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Mutations in *PMR1* Suppress Oxidative Damage in Yeast Cells Lacking Superoxide Dismutase

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Mutants of *Saccharomyces cerevisiae* lacking a functional *SOD1* gene encoding Cu/Zn superoxide dismutase (SOD) are sensitive to atmospheric levels of oxygen and are auxotrophic for lysine and methionine when grown in air. We have previously shown that these defects of SOD-deficient yeast cells can be overcome through mutations in either the *BSD1* or *BSD2* (bypass SOD defects) gene. In this study, the wild-type allele of *BSD1* was cloned by functional complementation and was physically mapped to the left arm of chromosome VII. *BSD1* is identical to *PMR1*, encoding a member of the P-type ATPase family that localizes to the Golgi apparatus. *PMR1* is thought to function in calcium metabolism, and we provide evidence that *PMR1* also participates in the homeostasis of manganese ions. Cells lacking a functional *PMR1* gene accumulate elevated levels of intracellular manganese and are also extremely sensitive to manganese ion toxicity. We demonstrate that mutations in *PMR1* bypass SOD deficiency through a mechanism that depends on extracellular manganese. Collectively, these findings indicate that oxidative damage in a eukaryotic cell can be prevented through alterations in manganese homeostasis.

A consequence of aerobic life is the generation of oxygen free radicals derived from the sequential one electron reduction of molecular oxygen (9, 28, 29). Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$) are generated as natural by-products of aerobic metabolism (8, 9, 28, 29, 44) and also result from the exposure to free-radical-generating compounds (39, 44, 47, 64). These reactive oxygen species are known to damage DNA, proteins, and cellular membranes (10, 21, 35, 38) and have been implicated in a number of human disorders, including heart and lung diseases (12, 17, 25, 34, 49), cancer (10, 12, 63), and the aging process (1, 36).

Aerobic organisms have evolved with a number of enzymatic and nonenzymatic antioxidant defense mechanisms designed to combat free-radical-mediated damage (9, 28, 58). A major defense is provided by three enzymes that act to scavenge the intermediates of oxygen reduction, namely, superoxide dismutase (SOD), which disproportionates two molecules of O_2^- to H_2O_2 and O_2, and catalase and glutathione peroxidase, which act to eliminate H_2O_2 (9, 58). Nearly all aerobic organisms from bacteria to humans possess one or more forms of a metal-containing SOD. Prokaryotic organisms typically have both iron- and manganese-containing SODs, while eukaryotic cells possess a manganese-containing enzyme located in the mitochondria and a cytosolic copper/zinc-containing SOD. Mutations in the corresponding SOD genes have been shown to cause overall oxygen intolerance and sensitivity to free-radical-generating compounds in *Escherichia coli* (11, 27), *Drosophila melanogaster* (52), and *Saccharomyces cerevisiae* (6, 13, 15, 31, 46, 65). Moreover, mutations in the human *SOD1* gene encoding copper/zinc SOD have been correlated with amyotrophic lateral sclerosis, a fatal disorder affecting the motor neurons (22, 55). Although SOD has been linked with protection against oxidative damage, the precise physiological role(s) of this enzyme and the critical targets of oxygen toxicity are not completely understood.

The unicellular eukaryote *S. cerevisiae* represents an excellent genetic system in which to explore the biological roles of SODs. Strains containing deletions in the *SOD1* (encoding copper/zinc SOD) and *SOD2* (encoding manganese SOD) genes have provided valuable tools for probing the biochemical defects associated with SOD deficiency. Mutations in the *S. cerevisiae SOD2* gene have been shown to confer sensitivity to hyperbaric, but not atmospheric, levels of dioxygen (65). In contrast, *sod1* mutant cells exhibit a variety of growth abnormalities even under normoxic conditions, including an increased mutation rate as well as auxotrophies for lysine and methionine when grown in air (6, 13, 15, 31, 46). These aerobic defects of yeast lacking copper/zinc SOD can be suppressed by treating the cells with high concentrations of the redox-active transition metal manganese or copper (4, 7, 14, 41, 61). The defects of *SOD1* mutants can also be reversed genetically, through the isolation of extragenic suppressors (32, 46). In this regard, we have reported that a recessive mutation in one of two genes identified as *BSD1* and *BSD2* (bypass SOD defects) will effectively suppress the aerobic growth defects and lysine and methionine auxotrophies of yeast cells lacking copper/zinc SOD (46). The *BSD2* gene was recently cloned and found to encode a novel 37.5-kDa protein that normally functions in transport and accumulation of copper ions (45). Mutations in *BSD2* evidently reverse oxidative damage by altering cellular pools of copper ions (45).

