.

Localization of GFP-tagged Yap1—Cells containing the GFP-Yap1 expression plasmid were concentrated to ~25 μl of medium and 5 μl spotted on to a glass slide. This spot was mixed on the slide with 5 μl of 0.4°C/liter (w/v) low melting temperature agarose (Invitrogen) containing an appropriate concentration of H₂O₂ or diamide to give the desired level of stress. A coverslip was placed on top, and GFP-tagged Yap1 was detected by exciting cells with 450–490 nm using a Zeiss Axioscope fluorescence microscope, with a ×40 oil immersion objective and an Axiovision digital imaging system.

Localization of Phk-tagged Ybp1—Cells were prepared essentially as described by Kilmartin and Adams (34) except that 5 μg/ml lyticase in 50% glycerol for 30 min at 30°C was used. Anti-Pk primary antibodies (Serotec) and Alexa 488-conjugated anti-mouse secondary antibodies (Molecular Probes) were used. DAPI-stained nuclei and anti-Pk immunofluorescence were visualized by excitation at 365 nm (DAPI) and 450–490 nm (Alexa 488) using a Zeiss Axioscope fluorescence microscope, with a ×63 oil immersion lens and an Axiovision digital imaging system.

RESULTS

Identification of a Novel Protein Involved in Peroxide Resistance—The deletion of the TSA1 gene, which encodes thioredoxin peroxidase, in the W303-1a strain reduces the H₂O₂-induced expression of the TRX2 promoter and increases peroxide sensitivity (19). A high copy suppressor screen revealed a previously uncharacterized ORF, YBR216c (named YBP1 from here on), that increased the peroxide resistance of the ska1Δ mutant and stimulated the H₂O₂-induced expression of the TRX2 gene in ska1Δ cells (data not shown). These results suggest that Ybp1 functions downstream of TsA1, or within a separate pathway, to protect cells against peroxide stress. Analysis of the predicted amino acid sequence of Ybp1 did not reveal homology to identified domains or provide clues to the biochemical activity of the protein.

Because the Yap1 and Skn7 transcription factors regulate peroxide-induced TRX2 gene expression (3, 4) the effect of expression of YBP1 from the Yep24 vector on H₂O₂ and BOOH resistance was examined in the W303-1a (YAPI SKN7), yap1Δ, and ska1Δ strains. Yap24-YBP1 greatly increased the resistance of W303-1Δ cells to these peroxides (Fig. 1A) but was unable to restore BOOH resistance to the yap1Δ and ska1Δ mutants (Fig. 1B). This suggests that the increased resistance associated with the presence of Yap24-YBP1 in W303-1a is dependent on Yap1 and Skn7 (Fig. 1B). Intriguingly, Yap24-YBP1 did not confer any increased resistance to the thiol-oxidizing agent diamide and, in fact, actually slightly increased the sensitivity of W303-1a (Fig. 1A). These data suggest that Ybp1 is a new protein involved in the peroxide-induced OSR but not the general response to oxidative stress.

Ybp1 Regulates H₂O₂-induced Gene Expression—Several genes, including those encoding proteins of the thioredoxin system, are induced by H₂O₂ in a Yap1- and Skn7-dependent
manner (3–5). RNA analysis of the W303–1a strain, either untreated or treated with H₂O₂, revealed that both the basal and H₂O₂-induced levels of TRX2 mRNA were increased in cells containing the high copy number plasmid, Yep24-YBP1 (Fig. 2A). Moreover, Yep24-YBP1 had no significant effect on the basal or H₂O₂-induced levels of TRX2 mRNA in the yap1Δ mutant and barely increased levels in the skn7Δ mutant (Fig. 2A). The expression of YBP1 from a high copy number plasmid also increased the expression of a reporter, where the TRX2 promoter was fused to the Escherichia coli lacZ gene, indicating that Ybp1 stimulates transcription from the TRX2 promoter (data not shown).

