The Yeast Copper/Zinc Superoxide Dismutase and the Pentose Phosphate Pathway Play Overlapping Roles in Oxidative Stress Protection* 

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In Saccharomyces cerevisiae, loss of cytosolic superoxide dismutase (Sod1) results in several air-dependent mutant phenotypes, including methionine auxotrophy and oxygen sensitivity. Here we report that these two sod1 Δ phenotypes were specifically suppressed by elevated expression of the TKL1 gene, encoding transketolase of the pentose phosphate pathway. The apparent connection between Sod1 and the pentose phosphate pathway prompted an investigation of mutants defective in glucose-6-phosphate dehydrogenase (Zwf1), which catalyzes the rate-limiting NADPH-producing step of this pathway. We confirmed that zwf1 Δ mutants are methionine auxotrophs and report that they also are oxygen-sensitive. We determined that a functional ZWF1 gene product was required for TKL1 to suppress sod1 Δ, leading us to propose that increased flux through the oxidative reactions of the pentose phosphate pathway can rescue sod1 methionine auxotrophy. To better understand this methionine growth requirement, we examined the sulfur compound requirements of sod1 Δ and zwf1 Δ mutants, and noted that these mutants exhibit the same apparent defect in sulfur assimilation. Our studies suggest that this defect results from the impaired redox status of aerobically grown sod1Δ and zwf1Δ mutants, implicating Sod1 and the pentose phosphate pathway as being critical for maintenance of the cellular redox state.

Aerobic organisms are constantly exposed to potentially harmful reactive oxygen species that are generated as by-products of cellular metabolism. These oxygen free radicals have the potential to damage critical cellular components such as DNA, proteins, and lipids (see Refs. 1 and 2 for review). However, aerobic organisms have evolved with both enzymatic and non-enzymatic antioxidant defense mechanisms that are designed for protection against such assaults. Non-enzymatic defenses include the tripeptide glutathione (GSH) and the small peptide thioredoxin (Trx), while enzymatic defenses include catalases, peroxidases, and superoxide dismutases (Ref. 1; review). Superoxide dismutases (SOD) are metallo-enzymes that scavenge the potentially harmful superoxide anion (O2·−) and represent a critical component of the oxidant stress protection system (3).

The unicellular eukaryotic Saccharomyces cerevisiae, or baker’s yeast, provides an ideal model system in which to study the metabolism of oxygen free radicals. Yeast contain the same oxidative stress defense mechanisms present in higher eukaryotes (see Ref. 4 for review), and are extremely amenable to genetic, biochemical, and molecular biological manipulation. S. cerevisiae contains two forms of SOD, encoded by the SOD1 and SOD2 genes. Sod2 is a mitochondrial, manganese-bound SOD (Mn-SOD), which is thought to provide protection from respiratory sources of oxygen radicals (5, 6). SOD1 encodes a copper- and zinc-bound SOD (Cu,Zn-SOD), which is reported to be cytosolic or peroxisomal (7–10), yet the precise function of this SOD is not completely understood. Yeast mutants that lack the Cu,Zn-SOD, due to gene deletion, exhibit several mutant phenotypes when grown in air. These sod1Δ mutants are auxotrophic for the amino acids lysine and methionine, but only when grown aerobically (see Ref. 11 for review). The molecular basis of these phenotypes remains elusive. The lysine auxotrophy may be due to a defect in lysine biosynthesis caused by oxidative inactivation of an, as yet undetermined, enzyme (12). The sod1 methionine auxotrophy is also not fully understood, but thought to be due to a defect in sulfur metabolism (13). In addition, the sod1Δ mutants are also sensitive to oxygen and exhibit extreme sensitivity to the O2·−-generating agent, paraquat (11).