In this paper, we report the cloning and characterization of the *BSD1* gene. Like *BSD2*, the *BSD1* gene appears to function in the proper metabolism of transition metals. *BSD1* was found to be equivalent to *PMR1*, encoding a P-type ATPase homolog that localizes to the Golgi apparatus (2, 56). We demonstrate

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TABLE 1. Yeast strains used in this study

Strain	Genotype	Reference of source
EG103	*MAT*α *leu2-3,112 his3*Δ*1 trp-289a ura3-52* Gal$^+$	46
EG133	*MAT*α *leu2-3,112 his3*Δ*1 sod1*Δ*a*::*URA3 sod2*Δ::*TRP1* Gal$^+$	46
VCSUP1	*MAT*α *leu2-3,112 his3*Δ*1 sod1*Δ*a*::*URA3 sod2*Δ::*TRP1* Gal$^+$ *bsd1-1*	46
DH4.6B	*MAT*α His$^-$ *ura3-52 ace1-1 trp1*	D. Hamer
XFSUP1	*MAT*α *leu2-3,112 his3*Δ*1 sod2*Δ::*TRP1 bsd1-1*	This study
XL103a	*MAT*α *leu2,3112 his3*Δ*1 trp-289a ura3052* Gal$^+$	46
PJKP-1	*MAT*α *leu2-3,112 his3*Δ*1 sod1*Δ*a*::*URA3 sod2*Δ::*TRP1* Gal$^+$ *pmr1*Δ::*LEU2*	This study
PJKP1-2	*MAT*α *leu2-3,112 his3*Δ*1* Gal$^+$ *pmr1*Δ::*LEU2*	This study
XL133Δc	*MAT*α *leu2-3,112 his3*Δ*1 sod1*Δ*a*::*URA3 sod2*Δ::*TRP1* Gal$^+$ *cup1*Δ::*URA3*	45
PJKP1-3	*MAT*α *leu2-3,112 his3*Δ*1 sod1*Δ*a*::*URA3 sod2*Δ::*TRP1* Gal$^+$ *cup1*Δ::*URA3 pmr1*Δ::*LEU2*	This study

that *PMR1* functions in manganese ion homeostasis and that through a manganese-dependent mechanism, complete loss of *PMR1* function eliminates all aerobic defects of cells lacking SOD.

MATERIALS AND METHODS

Yeast strains, genetic techniques, and media. The yeast strains used in this study are listed in Table 1. Strains EG103, EG133, XL103a, and VCSUP1 (46) and strain XL133Δc (45) are described elsewhere, and the *ace1-1* strain DH4.6D was a kind gift of Dean Hamer. The *pmr1*Δ strains PJKP1-1, PJKP1-2, and PJKP1-3 were obtained by deleting the chromosomal *PMR1* loci of EG133, EG103, and XL133Δc, respectively, using the *pmr1*Δ::*LEU2* construct pL119-3, as described previously (56). XFSUP1, a *bsd1-1 SOD1* strain, was constructed by crossing XL103a to VCSUP1 and by selecting for *ura3* segregants that failed to grow on ethanol and glycerol.

All genetic manipulations were done by the methods of Rose et al. (54). Since DNA sequencing showed that *ACE1* is adjacent to *PMR1*, linkage of *PMR1* and *BSD1* was assessed through a meiotic mapping test for *ACE1* and *bsd1*. An *ace1-1 BSD1* strain (DH4.6D) was crossed to a *bsd1-1* strain (VCSUP1), and the resultant diploids were induced to sporulate. The *ace1-1* segregants were scored by poor growth on medium containing 30 μM $CuSO_4$, and the *bsd1-1* segregants were scored by an ethanol/glycerol defect and by suppression of the *sod1*Δ-linked lysine auxotrophy. In 17 of 17 tetrads examined, *ace1-1* and *bsd1-1* genes gave only parental ditype segregation (i.e., no *ace1-1 bsd1-1* segregants were detected), demonstrating that *bsd1-1* and *ACE1* are tightly linked.

Stocks of yeast strains were maintained on standard YPD medium (54). Yeast strains were cultured either in air or in oxygen-free, CO_2-enriched anaerobic culture jars as previously described (46). Tests for amino acid requirements and metal ion resistance were conducted on cells grown in synthetic dextrose (SD) medium (54), sensitivity tests for paraquat (methyl viologen; Sigma Chemical Co.) were conducted in cells grown in YPD, and tests for metal ion dependence used a minimal defined medium depleted of essential ions as described by Dancis et al. (20). For each of these tests, fresh stocks of cells grown to confluence in YPD were seeded in 1.5-ml liquid cultures to an optical density at 600 nm (OD_{600}) of 0.025 to 0.05. The cultures were then incubated in Falcon tubes (17 by 100 mm) for 16 h at 30°C without shaking, and total growth was measured at OD_{600}. Standing cultures were necessary for all of these studies because *sod1*Δ yeast mutants grow poorly in shaken and well-aerated cultures (46). Tests for growth on the nonfermentable carbon sources ethanol and glycerol were conducted as previously described (46).

Tests for mutational frequency at the *can1* locus were carried out by a modification of the method of Sikorski and Boeke (59). Several independent colonies of either EG133 (*sod1*Δ *sod2*Δ) or VCSUP1 (*sod1*Δ *sod2*Δ *pmr1-1*) were plated to a final concentration of 2×10^8 cells per plate onto a minimal medium supplemented with arginine and 60 μg of canavanine sulfate per ml. Following 4 days of growth, the number of canavanine-resistant colonies was determined. The average numbers of canavanine-resistant clones per viable cell number were 8×10^{-6} and 1×10^{-6} for strains EG133 and VCSUP1, respectively.