To examine the role of Ybp1 in H₂O₂-induced expression of TRX2 RNA analysis was performed using a derivative of W303–1a where the YBP1 gene has been deleted. Less induction of TRX2 mRNA occurred after 20 and 40 min of H₂O₂ treatment in the yap1Δ mutants (SR1 and SR2) compared with the isogenic W303–1a strain (Fig. 2B) revealing that Ybp1 is normally required for the response to H₂O₂. However, the induction of expression by H₂O₂ was greater than that seen in the yap1Δ or skn7Δ mutants (Fig. 2B). As expected, a low copy number (CEN) plasmid carrying the library-encoded YBP1 gene, expressed from its own promoter, rescued the H₂O₂-induced expression of TRX2 in the yap1Δ strain (data not shown), confirming that Ybp1 is required for H₂O₂-induced TRX2 expression.

To determine whether Ybp1 affects Yap1 activity the effect of expression of YBP1 from a high copy number plasmid (YEplac181-YBP1) on the expression of a Yap1-specific lacZ reporter (regulated by SV40-derived AP-1 binding sites) was examined (3). Importantly, the presence of YEplac181-YBP1 stimulated H₂O₂-induced β-galactosidase activity (Fig. 2C) and had no detectable effect on the expression of the SV40AP1-lacZ reporter in a yap1Δ mutant treated with H₂O₂ (data not shown).

Collectively, these data suggest that Ybp1 is important for H₂O₂-induced gene expression and are consistent with the hypothesis that the increased peroxide resistance of W303–1a cells containing Yep24-YBP1 is attributed to increased Yap1-dependent antioxidant gene expression.

**Ybp1 Is Required for Resistance to Peroxide**—The role of Ybp1 in peroxide resistance was examined. The growth rates of the yap1Δ strain (SR1) containing either the low copy number (CEN) plasmid YCplac22 or YCplac22-YBP1 (carrying the library-encoded YBP1 gene expressed from its own promoter) were the same under non-stressed conditions (data not shown). However, in contrast, yap1Δ cells containing vector took four–five times longer to start growing again following treatment with a non-lethal concentration of H₂O₂ than those containing YCplac22-YBP1 (Fig. 3A), although they recovered much more quickly than yap1Δ cells (data not shown). Surprisingly, the W303–1a strain containing vector recovered from H₂O₂ treatment much more slowly than the isogenic ybp1Δ strain containing YCplac22-YBP1 (Fig. 3A). Furthermore, W303–1a was much more sensitive to peroxide in plate assays than the ybp1Δ mutant containing YCplac22-YBP1 (Fig. 3B) or a ybp1Δ mutant where the YBP1 gene from the library plasmid was integrated at the normal locus (Fig. 3C). Expression of YBP1 from a plasmid (see Fig. 1A and Fig. 3B) or from the normal genomic locus (Fig. 3C) did not increase the resistance to diamide but in fact resulted in increased sensitivity. These data suggest that the YBP1 gene in W303–1a and the Yap24 library are different. The sequence of the YBP1 gene from the Yep24 plasmid was found to be identical to the predicted amino acid sequence encoded by the YBR216c ORF in the genome sequence. However, in contrast, the sequence of the YBP1 gene in W303–1a contains four amino acid substitutions; isoleucine at position 7 to leucine, phenylalanine at position 328 to valine, lysine at position 343 to glutamate, and asparagine at position 571 to aspartate (see Fig. 9).

This analysis reveals that Ybp1 plays an important role in the normal cellular resistance to peroxide and that W303–1a contains an allele of the YBP1 gene, which we have named ybp1–1, that results in increased sensitivity to peroxide. In agreement with our data, a recent investigation of ~600 gene deletion mutants demonstrated that a deletion of the YBR216c ORF inhibited growth in response to H₂O₂, cumene hydroperoxide, and linoleic acid hydroperoxide (35).