Several suppressors of sod1 null mutations have been isolated in our laboratory that have the ability to reverse sod1 mutant phenotypes. The pmr1(bsd1) (14) and bsd2 (15) mutational suppressors were isolated as spontaneous suppressors of sod1Δ lysine auxotrophy. The ATX1 (16) and ATX2 (17) genes were isolated as multi-copy suppressors, also based upon their reversal of sod1Δ lysine auxotrophy. These suppressors share in common the ability to alleviate all sod1-related defects, including lysine and methionine auxotrophies, oxygen sensitivity, and paraquat toxicity. All of the BSD and ATX gene products have been demonstrated to function in metal ion homeostasis, and their sod1 suppression is mediated through hyperaccumulation of either copper or manganese ions (14–16). The metal ions are thought to relieve the requirement for Sod1 by acting as SOD “mimics” to detoxify oxygen radicals. To better understand the cellular function of the Sod1 protein and the consequences of its inactivation, we isolated multi-

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1 The abbreviations used are: SOD, superoxide dismutase; PAPS, phosphoadenylyl sulfate.

2 S. J. Lin and V. C. Culotta (1996) Mol. Cell. Biol. 16, in press.
copy suppressors that would rescue specific sod1Δ-related phenotypes. The work reported here describes the isolation of such a suppressor, the TKL1 gene, encoding the transketolase enzyme of the pentose phosphate pathway. The identification of TKL1 as a sod1 suppressor provided an opportunity to explore the role of the pentose phosphate pathway in protection against oxidative stress in yeast. This work has improved our understanding of the sod1 methionine auxotrophy and has provided insight into the relationships between the Cu,Zn-SOD, the rate-limiting enzyme of the pentose phosphate pathway, and the critical maintenance of the cellular redox state.

EXPERIMENTAL PROCEDURES

Yeast Strains, Media, and Growth Conditions—The S. cerevisiae yeast strains used in these studies are listed in Table I. Strain KS105 was constructed by introducing the SOD1 gene replacement plasmid pKS3 (18) into strain 1783. Strains KS113 and KS117 were constructed by introducing the ZWF1 replacement plasmid pBM2720 (19) into strains 1783 and KS105, respectively. Strain KS114 was obtained by deleting the TAL1 gene of 1783 using the gene replacement plasmid pATL1::LEU2 (20). Strains KS115 and KS116 were constructed by introducing the TKL1 or TKL2 replacement plasmids pTKL1::URA3 (21) or pTKL2::LEU2 (22), respectively, into strain 1783. Strains KS109, KS121, and KS122 were obtained by deleting the PMR1 gene of strains KS105, KS113, and KS117, respectively, using the PMR1 replacement plasmid PL119 (14). Plasmids were introduced into yeast by electroporation (23) or lithium acetate transformation (24). Gene deletions were all verified by Southern blot analysis.

Stocks of all strains were maintained on YPD medium, and tests for methionine auxotrophy, lysine auxotrophy, and paratov sensitivities (methyl violagen; Sigma) were carried out using a synthetic dextrose methionine auxotrophy, lysine auxotrophy, and paraquat sensitivity liquid test for sensitivity to atmospheric oxygen (BBL Gas Pak) (12). Liquid tests for sensitivity to the sod1Δ yeast strain KS105, and transformants were grown anaerobically prior to replica platting in triplicate onto medium lacking lysine, medium lacking methionine, and medium supplemented with 0.5 mM paraquat. The parental sod1Δ strain was unable to grow in air under any of these conditions, and transformants were selected that exhibited suppression of one or more of these sod1Δ mutant phenotypes. This strategy resulted in the isolation of the SOD1 gene, the ATP1 gene (16), and a 5.3-kilobase genomic fragment not corresponding to any known suppressor of sod1Δ (as determined by Southern blot analysis, not shown). The SOD1 and ATP1 genes reversed all three sod1Δ-linked phenotypes, while the new suppressor appeared specific for methionine auxotrophy. The genomic insert corresponding to this sod1Δ suppressor was partially sequenced, and used to search the GenBank™ data base for identity of known yeast genes. This search revealed 100% identity to a region of the genome on chromosome XI that included the TKL1 gene, and deletion analysis revealed that the TKL1 gene itself was responsible for the suppression of sod1Δ (data not shown).