Gene cloning and gene analysis techniques. A genomic library present on YCP50-LEU (kind gift of F. Spencer) was used to transform approximately 40,000 VCSUP1 (*sod1*Δ *sod2*Δ *bsd1-1*) cells via electroporation (5). Transformants were plated onto SD plates containing 1 M sorbitol and lacking leucine and were incubated for 4 days at 30°C in anaerobic culture jars. The Leu$^+$ transformants were then replica plated onto SD plates lacking both leucine and lysine, and following 2 days of aerobic growth, a number of lysine auxotrophic clones were isolated. From these, a single clone (designated 74C) which additionally exhibited methionine auxotrophy and sensitivity to atmospheric oxygen was isolated. The transforming plasmid (p74C) was isolated from this lysine auxotroph by plasmid rescue (50), and the genomic DNA insert was mapped through the use of commercially available restriction enzymes (57).

Physical mapping of the chromosomal position of p74C was conducted by using both a commercially obtained *S. cerevisiae* chromosome blot (Chromoblot Inc.) and genomic filters prepared in the Olson Laboratory as described previously (43, 53). The probe for these experiments consisted of a ^{32}P-radiolabelled 1.0-kb *Bam*HI-*Eco*RI fragment of p74C genomic DNA (see Fig. 2).

Plasmids. Constructs p74C and H6 represent overlapping segments of yeast genomic DNA spanning *PMR1*. The p74C clone contains a 10-kb *Sau*3A fragment inserted into the *Bam*HI site of YCP50-LEU, while H6 possesses a 7.5-kb segment inserted into the *Bam*HI and *Sal*I sites of pRS202, a 2 μm *URA3*-based vector (60). H6ΔBam was constructed by deleting a 4.0-kb *Bam*HI fragment of H6, and plasmids pKC7, pKC83, and pKC11 contain the DNA segments indicated in Fig. 2 inserted into B2205, a 2 μm derivative of pRS306 (60). The *pmr1*Δ::*LEU2* gene replacement plasmid pL119-3 has been previously described (56).

Metal accumulation studies. Measurements of metal ion accumulation were conducted in 15-ml cultures of yeast cells grown in SD medium to an OD_{600} of 1.0. In duplicate or triplicate samples, cells were harvested, washed two times in 50 ml of ice-cold 50 mM Tris-HCl–10 mM EDTA (pH 6.5), and analyzed for metal content through graphite furnace atomic adsorption spectrophotometry as described previously (42, 45). Total cellular accumulation of manganese, copper, iron, or zinc was converted to nanomoles of metal per 10^9 cells.

RESULTS

Strains of *S. cerevisiae* containing null mutations in the *SOD1* (encoding Cu/Zn cytosolic) and *SOD2* (encoding Mn mitochondrial) genes are sensitive to oxygen stress and are unable to grow in air without supplements of lysine and methionine (6, 13, 15, 31, 46, 65). We previously reported that these aerobic defects of *sod1*Δ *sod2*Δ yeast strains can be reversed through additional recessive mutations in the *BSD1* gene (46). In the present study, the *BSD1* gene was cloned by functional complementation of the *bsd1*-linked lysine prototrophy. A *sod1*Δ *sod2*Δ *bsd1-1* strain was transformed with a yeast genomic centromere library; following 4 days of anaerobic growth, transformants were replica plated onto medium lacking lysine and allowed to grow in air. Clones exhibiting an aerobic lysine auxotrophy were selected, and of these, a single transformant (designated 74C) which additionally exhibited reversal of the *bsd1*-linked methionine prototrophy was isolated. The p74C transforming plasmid was rescued from this lysine auxotroph and was found to contain a 10-kb genomic DNA insert that was capable of reversing the oxygen resistance (Fig. 1A) and amino acid prototrophies (Fig. 1B) of *bsd1-1* cells. The p74C construct also reversed the previously reported growth defect of *bsd1* mutants on nonfermentable carbon sources (reference 41 and legend to Fig. 2). These data suggested that p74C may contain the wild-type allele of *BSD1*).

The localization of the p74 insert on the yeast physical DNA map was determined by hybridization to an ordered set of chromosomal fragments carried on lambda phage (43, 53). A fragment of p74C hybridized uniquely to a region of chromosome VII that has been well characterized. The 10-kb insert of p74C spans the complete open reading frames of *54B* (24), *ACE1* (30, 62, 66), and *PMR1* (56). An *ace1-1* mutation (62) did not suppress SOD deficiency but was found to be tightly linked to *BSD1* in genetic crosses (100% linkage in 17 tetrads; see Materials and Methods). The region of p74C that complements the *bsd1* mutant was further defined by using a series of