**Ybp1 Forms a Complex with Yap1**—The relationship between Ybp1 and the Yap1/Skn7 pathway suggested that Ybp1 may form a complex with one or both of these proteins. Moreover, large-scale studies of the yeast proteome have identified potential interactions between Yap1 and Ybp1 by two-hybrid (36) and affinity precipitation (37) approaches. Hence, to further investigate the relationship between Ybp1 and Yap1/Skn7, the wild type YBP1 gene was fused in-frame with the DNA binding domain of the E. coli LexA protein (LexA:Ybp1) in plasmid pBTM-YBP1. This plasmid was introduced into the
two-hybrid reporter strain CTY10–5d in conjunction with derivatives of the pGAD-C2 vector (29) expressing either full-length Yap1 (pGAD-Yap1 (1–650)) or Skn7 (pGAD-Skn7) fused in-frame with the Gal4 acid activation domain. Several functional domains of Yap1 have been defined: a bZip domain that is important for DNA binding, two separate transcriptional activation domains (I and II), and two cysteine-rich regions, located toward the middle of the protein (n-CRD) and at the C terminus (c-CRD), that are important for the stress-induced accumulation of active Yap1 in the nucleus (see Ref. 8 for a review, and see Fig. 4). To determine the region(s) of Yap1 that are required for the interaction with Ybp1, the Gal4 acid activation domain was fused in-frame with several deletion derivatives of Yap1, and the ability of the LexA:Ybp1 fusion protein to interact with these was analyzed (Fig. 4A). These data revealed that a region near the C terminus (amino acids 379–650), containing part of transcriptional activation domain II and the c-CRD, is sufficient to form a complex with Ybp1 (Fig. 4A).

To further investigate the role of this Yap1- and Ybp1-containing complex in the regulation of Yap1, we examined the effect of H2O2 on the interaction of Ybp1 and Yap1 in vivo. Myc-tagged Yap1 (pRS316-Yap1-Myc) (15) and Ybp1, tagged with six histidine residues and a FLAG epitope (YCplac111-YBP1-His6FLAG), were co-expressed in a yap1Δ ybp1Δ mutant (SR8), and cell extracts were prepared before and after treatment with H2O2. When His6FLAG-tagged Ybp1 was partially purified from these cell extracts using nickel-nitrilotriacetic acid-agarose, analysis of the precipitates revealed that, although a small amount of Yap1-Myc was co-purified with Ybp1-His6FLAG from unstressed cells (data not shown), H2O2 treatment greatly increased the amount of Yap1-Myc (Fig. 4B). This suggests that the Yap1- and Ybp1-containing complex is stabilized following H2O2 treatment and raises the possibility that the formation of a complex between Ybp1 and Yap1 is important for the regulation of Yap1 by oxidative stress.

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Ybp1 Is Located in the Cytoplasm—To understand further the role of Ybp1, its cellular localization was examined. We were able to detect Pk epitope-tagged Ybp1 (Ybp1-Pk) by indirect immunofluorescence when expressed from a high copy but not from a low copy number vector (Fig. 5) (data not shown). Analysis of the subcellular localization of Ybp1-Pk, expressed
in ybp1Δ cells (SR1), revealed that Ybp1-Pk was distributed throughout the cytoplasm but excluded from the nucleus. This pattern was unchanged following H₂O₂ treatment (Fig. 5). Although, as expected, the use of a high copy number plasmid gave rise to some intercellular variation in the levels of Ybp1-Pk, reflected in the varying brightness of the immunostained cells, the distribution was the same in all cells. These data indicate that, in contrast to Yap1, Ybp1 does not accumulate in the nucleus following stress.

Ybp1 Is Important for the H₂O₂-induced Nuclear Accumulation of Yap1

To examine whether Ybp1 is important for the oxidative stress-induced nuclear accumulation of Yap1, pRS-cp-GFP-YAP1, which expresses a GFP-tagged version of Yap1 (11), was introduced into the yap1Δ/H9004 ybp1Δ/H9004 (SR4) and the yap1Δ/H9004 YBP1 (SR6) strains were grown to mid-log, and 10-fold serial dilutions were spotted on to YPD media containing the indicated concentrations of tBOOH and diamide.