S. cerevisiae TKL1 encodes the primary transketolase of the pentose phosphate pathway, depicted in Fig. 1 (31–33). This pathway has both an oxidative portion that directly produces NADPH and a non-oxidative portion that can ultimately feed back to the oxidative segment via glucose-6-phosphate (32). As seen in Fig. 2A, the aerobic methionine auxotrophy of sod1Δ mutants was fully rescued by overexpression of TKL1, but not by other genes in the non-oxidative half of the pentose phosphate pathway. Overexpression of TKL2 (Fig. 2A), which encodes a secondary transketolase in yeast (22), or TAL1, encoding transaldolase (data not shown), had no effect on the sod1Δ mutant. Consistent with these findings, studies by Berthon et al. (32) have shown that Tkl1 is rate-limiting for the non-oxidative portion of the pentose phosphate pathway.

Overexpression of TKL1 also improved the aerobic growth of the sod1Δ mutant in the presence of methionine (Fig. 2A), and this effect of TKL1 was confirmed in a liquid culture test for oxygen sensitivity. Yeast strains harboring a sod1Δ mutation grew poorly in a methionine-free defined liquid medium under well aerated conditions (12), a phenotype that is reversed by overexpression of ATX1 (Fig. 2B) and the ATX1, pmr1, and sod2 suppressors of Sod1 deficiency (12). In this same test for sensitivity to atmospheric oxygen, elevated expression of TKL1 also facilitated efficient growth of the sod1Δ mutant strain under well aerated

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TABLE I

| Strain | Genotype | Source |
|--------|----------|--------|
| 1783   | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr | (17) |
| KS105  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP | This study |
| KS109  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP1, pmr1Δ::LEU2 | This study |
| KS113  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP, pmr1Δ::LEU2 | This study |
| KS114  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, trk1Δ::LEU2 | This study |
| KS115  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, trk2Δ::LEU2 | This study |
| KS116  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, trk3Δ::LEU2 | This study |
| KS117  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP1, zwf1Δ::URA3 | This study |
| KS118  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP, zwf1Δ::URA3 | This study |
| KS119  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP1, zwf1Δ::URA3, pmr1Δ::LEU2 | This study |
| KS120  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP1, zwf1Δ::URA3, pmr1Δ::LEU2 | This study |
| KS121  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP1, zwf1Δ::URA3, pmr1Δ::LEU2 | This study |
| KS122  | Mata, leu2-3,112, ura3-52, trp1-1, his4, canlr, sod1Δ::TRP1, zwf1Δ::URA3, pmr1Δ::LEU2 | This study |
| KS107  | Mata, leu2-3,112, his3Δ1, GAL+, trp1-288a, ura3-52, sod1Δ::TRP1 | This study |
| EM56-4B | Mata, ade2-10c, ade3Δ-100, trp1-1, leu2-3,112, lys2Δ::HIS3, ura3-1, his3-11, can1-100, trx1Δ::LYS2, trx2Δ::LEU2 | E. Muller |
Hence, Sod1 deficiency by the oxidative segment. TKL1 and TAL1 encode transketolase and transaldolase, respectively, of the non-oxidative segment. The two NAPDH-producing steps of the oxidative branch are indicated.

![Diagram of the pentose phosphate pathway](image)

**Fig. 1.** Diagram of the pentose phosphate pathway. ZWF1 encodes glucose-6-phosphate dehydrogenase, catalyzing the first reaction of the oxidative segment. TKL1 and TAL1 encode transketolase and transaldolase, respectively, of the non-oxidative segment. The two NAPDH-producing steps of the oxidative branch are indicated.