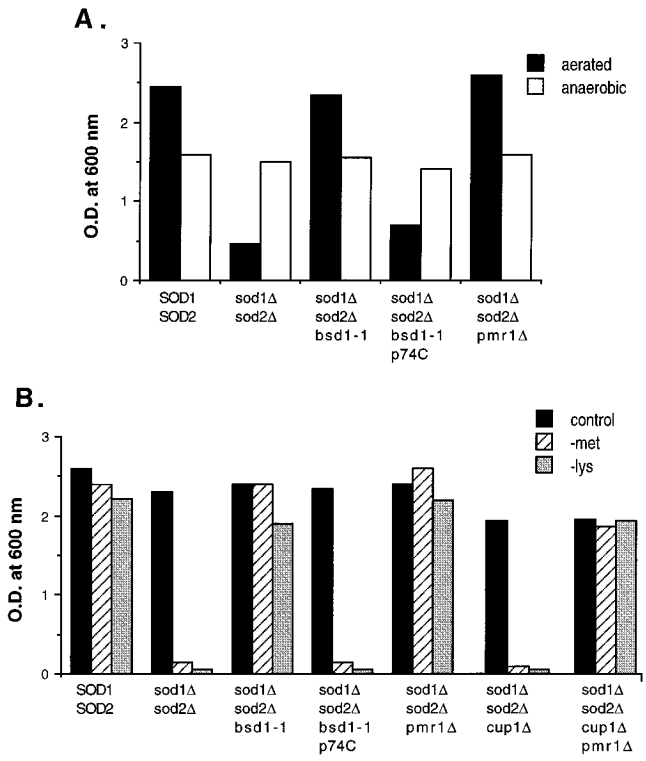

FIG. 1. The oxygen sensitivity and amino acid defects of yeast cells lacking SOD. (A) The designated yeast strains were tested for sensitivity to atmospheric oxygen by culturing in either well-aerated flasks (black bars) or anaerobic culture jars (gray bars) as previously described (46). (B) The designated yeast strains were tested for lysine/methionine auxotrophy as described previously (46) by culturing in minimal medium supplemented with either lysine (-met), methionine (-lys), or both amino acids (control). Cells were seeded at an OD_{600} of 0.025, and total cell growth following a 16-h incubation at 30°C was determined turbidimetrically. Strains: *SOD1 SOD2*, EG103; *sod1*Δ *sod2*Δ, EG133; *sod1*Δ *sod2*Δ *bsd1-1*, VCSUP1; *sod1*Δ *sod2*Δ *bsd1-1* p74C, VCSUP1 transformed with genomic clone p74C; *sod1*Δ *sod2*Δ *pmr1*Δ, PJKP1-1; *sod1*Δ *sod2*Δ *cup1*Δ, XL133Δc; *sod1*Δ *sod2*Δ *cup1*Δ *pmr1*Δ, PJKP1-3.

plasmids containing smaller inserts (Fig. 2). Plasmids containing only the *54B* and *ACE1* genes failed to complement the *bsd1* mutant, whereas the plasmid containing only *PMR1* complemented the phenotypes of the *bsd1* mutant.

To conclusively determine whether *BSD1* is identical to *PMR1*, the effects of introducing a *pmr1* null mutation into the *sod1*Δ *sod2*Δ double mutant were examined. The *pmr1* null mutation suppressed the aerobic lysine and methionine auxotrophies and oxygen sensitivity associated with SOD deficiency, identical to the effects of *bsd1-1* (Fig. 1). Therefore, *BSD1* is identical to *PMR1*, and the complete knock out of this gene functions to reverse the effects of SOD deficiency.

PMR1 encodes a 104-kDa polypeptide homologous to mammalian Ca^{2+} ATPases found in the sarcoplasmic/endoplasmic reticulum (56) and is most similar to a putative Ca^{2+} ATPase identified in rats (33). The *PMR1* product has been localized to the Golgi complex in *S. cerevisiae* and is thought to act in the transport and processing of secretory proteins (2, 56). Mutations in *PMR1* have previously been shown to cause protein secretory defects, and these defects can be reversed through calcium supplements to the growth medium (56). To test whether the *pmr1* bypass of SOD deficiency is mediated through these secretory defects, we attempted to reverse the oxygen resistance of a *sod1*Δ *sod2*Δ *pmr1*Δ strain through calcium ion supplements. As shown in Fig. 3A, addition of millimolar concentrations of calcium to the growth medium failed to reverse the lysine prototrophy of *sod1*Δ *sod2*Δ *pmr1*Δ cells, indicating that the secretory defects of *pmr1* mutants are unrelated to the bypass of *SOD*.

PMR1 is also thought to function in controlling calcium homeostasis (18); we therefore explored the effects of very high and very low calcium concentrations on SOD defects. As shown in Fig. 3A, culturing cells in high concentrations of $CaCl_2$ failed to bypass the lysine biosynthetic defect of *sod1*Δ *sod2*Δ cells. We also found that *pmr1* mutations continued to reverse SOD defects even when calcium ions were depleted from the growth medium (see Fig. 5). Together, these obser-