FIG. 4. Ybp1 forms a complex with Yap1. A, Ybp1 interacts with Yap1 in two-hybrid assays. β-Galactosidase assays were performed on cultures of the two-hybrid strain (CTY10–5d) containing pBTM-YBP1 and either the pGAD-C2 vector (pGAD), pGAD-Yap1 (1–650), pGAD-Yap1 (157–650), pGAD-Yap1 (379–650), or pGAD-Yap1 (460–650). LacZ activities were calculated as (A₄₂₀ × 1000)/A₆₀₀ × time of incubation [min] × cells [ml]). The positions of the Gal4 acid activation domain (GAD) in the fusion proteins are shown. The previously identified domains of Yap1, including the bZip domain, the n-CRD, the c-CRD, and transcription activation domains I and II, are also indicated. B, Ybp1 forms a stress-induced complex with Yap1 in vivo. Cell lysates were prepared from yap1Δ/ybp1Δ (SR8) cells containing pRS316-Yap1-Myc, expressing Myc epitope-tagged Yap1 (Yap1-Myc) (15), and either YCplac111-YBP1-His₆FLAG, expressing Ybp1 tagged with six histidine residues and a FLAG epitope (Ybp1-6xHisFlag), or vector control, following treatment with 0.8 mM H₂O₂ for 0, 5, or 10 min. Ybp1 was partially purified from these cell lysates using nickel-nitrilotriacetic acid-agarose, and precipitated proteins (Ni²⁺ pull down) were analyzed by SDS-PAGE and Western blotting using anti-Myc and anti-FLAG antibodies. The amount of Yap1-Myc co-purified with Ybp1 is shown relative to the amount of Yap1-Myc present in 2% of the cell lysate used in the pull down.
the yap1ΔYBP1 strain suggesting that the ybp1−1 allele is a loss of function mutant (data not shown).

The nuclear accumulation of Yap1 is also stimulated by diamide though in this case the c-CRD is important for regulation (11, 14, 16). In contrast to H2O2 treatment, the diamide-induced nuclear accumulation of GFP-tagged Yap1 was similar in yap1ΔYBP1 and yap1Δybp1Δ cells (Fig. 6). These data clearly demonstrate that Ybp1 affects the localization of Yap1 in response to specific oxidative stress.

Ybp1 Is Important for the H2O2-induced Oxidation of Yap1—To examine whether Ybp1 affects the redox regulation of Yap1, cells of the yap1Δybp1Δ (SR8) strain expressing Myc epitope-tagged Yap1 (Yap1-Myc), and containing either the low copy number CEN plasmid YCPlac111-YBP1 (the wild type YBP1 gene is expressed from its own promoter) or YCPlac111 vector, were treated with H2O2. The redox changes in Yap1-Myc were preserved with iodoacetamide and then examined by non-reducing SDS-PAGE and Western blotting for characteristic changes in protein mobility (15). A more mobile form of Yap1-Myc was detected following H2O2 treatment of cells containing the wild type YBP1 gene, similar to previous studies of Yap1 oxidation (Fig. 7A) (15, 18). Treatment with β-mercaptoethanol confirmed that the more mobile form of Yap1-Myc is oxidized Yap1 (data not shown). Interestingly, deletion of the YBP1 gene results in a significant inhibition of the H2O2-induced oxidation of Yap1 demonstrating that Ybp1 is required for this process (Fig. 7A).

