Null Mutants of Saccharomyces cerevisiae Cu,Zn Superoxide Dismutase: Characterization and Spontaneous Mutation Rates

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Deletion-replacement mutations of the Saccharomyces cerevisiae Cu,Zn superoxide dismutase gene were constructed. They were exquisitely sensitive to redox cycling drugs and showed slight sensitivity to other agents. The aerobic spontaneous mutation rate was three- to fourfold higher in sod1Δ1 mutants, while the anaerobic rate was similar to that of the wild type.

The proposals that superoxide might be a major agent of dioxygen toxicity and that the primary function of superoxide dismutases (SODs) is to protect against superoxide-induced damage (7–9, 12) have been difficult to confirm, because the targets of such damage have not been characterized (6, 16). However, SOD-deficient mutants of both Escherichia coli and Saccharomyces cerevisiae are highly sensitive to dioxygen (2, 3), suggesting strongly that SODs play an important role in enabling the cells to grow aerobically. In Drosophila melanogaster, Cu,Zn SOD may protect against ionizing radiation (13) and increase longevity (14). To investigate the possibility that DNA is a primary target for superoxide-induced damage that is inhibitable by Cu,Zn SOD, we constructed and characterized Cu,Zn SOD-deficient mutants in the yeast S. cerevisiae. There is some disagreement in the literature about the nomenclature of SOD genes in S. cerevisiae. We use SOD1 for Cu,Zn SOD for consistency with the convention used for humans and other eucaryotes (18). We report here our results with respect to mutation rates in sod1 (Cu,Zn SOD−) null mutants and our conclusions concerning the role of SOD in preventing DNA damage.

Deletion-replacement mutations. Deletion-replacement mutations in the Cu,Zn SOD gene were made by the one-step gene disruption procedure (15). The 3.7-kb EcoRI-PstI fragment of pUC-SOD4.3 (1) was recloned in the same vector to yield pUC-SOD3.7. A 0.7-kb AsuII fragment containing the C-terminal 75% of the Cu,Zn SOD-coding region was removed and replaced by a HindIII fragment containing the yeast URA3 gene, following end filling, to yield pUC-SODΔA::URA3. Standard methods were used (11, 17).

S. cerevisiae DBY746 (MATa leu2-3,112 his3Δ1 trp1-289a ura3-52) was transformed (10) with HindIII-digested pUC-SODΔA::URA3 DNA, and URA3− colonies were isolated on plates containing synthetic complete medium (SC) minus uracil (17). To screen for sod1 mutants, transformants were tested for oxygen and paraquat sensitivity. Of 18 URA3− isolates tested, 5 were paraquat and oxygen sensitive. Southern blot analyses (data not shown) revealed that the other colonies retained the wild-type SOD1 gene. The mutation is called sod1Δ1::URA3, sod1Δ1 for short. Extracts from strains carrying this mutation showed no Cu,Zn SOD activity or immunoreactive material (data not shown). The sod1 point mutants described previously (2) were selected on the basis of paraquat sensitivity. Our deletion-replacement mutants showed the same greatly elevated sensitivity without previous selection, indicating that paraquat sensitivity is a direct consequence of the sod1 mutation. EG1 (MATa leu2-3,112 his3Δ1 trp1-289 ura3-52 sod1Δ1::URA3) and EG3 (MATa leu2-3,112 his3Δ1 trp1-289 ura3-52 ade2::hisG gal2 sod1Δ1::URA3) were isolated after two backcrosses to SLAA (MATa leu-2,112 his3Δ1 trp1-289 ura3-52 ade2::hisG), a related wild-type strain. As the work progressed, it became clear that drug resistance phenotypes varied between the wild-type parent strains. Therefore, other sod1Δ1 strains, EG-AC1, EG-AC2, and EG-301, were produced subsequently in SLAA, DBY746, and DBY747 (MATa leu2-3,112 his3Δ1 trp1-289 ura3-52 Can′ gal2), respectively, and were not backcrossed. To help prevent second-site pseudoreversion events, these strains were maintained microaerobically (CampyPak; BBL).

Sensitivity to oxidative conditions. The sod1Δ1 mutants were viable in aerobic incubation but grew more slowly than wild-type cells with glucose as the carbon source (Table 1). The difference was not as pronounced with 3% glycerol as the carbon source, perhaps because the sod1Δ1 strains retain the functional gene for mitochondrial Mn SOD, which is expressed during growth on glycerol but not during growth on glucose (20).