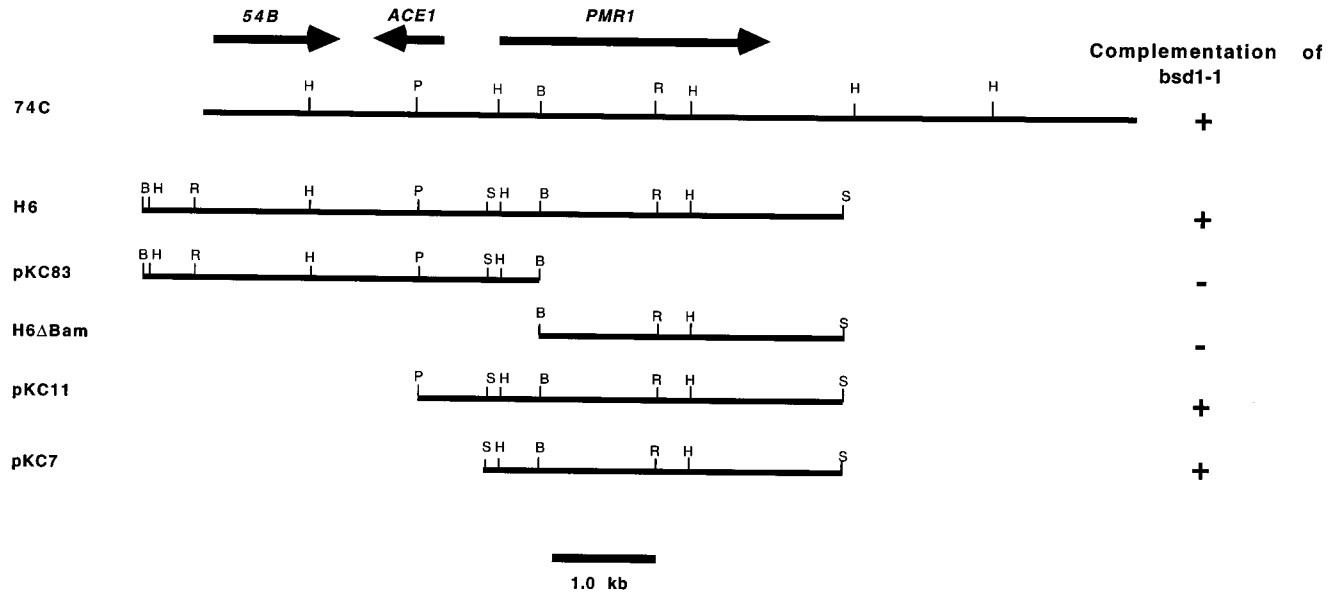

FIG. 2. Functional mapping of the *bsd1-1*-complementing clone. Shown are a series of overlapping genomic clones spanning the open reading frames of *54B*, *ACE1*, and *PMR1*, as indicated. These constructs were used to transform a *bsd1-1 ura3* strain (XFSUP1) and were tested for the ability to reverse the *bsd1*-linked growth defect on ethanol and glycerol as previously described (46). +, positive growth on ethanol/glycerol-containing medium, equivalent to that of corresponding *BSD1* strains; −, negative growth on ethanol/glycerol plates, equivalent to that of the *bsd1-1* mutant transformed with control vector. Restriction enzyme cutting sites are indicated by B (*Bam*HI), H (*Hin*dIII), P (*Pvu*II), R (*Eco*RI), and S (*Spe*I).