Recently, the thiol peroxidase, Gpx3, was found to be important for the oxidation of Yap1 in response to H2O2 (18). To investigate the relationship between Gpx3 and Ybp1 the effects of the absence of Ybp1 and/or Gpx3 on peroxide-induced Yap1 oxidation were examined (Fig. 7B). As can be seen, the loss of either Ybp1 or Gpx3 results in a loss of Yap1 oxidation following peroxide treatment, although a small but reproducible level of oxidation was detected (Fig. 7B). Interestingly, a small level of Yap1 oxidation also occurred in the absence of both Ybp1 and Gpx3. These data suggest that Ybp1 and Gpx3 may act in the same pathway to regulate peroxide-induced Yap1 oxidation. Previously, Delaunay and colleagues (18) revealed that a gpx3Δ mutant is more sensitive than a Gpx3 strain to peroxide although not as a sensitive as a yap1Δ mutant, suggesting that an alternative pathway(s) for Yap1 activation acts in the absence of Gpx3. Indeed, our studies in the W303-1a genetic background where the ybp1−1 allele had been replaced with the wild type YBP1 gene also suggested the presence of alternative pathway(s) (Fig. 7C). Hence, it was possible that Ybp1 functions in an alternative pathway to Gpx3. However, a gpx3Δybp1Δ double mutant was no more sensitive to peroxide than either of the single mutants (Fig. 7C). These data are consistent with the hypothesis that Gpx3 and Ybp1 act in the same pathway to regulate the peroxide-induced oxidation of Yap1.

These data strongly suggest that Ybp1 is required for the efficient H2O2-induced oxidation of Yap1 that results in the nuclear accumulation of active Yap1. Furthermore, Ybp1 likely acts in the same pathway as the Gpx3 thiol peroxidase to regulate Yap1. Thus, Ybp1 is a novel regulator of the OSR.

**DISCUSSION**

Here, we describe the identification of Ybp1 as a novel regulator of the OSR in *S. cerevisiae*. Ybp1 is required for resistance to peroxide (Fig. 3). Moreover, Ybp1 regulates the H2O2-induced expression of the TRX2 (Fig. 2) and TRR1 (data not shown) genes in a Yap1-dependent manner suggesting that Ybp1 is required for Yap1 activity. Indeed, Ybp1 specifically forms a stress-induced complex with Yap1 and stimulates the peroxide-induced oxidation, nuclear accumulation, and activity of this transcription factor.

The main mechanism by which oxidative stress activates Yap1 is by stimulating its accumulation in the nucleus (11). In unstressed cells Yap1 is exported from the nucleus by Crm1. However, oxidative stress induces changes in the redox status of cysteines in the n-CRD and c-CRD of Yap1 (15, 16, 18) that inhibit the interaction of Yap1 with Crm1 (12, 13). Yap1 stimulates the H2O2-induced nuclear accumulation (Fig. 6) and activity (Fig. 2C) of Yap1 but does not affect the ability of a constitutively nuclear and active derivative of Yap1 to activate a Yap1-dependent reporter (data not shown). Furthermore, Ybp1 did not affect the nuclear localization of GFP-tagged Yap1 where the c-CRD and nuclear export sequence were replaced with a non-redox regulated, Crm1-dependent nuclear export sequence (12) (data not shown). Thus, the c-CRD is required for redox regulation of Yap1 and for stimulation of nuclear accumulation by Ybp1.

Recently, large scale analyses of yeast protein complexes, by affinity precipitation and two-hybrid studies, revealed that Ybp1 forms a complex with Yap1 (36, 37). We have found that Ybp1/Yap1 complex formation is further stimulated by prior treatment of cells with H2O2. Different domains of Yap1 have specific regulatory roles; the c-CRD is required for diamide-induced regulation, whereas both the n-CRD and the c-CRD are required for H2O2-induced regulation (14, 38). Our two-
hybrid interaction data suggest that the region of Yap1 containing the c-CRD is sufficient to support the interaction of Yap1 and Ybp1. However, Ybp1 is required for the H2O2-induced, but not the diamide-induced, nuclear accumulation of Yap1. Moreover, ybp1/H9004 and ybp1–1 strains were more resistant to diamide than strains containing the YBP1 gene. The basis of this diamide resistance is unclear, but together these results suggest that Ybp1 is required for peroxide-specific aspects of Yap1 regulation.

