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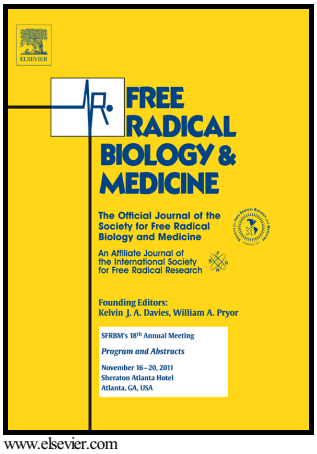

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Superoxide dismutase protects ribonucleotide reductase from inactivation in yeast

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Abstract

Ribonucleotide reductase (RNR) catalyses the rate limiting step of DNA synthesis utilising a mechanism that requires a tyrosyl radical. We have previously shown that superoxide can quench protein tyrosyl radicals *in vitro*, either by oxidative addition, or reduction of the radical to tyrosine. Here, we observe that *Saccharomyces cerevisiae* strains lacking either copper-zinc SOD (SOD1) or manganese SOD (SOD2) had decreased RNR activity compared to SOD competent yeast. When superoxide production was increased by treatment with paraquat, RNR activity was further decreased, with yeast lacking SOD1 being the most sensitive. The growth of yeast lacking SOD1 was also the most sensitive to paraquat treatment. Using expressed recombinant RNR, superoxide addition was not detectable using mass-spectrometry. This suggests that oxidative addition is not the major route of inhibition in our system, but does not rule out reduction by superoxide as a possible mechanism. Our results demonstrate that protection of RNR from inactivation by superoxide is an important function of SOD, particularly cytoplasmic SOD1.

Abbreviations:

dNDP, deoxyribonucleotide diphosphates; DTT, dithiothreitol; EPR, electron paramagnetic resonance; GSH, glutathione; IAM, iodoacetamide; MS, mass spectrometry, NDP, ribonucleotide diphosphates; RNR, ribonucleotide reductase; SOD, superoxide dismutase