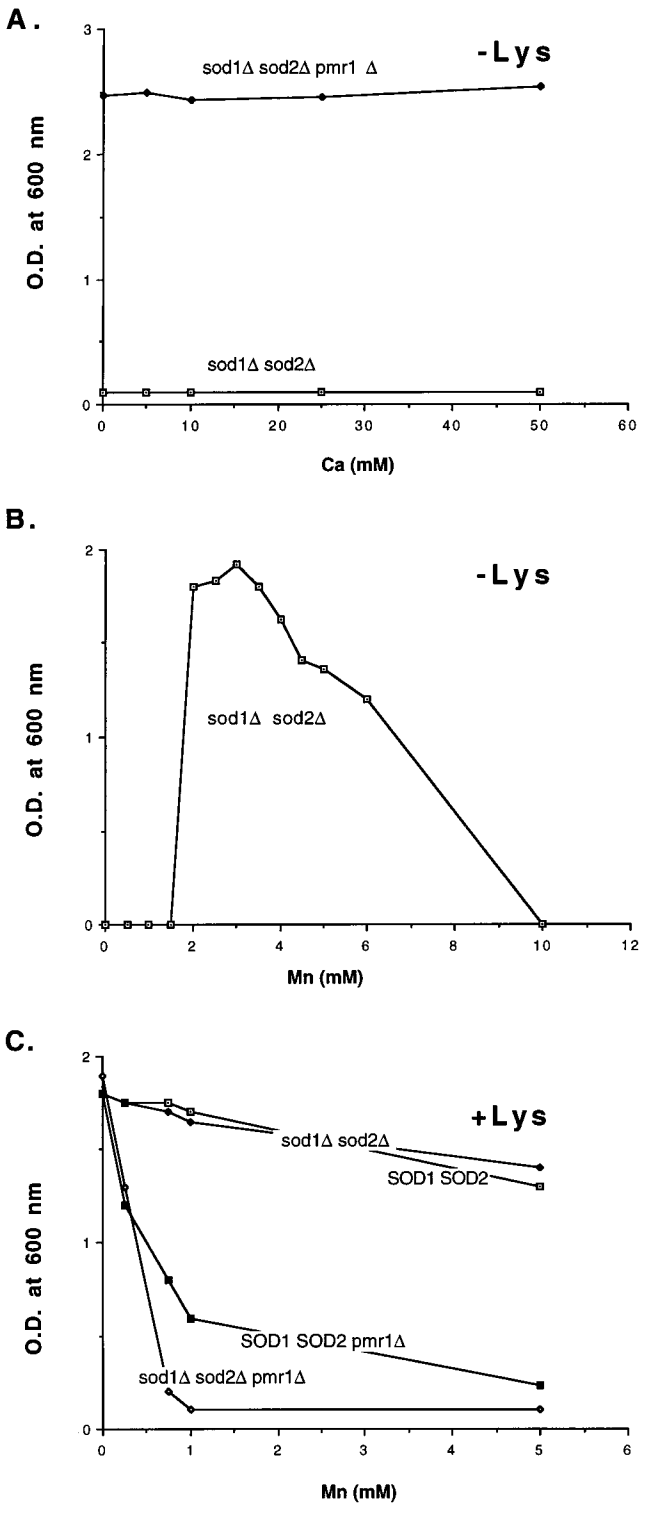

FIG. 3. Calcium and manganese treatment of yeast cells lacking SOD and PMR1. (A and B) The effect of calcium and manganese ions on the lysine biosynthetic defect of SOD mutants was tested by growing the indicated strains in medium lacking lysine (as in Fig. 1B) supplemented with the designated concentrations of $CaCl_2$ (A) or $MnCl_2$ (B). (C) Tests for sensitivity to manganese toxicity were conducted by growing the indicated yeast strains in complete SD medium (as defined in the legend to Fig. 1B) supplemented with the given concentrations of $MnCl_2$. Strains: *sod1*Δ *sod2*Δ *pmr1*Δ, PJKP-1; *SOD1 SOD2 pmr1*Δ, PJKP-2. Descriptions of remaining strains and growth conditions are provided in the legend to Fig. 1.

vations suggest that the effects of *pmr1* mutations on calcium homeostasis cannot explain the oxygen resistance of *sod1*Δ *sod2*Δ *pmr1*Δ cells.

Oxygen toxicity in yeast strains lacking copper/zinc SOD can be suppressed by treating cells with elevated levels of the transition metal copper (61) or manganese (14). Furthermore, *bsd2* mutants are associated with increased levels of copper (45). To test whether *pmr1* mutations affect accumulation of ions other than calcium, the cellular levels of manganese, copper, and zinc were measured in *pmr1*Δ cells and in isogenic *PMR1* strains. As shown in Fig. 4, the *pmr1* mutants accumulated 3 to 4.5 times more manganese than did *PMR1* cells, and this result was observed in the background of both *sod1*Δ *sod2*Δ and *SOD1 SOD2* strains. This increase in manganese ion accumulation is associated with an increase in manganese ion toxicity, as *pmr1* mutants exhibit a striking sensitivity to added manganese in the growth medium (Fig. 3C). Strains containing *pmr1* mutations were also seen to accumulate slightly higher levels of copper but not of zinc (Fig. 4), yet the increase in copper accumulation was not associated with a concomitant increase in sensitivity to copper toxicity (not shown).

Elevated levels of copper ions have previously been shown to suppress oxygen toxicity in *sod* mutants through the induction of *CUP1*, an O_2^--scavenging metallothionein (61). Since *pmr1* mutants exhibit a slight increase in copper ion accumulation, we tested whether the *pmr1* suppression of SOD deficiency is mediated through the *CUP1* metallothionein. For these studies, we used a *sod1*Δ *sod2*Δ *cup1*Δ strain lacking a functional *CUP1* gene (45). As shown in Fig. 1B, a *pmr1* null mutation is still capable or suppressing the lysine and methionine auxotrophies of yeast strains lacking SOD and CUP1, demonstrating that *pmr1* mutations reverse oxygen toxicity in a manner that is independent of *CUP1* metallothionein.

The striking effect of *PMR1* mutations on manganese ion accumulation and resistance to manganese toxicity suggested a link between this transition metal and the oxygen resistance of *sod1*Δ *sod2*Δ *pmr1*Δ cells. Treating *sod1*Δ *sod2*Δ double mutants with millimolar concentrations of manganese suppressed the lysine auxotrophy of these cells (Fig. 3B), and similar findings have been reported for *sod1-1* single mutants (14). To explore the role of manganese in the *pmr1* suppression of SOD defects, the growth of a *sod1*Δ *sod2*Δ *pmr1*Δ strain was examined in manganese-depleted medium. As demonstrated in Fig. 5, *pmr1* mutations failed to bypass the lysine auxotrophy of *sod1*Δ *sod2*Δ cells when manganese was depleted from the medium, whereas a similar depletion of copper or calcium had no effect. These observations demonstrated that *pmr1* mutations work to reverse oxygen toxicity in *sod* mutants through a manganese-dependent mechanism.

A number of biochemical and genetic studies have implicated a free-radical-scavenging function for manganese ions both in vivo and in vitro (3, 4, 7, 14, 41, 47). It was therefore conceivable that manganese accumulation in *pmr1* mutants might reverse oxygen toxicity through the neutralization of oxygen free radicals. Reactive oxygen species are known to cause damage to DNA which can lead to gene mutations. Notably, *sod1*Δ mutants are associated with an increased mutation rate (31), and we find that inactivation of *PMR1* suppresses this high mutation frequency (see Materials and Methods). To study the antioxidant capacity of *pmr1* mutants further, we examined the sensitivity of *pmr1* mutants to paraquat, a known generator of intracellular O_2^-. Although *pmr1* mutations did not augment the strong paraquat resistance of *SOD1 SOD2* strains (not shown), these mutations effectively increased paraquat resistance in *sod1*Δ *sod2*Δ yeast cells by approximately 10-fold (Fig. 6). This elevated resistance to