Several aspects of the mechanism of H2O2-dependent regulation of Yap1 have been characterized. In particular, the thiol peroxidase Gpx3 is important for the H2O2-induced oxidation of Yap1 (18) whereas reduced thioredoxin can reverse H2O2-induced oxidation (15). The effects of loss of Ybp1 (this study) or Gpx3 (18) function on the H2O2-induced oxidation and nuclear accumulation of Yap1 are very similar. Furthermore, a gpx3/H9004 ybp1/H9004 double mutant is no more sensitive to peroxide than either of the single mutants. These data suggest that Ybp1 and Gpx3 act in the same pathway to regulate Yap1. The precise nature of the relationship between Gpx3 and Ybp1 is under investigation. However, Delaunay et al. (18) demonstrated that a transient intermolecular disulfide bond forms between Gpx3 and Yap1 following H2O2 treatment, and it is possible that Ybp1 may have a role in this process. The H2O2-induced stimulation of Yap1/Ybp1 complex formation, together with the observation that Ybp1 is located predominantly in the cytoplasm, both before and after peroxide stress, support a model where stress-induced binding of Ybp1 is important for the H2O2-induced oxidation of Yap1 by Gpx3 in the cytoplasm whereupon Yap1 accumulates in the nucleus (Fig. 8).

In this study W303–1a was found to contain a mutant allele of YBP1, ybp1–1, encoding four amino acid substitutions, that results in increased peroxide sensitivity. This increased sensitivity correlated with reduced H2O2-induced oxidation and nuclear accumulation of Yap1, compared with cells containing the YBP1 gene and previous studies in other strains of S. cerevisiae. However, ybp1–1 is not a complete loss of function allele as it is more resistant to peroxides than the ybp1Δ strain (Fig. 3). Many laboratories have investigated diverse biological processes, including the OSR, in the W303 genetic background. Furthermore, the identification of an allele of YBP1 in a com-
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commonly used laboratory strain has important implications for studies of Yap1 regulation. Indeed, the very fact that this strain had a mutant allele that affected the OSR to peroxide allowed us to identify YBP1. We previously utilized this strain to identify a mutation in the YBP1 gene into the YBP1−1a is not understood. However, the introduction of the YBP1−1a. We recently utilized this strain rescued the peroxide-induced expression of the TRX2 gene, encoding a thioredoxin peroxidase, that reduced the Yap1 protein with 35% identity. Interestingly, the expression of TRX2 is induced by the DNA damaging agent MMS (39), which raises the possibility that Tsa1 may affect Yap1 oxidation in other examples. The S. cerevisiae strain had a mutant allele that affected the OSR to peroxide. In contrast, the human pathogen C. albicans has also identified homologues of Ybp1 in other fungi, including the development of effective anti-fungal treatments.

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Ybp1 Is Required for the Hydrogen Peroxide-induced Oxidation of the Yap1 Transcription Factor
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Page 30903: In the original paper, Fig. 9 indicated that the protein encoded by the ybp1-1 allele was predicted to contain four amino acid changes. We have subsequently discovered another change in the ybp1-1 allele; a “C” is inserted in the coding strand (see below). This insertion alters the third base of the codon for Pro-243, and the codon for Asp-244 is changed to a Stop codon (TGA). Importantly, this additional change does not affect the conclusions of the paper with regard to the role of Ybp1 in the regulation of Yap1 or that W303-1a contains a mutant allele of the YBP1 gene. Interestingly, the phenotypes associated with the ybp1-1 allele (see paper) suggest that the mutant protein retains some activity. Thus, the insertion of a premature stop codon suggests that this residual activity is located in amino acids 1-243. We thank Dr. S. Kuge for bringing this error to our attention, and we apologize to the readers of the Journal for any confusion or inconvenience this error may have caused.

The predicted amino acid sequence (original Fig. 9) of the ybp1-1 allele between amino acids 241 and 245 was:

....(aa241) I P P D P(aa245)....
.....ATT CCC CCT GAT CCA....

The corrected predicted sequence of the protein encoded by the ybp1-1 allele is:

....(aa241) I P P Term
.....ATT CCC CCC TGA....

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