The sod1Δ1 strains were extremely sensitive to paraquat and menadione, as measured by disk assays or killing by challenge in liquid culture (data not shown). Gradient plates (Table 2) were formed in square petri dishes by pouring two layers of YPD (17) agar—the first a slanted one containing the agent at the indicated concentration and the second without the agent filling in the slope. Yeast cultures were diluted 1:100 in 0.5% agar, and even lines of cells were inoculated along the gradient by using a bent glass rod. The length of the growth area was measured after 3 days. The lowest level of paraquat tested (10 μM in the bottom layer) was sufficient to prevent all growth of sod1Δ1 cells, while SOD1 (wild-type) strains were resistant to nearly 1,000 μM (>90% growth on a 1,000 μM plate). This represents a more-than-100-fold difference in sensitivity. The extreme sensitivity to redox cyclers was observed only in the presence of dioxygen (Table 2). sod1Δ1 strains also exhibited dioxygen-dependent lysine auxotrophy and inability to survive in 100% dioxygen (Table 2) that was observed with the previously isolated point mutants (2, 4).

Sensitivity to other agents. Aerobic growth slightly but...
consistently increased the sensitivity of the already stressed sod1Δ1 strains to certain other DNA-damaging agents, such as methyl methanesulfonate and tert-butyl hydroperoxide (Table 2). However, the seemingly unrelated agent canavanine (an arginine analog) gave a similar result (data not shown), leading us to believe that this is a "straw that broke the camel's back" effect rather than a specific sensitivity. These differences were not evident in anaerobic conditions. The increase in anaerobic tert-butyl hydroperoxide resistance in the sod1Δ1 strain was not reproducibly observed in all genetic backgrounds.

**Spontaneous mutation rates.** Spontaneous mutation rates were measured in sodl and parent strains by using the fluctuation test method described by Von Borstel (19). The strains used in this work contained the trpl-289 allele, a revertible amber mutation of the TRPI gene. Individual cultures were grown in 24-well tissue culture dishes in SC medium (17) limited in tryptophan (1.25 μM) and increased fourfold in other required nutrients (Ade, Leu, His, Ura, Met, and Lys) to prevent growth limitation by their depletion. Growth proceeded until the Trp supply was exhausted, after which only cells that had reverted to TRP+ grew and formed large colonies over a thin background. The concentration of Trp, and thus the final nonrevertant cell density, was adjusted so that revertants arose in about half of the wells. The reversion rate was calculated from the zeroth term of the Poisson distribution. For experiments in which anaerobic conditions were used, 0.1% Tween 80 and 30 mg of ergosterol per liter were added to the medium. Supplementation did not affect aerobic mutation rates. The results are shown in Table 3. The sod1Δ1 strains had mutation rates during aerobic growth about threefold greater than that determined for wild-type cells. Under anaerobic conditions, the rates were similar for sod1Δ1 and wild-type strains. Essentially the same results were obtained with sod1Δ1 and SOD1+ strains in the various backgrounds tested. Aerobic mutation rates differed with different growth temperatures, but the ratio between sod1Δ1 and SOD1+ mutation rates remained similar.

**E. coli** strains lacking both Mn SOD and Fe SOD have dramatic increases in aerobic mutation rates. Those lacking only Mn SOD have somewhat lower increases, while those lacking only Fe SOD show no change (5). Work with *D. melanogaster* has implied a possible connection between SOD and ionizing radiation damage (13). However, ours is the first demonstration of the role of Cu,Zn SOD in a eucaryote as a protector against mutational change. The increase measured here is modest but nonetheless significant. While recognizing that any increase in the accumulation of mutations in a species is significant for the long-term survival of the species, we believe that the effect of sod1Δ1 on mutation rates is unlikely to account for the other phenotypes observed in these mutants. In particular, the acute toxicity of redox cycling drugs and the reduced growth rates are probably best accounted for by other mechanisms. The effect might be more dramatic in strains lacking mitochondrial Mn SOD as well as Cu,Zn SOD. Work is under way in our laboratory to isolate and characterize such mutants. Nevertheless, the strong phenotype of the sod1Δ1 mutant, even in the presence of Mn SOD, argues for separate roles for these two proteins, and in particular for the importance of cytoplasmic Cu,Zn SOD.

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### REFERENCES