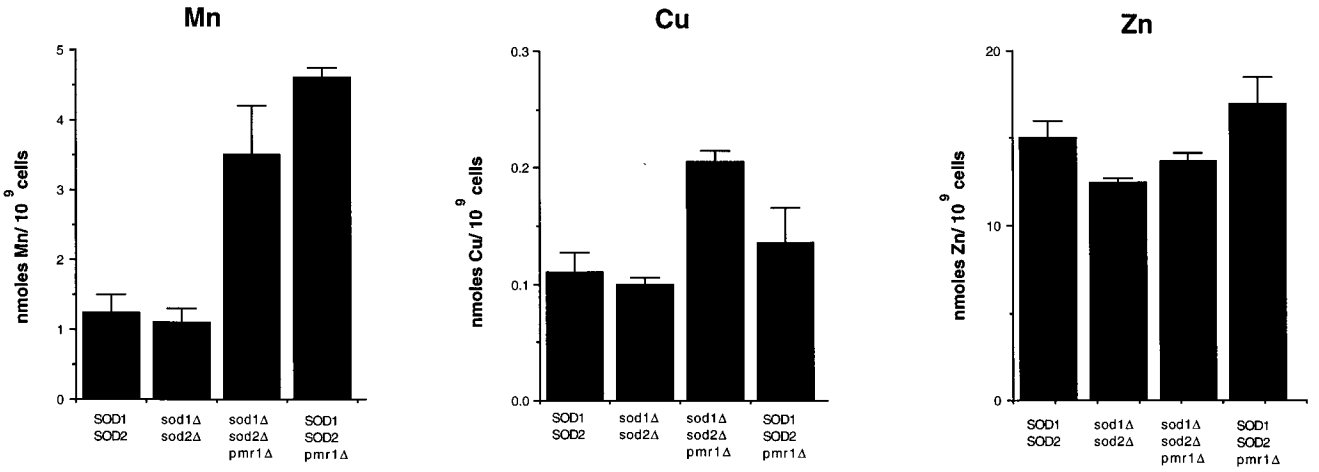

FIG. 4. Metal ion accumulation. Accumulation of manganese, copper, and zinc in the indicated yeast strains was determined through atomic absorption spectroscopy as previously described (46). Results represent averages of duplicate samples and one to three independent trials. Error bars indicate total range. Descriptions of strains are provided in the legends to Fig. 1 and 3.

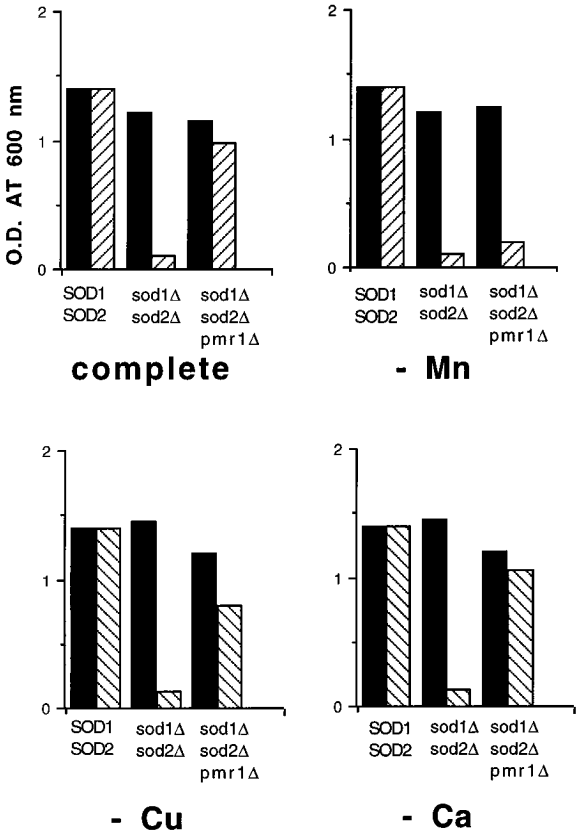

FIG. 5. Metal ion dependence of *pmr1* mutants. The indicated strains were grown aerobically in the presence (closed bars) or absence (hatched bars) of lysine in a minimal defined medium (20) supplemented with 0.15 μM $CuCl_2$, 6.0 μM $MnCl_2$, and 2.0 mM $CaCl_2$ (complete) or in analogous medium specifically depleted of manganese (-Mn), copper (-Cu), or calcium (-Ca) ions, as indicated. Descriptions of yeast strains and growth conditions are provided in the legends to Fig. 1 and 3.

paraquat together with the manganese dependency studies outlined above suggested that the increased manganese accumulation observed in *pmr1* mutants bypasses SOD deficiency through a free-radical-scavenging mechanism.

DISCUSSION

SOD is thought to play an important role in protecting cells against oxidative damage, and in *S. cerevisiae*, the aerobic requirement for this enzyme can be circumvented by mutations in either the *BSD1* or *BSD2* gene (46). The *BSD2* gene has been shown to encode a 37.5-kDa protein that functions in copper ion transport and accumulation (45). In this study, we report that *BSD1* is identical to the previously characterized *PMR1* gene, predicted to encode a well-conserved member of the P-type ATPase family. A complete inactivation of *PMR1* function was found to reverse all aerobic defects of yeast strains lacking SOD.