Furthermore, sod1 deficiency was still capable of reversing the methionine auxotrophy and poor aerobic growth defects of yeast lacking Sod1. A likely explanation for the TKL1 suppression of Sod1 deficiency is an increased flux through the pentose phosphate pathway, activating the oxidative portion and leading to enhanced production of the cellular reductant, NADPH. To test this, we asked whether blocking the oxidative portion of the pentose phosphate pathway would prevent TKL1-mediated suppression of sod1Δ. Such a blockage was imposed by deleting the ZWF1 gene, encoding glucose 6-phosphate dehydrogenase, the rate-limiting step in the oxidative segment of the pathway that produces NAPDH (Fig. 1). In a strain containing double mutations in sod1Δ and ZWF1, TKL1 overexpression no longer had the ability to reverse the methionine auxotrophy or poor aerobic growth specific to sod1Δ mutations (Fig. 3). This result strongly indicated that TKL1 suppresses Sod1 deficiency through a mechanism dependent upon Zwf1 and the oxidative portion of the pentose phosphate pathway.

**Table II**

| Genotype         | Lysine | Methionine | Paraquat | Aerobic growth |
|------------------|--------|------------|----------|---------------|
| sod1Δ            | Auxotrophic | Methionine | Sensitive | Poor          |
| zwf1Δ            | Auxotrophic | Auxotrophic | Resistant | Poor          |
| sod1Δ pmr1Δ      | Prototrophic | Auxotrophic | Resistant | Good          |
| sod1Δ TKL1Δ      | Auxotrophic | Sensitive | Good      |

* Gene overexpressed.

conditions (Fig. 2B). However, TKL1 was incapable of reversing the lysine auxotrophy of sod1Δ yeast (Fig. 2A), and also did not appreciably suppress the paraquat sensitivity of these cells (Table II). These results demonstrate the specificity of TKL1 for relieving the methionine auxotrophy and poor aerobic growth of sod1Δ cells. This pattern of suppression is unlike that of the previously identified sod1Δ suppressors ATX1, ATX2, bsd2, or pmr1, which indiscriminately reverse all aerobic defects associated with loss of Sod1 (14–16). The suppression of sod1Δ by ATX1, ATX2, pmr1, and bsd2 has been linked to altered metal ion homeostasis resulting in elevated cellular accumulation of either copper or manganese ions (14–16). However, these metals do not appear to play a role in TKL1-mediated suppression of sod1Δ, as atomic absorption analysis indicated no change in cellular levels of copper or manganese ions with elevated TKL1 expression (data not shown). Furthermore, TKL1 was still capable of reversing sod1Δ defects when cells were grown in a medium depleted of copper and manganese (data not shown), a treatment known to block the suppression of Sod1 deficiency by the ATX genes and pmr1 (14, 16). Hence, TKL1 operates through a mechanism not involving metals to specifically suppress the methionine auxotrophy and poor aerobic growth defects of yeast lacking Sod1.

The Pentose Phosphate Pathway and Oxidative Stress—Since overexpression of TKL1 provided some amelioration of oxidative stress in an sod1Δ mutant, we wanted to determine whether disruption of the pentose phosphate pathway would result in oxygen sensitivity. To address the effects of enzymes in the non-oxidative portion of the pathway, we deleted the genes encoding Tkl1, Tkl2, and TAL1, alone and in combination with sod1Δ mutations. These gene deletions did not cause a dependence on methionine or lysine for aerobic growth (data not shown), and also did not enhance sensitivity toward paraquat (data not shown) or atmospheric oxygen (Fig. 4B). In contrast to these results obtained with gene deletions of the non-oxidative segment, oxygen resistance was substantially compromised by blocking the oxidative portion of the pentose phosphate pathway through a ZWF1 deletion. In confirmation of earlier reports (34), zwf1Δ strains are partially auxotrophic for methionine, yet this defect is not as severe as that of sod1Δ strains (Fig. 4A). Like sod1Δ, the methionine independent growth of zwf1Δ strains was improved by culturing under anaerobic conditions (Fig. 4A). The zwf1Δ strain also exhibited impaired aerobic growth even in medium supplemented with methionine, which was demonstrated in a liquid test for oxygen sensitivity. In this experiment, both the zwf1Δ strain and the sod1Δ strain exhibited striking sensitivity toward atmospheric levels of oxygen in well aerated cultures (Fig. 4B). In parallel aerobic cultures, these mutant strains exhibited wild type levels of growth (data not shown). Interestingly, the combination of null mutations in ZWF1 and SOD1 resulted in a profound aerobic growth defect more severe than that of either mutant alone, that could even be observed using solid medium (Fig. 4A). This apparent “synthetic lethal” effect of sod1Δ and zwf1Δ mutations was rescued by growing the double mutant strain under anaerobic conditions (Fig. 4A). A further comparison of sod1Δ and zwf1Δ mutant phenotypes revealed that, while both are methionine auxotrophs, only sod1Δ is auxotrophic for lysine and sensitive to paraquat (Table II).

Because sod1Δ and zwf1Δ mutants both exhibit a defect in methionine biosynthesis and a sensitivity to oxygen, it was of
Interest to determine whether known suppressors of sod1Δ also have the ability to suppress zwf1Δ. We have divided these suppressors into two classes: those that work through transition metal homeostasis (i.e. pmr1, bsd2, and the ATX genes), and TKL1, which works through the pentose phosphate pathway. Expressing the TKL1 gene on a multi-copy plasmid did not suppress the methionine auxotrophy of zwf1Δ (data not shown), while TKL1 completely suppressed the methionine auxotrophy of sod1Δ (Fig. 2A). As a representative of the metal transport suppressors, we investigated the effect of pmr1 mutations (14). A pmr1Δ mutation did not rescue methionine auxotrophy in a zwf1Δ strain, although this mutation was fully capable of reversing the sod1Δ defect (Fig. 5). In the background of the sod1Δ zwf1Δ double mutant, the pmr1Δ mutation rescued methionine auxotrophy and poor aerobic growth to precisely the same level observed with the zwf1Δ mutant alone (Fig. 5). Hence, pmr1 mutations and TKL1 overexpression are highly specific for suppressing Sod1 deficiency and cannot reverse the oxidative stress effects of a zwf1Δ mutation.

Examination of the Sulfur Assimilation Defect in sod1Δ and zwf1Δ Strains—The finding that elevated expression of the pentose phosphate pathway gene, TKL1, rescued sod1Δ methionine auxotrophy strongly suggested that the methionine requirement of sod1Δ and zwf1Δ mutants may be intimately related. NADPH is presumably the common denominator. Mutants of sod1Δ are known to possess lowered levels of NADPH under aerobic, but not anaerobic conditions (13). In addition, we recently found that NADPH levels are lowered in aerobically grown sod1Δ and zwf1Δ mutants to levels that are 59% and 67% that of an isogenic wild type strain, respectively (high performance liquid chromatography analysis of pyridine nucleotide levels; data not shown). We therefore wanted to determine whether the NADPH requiring steps in methionine biosynthesis were similarly affected in sod1Δ and zwf1Δ strains.

As shown in Fig. 6, the PAPS reductase and sulfite reductase steps of sulfur assimilation both utilize NADPH, and in the case of PAPS reductase, NADPH is needed to reduce the Trx co-factor (35). It is noteworthy that a trx1Δ trx2Δ double mutant, which lacks both thioredoxin genes, is also auxotrophic for methionine (36). To address whether the two reductase steps in sulfur assimilation are similarly affected by loss of Sod1, Zwf1, or Trx, supplementation experiments were performed. Intermediates of the sulfur assimilation pathway were added to sulfur-free growth medium, and were examined for the ability to rescue the methionine auxotrophy of sod1Δ, zwf1Δ, and trx1Δ trx2Δ mutants in parallel. The supplementation of either sulfite (0.05–0.3 mM) or sulfide (0.05–0.15 mM) alone did not significantly improve methionine independent growth of any of these mutant strains (data not shown). However, as reported previously (13, 37), sulfite and sulfide proved to be toxic at relatively low concentrations in these experiments, which may have masked any capacity these single agents might have for rescuing the methionine defects. In an attempt to detect a possible additive effect of these agents, sulfite and sulfide were supplied in combination to the growth medium at very low concentrations. In this case, methionine auxotrophy was partially reversed in all three mutant strains examined (Fig. 7). We also tested the effect of thiosulfate, which is far less toxic to the yeast strains, and can feed into sulfide (and perhaps sulfite as well) through the action of thiosulfate reductase (38). Thiosulfate has previously been reported to rescue the methionine auxotrophy of yeast strains containing a point mutation in SOD1 (13), and we confirmed this finding with our sod1Δ strain (Fig. 7). Significantly, the addition of thiosulfate also completely relieved the methionine growth requirement of strains lacking Zwf1 and Trx (Fig. 7). The striking resemblance between the zwf1Δ sod1Δ and trx1Δ trx2Δ strains in their response to sulfur compound supplementation strongly suggests that the same biosynthetic step is affected in all three mutants. Our findings demonstrate that this common block in sulfur
assimilation occurs upstream of sulfide, and that the PAPS reductase and/or the sulfite reductase steps may be affected by the loss of Sod1 and Zwf1 activity.