PMR1 encodes a P-type ATPase homolog that is most sim-

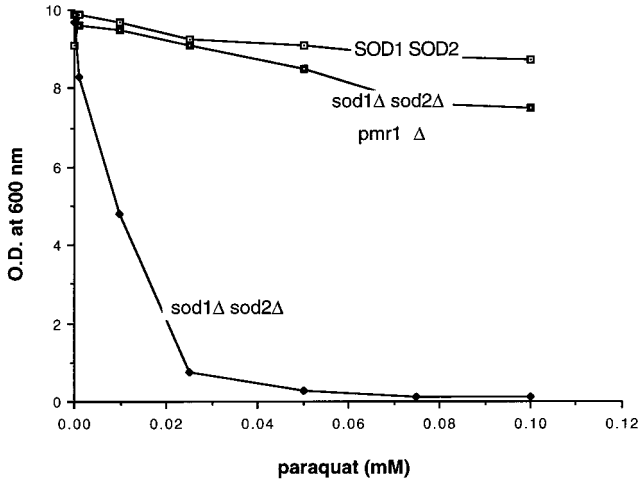

FIG. 6. Sensitivity to paraquat. The designated yeast strains were tested for paraquat sensitivity by culturing cells in the presence of the specified concentrations of paraquat as discussed in Materials and Methods. Descriptions of yeast strains are provided in the legends to Fig. 1 and 3.

ilar to mammalian calcium ATPases found in the sarcoplasmic/endoplasmic reticulum (2, 18, 56). In *S. cerevisiae*, the *PMR1* gene product localizes to the Golgi apparatus and is believed to function both in calcium ion metabolism and in secretory protein transport and processing (2, 56). The *pmr1* mutants have been shown to accumulate elevated levels of cytosolic calcium (18) and exhibit calcium-dependent alterations in protein secretion (56). However, we provide several lines of evidence that these calcium-related defects do not contribute to the oxygen resistance of *sod1*Δ *sod2*Δ *pmr1*Δ yeast cells. First, treatment of *sod1*Δ *sod2*Δ mutants with elevated levels of calcium failed to reverse the aerobic defects of these cells. Second, *pmr1* mutations continued to suppress SOD defects even when calcium ions were depleted from the medium. We also found that reversing the *pmr1* secretory defects through calcium treatments did not alter the oxygen resistance of *sod1*Δ *sod2*Δ *pmr1*Δ yeast cells. Hence, the biological roles of *PMR1* in calcium ion metabolism cannot totally account for the suppression of SOD deficiency, suggesting an auxiliary function for this putative cation pump.

We provide evidence that *PMR1* participates in the homeostasis of certain transition metal ions. Strains containing *pmr1* mutations were seen to accumulate elevated levels of intracellular manganese and copper and also displayed an unusual sensitivity to manganese toxicity. Moreover, our studies showed that the oxygen resistance of *sod1*Δ *sod2*Δ *pmr1*Δ cells is absolutely dependent on the presence of manganese ions. A number of biochemical studies have demonstrated that free manganese or complexes of manganese can scavenge superoxide anions in vitro (4, 7, 41). Furthermore, high intracellular levels of this metal have been shown to substitute functionally for SOD in both *Lactobacillus plantarum* and *S. cerevisiae* (3, 14). We also show here that *pmr1* mutations can be mimicked through an elevation in intracellular manganese, yet the metal concentration needed to reverse oxygen toxicity in *PMR1* wild-type yeast cells is two- to three-fold greater than the intracellular level of manganese that accumulates in *pmr1* mutants (not shown). We therefore propose that *pmr1* mutations reverse oxidative damage in *sod1*Δ *sod2*Δ cells by causing a local rise in the level or availability of a manganese-dependent antioxidant(s) rather than a global increase in total cellular manganese.

How do *pmr1* mutations alter manganese ion homeostasis? A simple possibility is that the *PMR1* gene product functions as a manganese pump. In this regard, microsomal Ca^{2+} ATPases of mammalian cells have been reported to also function in manganese ion transport (16, 19, 40). In the case of *S. cerevisiae PMR1*, manganese ions may be delivered directly to the Golgi apparatus, where they are needed to activate the various manganese-dependent enzymes involved in protein processing and secretion (37, 48, 51). However, to date, there has been no available biochemical evidence that directly supports ion transport by *PMR1*. Using the standard ion transport methods of Dunn et al. (23), our recent attempts to measure *PMR1*-dependent pumping of calcium and manganese ions in vitro have not been successful. Despite the lack of biochemical evidence, several observations are consistent with a role for *PMR1* in the homeostasis of manganese ions. First, *PMR1* has been localized to the Golgi apparatus, and *pmr1* mutants accumulate elevated levels of manganese. This result would be expected if *PMR1* indeed acts to deliver manganese to the Golgi apparatus, where the metal can normally exit the cell via the secretory pathway. Also consistent with this model, a number of Golgi processing enzymes require manganese as a cofactor (37, 48, 51), and *pmr1* mutants have been associated with protein processing and secretory defects. Moreover, a manganese-dependent mannosyltransferase located in the yeast Golgi apparatus (48) appears to be defective in *pmr1* mutants (56). Therefore, we propose that *PMR1* serves a physiological role in the transport of manganese into the Golgi apparatus and that the inactivation of PMR1 suppresses oxygen toxicity in *sod1*Δ yeast cells as a result of the accumulation of redox-active manganese ions in the cytosolic compartment(s) where copper/zinc SOD becomes critical.

It is noteworthy that oxidative damage in SOD-deficient yeast cells is additionally suppressed by *bsd2* mutations, causing the cell to accumulate elevated levels of yet another redox-active transition metal, copper (45). Oxygen toxicity in *SOD* mutants is also suppressed by overexpression of the *ATX1* gene, encoding a well-conserved polypeptide controlling copper ion accumulation in yeast cells (42a). These observations together with our present studies on *PMR1* and manganese have established important biological links between transition metal homeostasis and oxygen radical metabolism in aerobic cells.

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