DISCUSSION

The pentose phosphate pathway is considered to be a major source of cellular reducing power, with the ZWF1 gene product (glucose-6-phosphate dehydrogenase) catalyzing the key NADPH-producing step. It is well documented that this pathway and, specifically, glucose-6-phosphate dehydrogenase play a protective role during oxidative stress (39, 40). Here we report experiments designed to further understand the role of another key player in oxidative stress protection, Sod1, and find an interesting overlap between Sod1 and the pentose phosphate pathway. We discovered that elevated expression of the S. cerevisiae transketolase gene of the pentose phosphate pathway had the ability to suppress specific oxidative stress phenotypes caused by Sod1 deficiency. In studying the mechanism of this TKL1-mediated suppression, we uncovered an intriguing connection between SOD1, NADPH, and the pentose phosphate pathway.

Our studies show that the ability of TKL1 to suppress Sod1 deficiency is absolutely dependent on Zwf1 and the oxidative portion of the pentose phosphate pathway that produces NADPH. We therefore propose that expression of TKL1 results in an increased flux through the pathway, generating more NADPH, which relieves specific sod1 mutant phenotypes. This model is based upon the knowledge that TKL1 is the rate-limiting enzyme in the non-oxidative reactions of the pathway, and that cells that are in demand for NADPH convert pentose phosphate pathway-generated fructose 6-phosphate to glucose 6-phosphate, for recycling through the oxidative reactions (32). A prediction of our TKL1 suppression model would be that elevated expression of ZWF1 should also create more flux through the oxidative reactions of the pentose phosphate pathway, and should also suppress Sod1 deficiency. However, we were unable to test this possibility since ZWF1 is known be under tight control in yeast, with no increase in glucose-6-phosphate dehydrogenase activity detected with increased ZWF1 expression (19). We nevertheless were able to evaluate the association between Zwf1 and Sod1 through ZWF1 gene deletion experiments.

Our gene deletion studies show that Zwf1 and Sod1 play overlapping, but not redundant, roles in oxidative stress resistance. Cells lacking either one of these factors share in common an auxotrophic requirement for methionine and poor aerobic growth (Table II). The sod1Δ mutants are additionally auxotrophic for lysine and are sensitive to paraquat, but zwf1Δ cells are not. Significantly, the same two phenotypes that are shared by sod1Δ and zwf1Δ mutants are those that are specifically suppressed in a sod1Δ strain by overexpression of TKL1 (Table II). These findings strongly indicate that the methionine dependence and poor aerobic growth of sod1Δ mutants result from a compromise in aerobic NADPH levels. In support of this model, we found that aerobically grown sod1Δ and zwf1Δ mutants have total NADPH levels that are 59–67% that of an isogenic wild type strain. The reduction in NADPH levels in sod1Δ mutants presumably reflects the increased consumption of this pyridine nucleotide during oxidative stress by molecules such as glutathione. This same demand for NADPH under aerobic conditions helps to explain why the methionine auxotrophy and poor aerobic growth of zwf1Δ strains are reversed by anaerobiosis.

We conclude that sod1Δ and zwf1Δ mutations affect the same step(s) in methionine biosynthesis. In a previous study by Thomas et al. (34), it was proposed that the methionine auxotrophy of these two strains must be distinct since the zwf1Δ defect was not rescued by sulfide supplementation, a treatment that had been reported by Chang and Kosman (15) to partially reverse the methionine auxotrophy of a sod1 mutant. However, the former study did not experimentally compare sod1Δ and zwf1Δ mutants side by side (34), and in doing this, we noted that sulfide treatment would not correct either the sod1Δ- or zwf1Δ-related defects, apparently due to sulfide toxicity. Sulfide has been reported to be quite toxic to certain yeast strains (37), which helps to explain the discrepancy in results obtained by different laboratories. Nevertheless, the sod1Δ and zwf1Δ strains responded identically in our studies to supplementation with other sulfur compounds, demonstrating that the block in sulfur assimilation in both zwf1Δ and sod1Δ mutants exists upstream of sulfide. We propose that this common block is mediated through lowered NADPH availability for the Trx-dependent PAPS reductase and/or the sulfite reductase steps of sulfur assimilation. Although total cellular NADPH levels were lowered by no more than 30–40% in sod1Δ and zwf1Δ mutant strains, the local effect on NADPH levels may be more dramatic. In this regard, it is worth noting that Sod1, the pentose phosphate pathway, and the sulfur assimilation pathway all appear cytosolic (8, 41, 42).

Aside from methionine auxotrophy, the overall sensitivity to atmospheric oxygen observed with zwf1Δ and sod1Δ strains is not well understood. However, this phenotype should also involve a reduction in NADPH since this sod1Δ defect is alleviated by TKL1 overexpression. During anaerobic growth, NADPH can be utilized entirely for essential cellular functions such as DNA synthesis, fatty acid synthesis, and other reduc-
tive biosynthetic processes. However, when cells are grown in air, NADPH must also be used to maintain a level of reduced glutathione to combat oxidative damage. It is therefore quite conceivable that zwf1Δ and sod1Δ mutants grow poorly in air because they are limited for NADPH and their redox state is compromised under these conditions, drawing NADPH away from essential reductive processes. In this manner, the additive effect of a double sod1Δ zwf1Δ mutation should severely compromise the cellular redox state and drastically inhibit aerobic growth, and this is precisely what we have observed.

Since its discovery nearly 3 decades ago (43), SOD has been thought to directly protect biomolecules from oxidative damage by scavenging O$_2^-$. The parquat sensitivity of sod1Δ yeast is certainly consistent with this model, and, in bacteria, loss of SOD has been associated with O$_2^-$-mediated damage to iron-sulfur-containing enzymes (44–46). The lysine biosynthetic defect of sod1Δ yeast may also be attributed to direct attack by O$_2^-$ or another reduced oxygen species, since this defect is not rescued by the pentose phosphate pathway TKL1 gene. Yet all of the oxygen-related phenotypes of sod1Δ mutants, including lysine auxotrophy, are fully rescued by the bsd and ATX metal transport suppressors that cause increased accumulation of copper or manganese ions (14–16).

We have proposed that these suppressors work on the cause of oxidative damage, and through reactions involving copper or manganese, help to neutralize the O$_2^-$ or other reduced oxygen species that predominate in sod1Δ strains (14–16). Elevated levels of O$_2^-$ should not prevail in zwf1Δ strains, and these mutants are therefore refractory to suppression by transition metals (Ref. 34 and this report). In comparison to the metal transport suppressors, the TKL1 pentose phosphate suppressor of sod1Δ alleviates a specific effect of oxidative damage and helps to correct the imbalance in the redox state of the cell. Overall, our studies indicate that deleting the S. cerevisiae SOD1 gene has broader ramifications than mere loss of O$_2^-$ scavenging. Together with the pentose phosphate pathway, Cu/Zn-Sod1 helps to maintain the reducing environment of the cell.

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