Effects of Deleting Mitochondrial Antioxidant Genes on Life Span

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ABSTRACT: Reactive oxygen species (ROS) damage biomolecules, accelerate aging, and shorten life span, whereas antioxidant enzymes mitigate these effects. Because mitochondria are a primary site of ROS generation and also a primary target of ROS attack, they have become a major focus area of aging studies. Here, we employed yeast genetics to identify mitochondrial antioxidant genes that are important for replicative life span. In our studies, it was found that among the known mitochondrial antioxidant genes (*TTR1*, *CCD1*, *SOD1*, *GLO4*, *TRR2*, *TRX3*, *CCS1*, *SOD2*, *GRX5*, *PRX1*), deletion of only three genes, *SOD1* (Cu, Zn superoxide dismutase), *SOD2* (Manganese-containing superoxide dismutase), and *CCS1* (Copper chaperone), shortened the life span enormously. The life span decreased 40% for Δ*sod1* mutant, 72% for Δ*sod2* mutant, and 50% for Δ*ccs1* mutant. Deletion of the other genes had little or no effect on life span.

KEYWORDS: aging; antioxidant genes; mitochondria; ROS; CCS1

INTRODUCTION

Free radicals generated by aerobic metabolism cause oxidative damage to cell components, such as DNA, proteins, and lipids. Such damage results in altered structure and loss of biological function, which leads to aging and cell death.[1] The mitochondrial respiratory chain on the inner mitochondrial membrane is a major intracellular source of reactive oxygen species (ROS) in eukaryotes. Since ROS are highly reactive and short-lived, mitochondria are continuously exposed to them and accumulate oxidative damage more rapidly than the rest of the cell.[2] Various defense mechanisms operate in mitochondria to minimize the deleterious effects of oxidative stress and maintain the mitochondrial function. Some protective enzymes have direct role in scavenging free radicals, for example, superoxide dismutase is involved in the conversion of superoxide anion to H_2O_2, which is further detoxified by catalases or peroxidases.[3] Enzymes that repair the products of oxidatively damaged

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TABLE 1. List of mitochondrial antioxidant genes

ORF (gene)	Gene product	Subcellular localization
SOD2	Mn superoxide dismutase	mitochondrial matrix
GRX5	glutaredoxin	mitochondrial matrix
PRX1	peroxiredoxin	mitochondrion
TRX3	thioredoxin	mitochondrion
CCP1	cytochrome c peroxidase	mitochondrial intermembrane space
SOD1	Cu, Zn superoxide dismutase	cytosol, mitochondrial intermembrane space
GLO4	glyoxylase-II	mitochondrial matrix
TRR2	thioredoxin reductase	mitochondrion
GRX2	glutaredoxin	cytosol, mitochondrion
CCS1	copper chaperone	cytosol, mitochondrial inner membrane

components form the second line of antioxidant defense.[4] If oxidative stress defense mechanisms are compromised or overwhelmed, disease states may develop. It has been proposed that mitochondrial oxidative damage contributes to neurodegenerative disorders, cancer, and the aging process in humans.[5] Yeast (*Saccharomyces cerevisiae)* cell possesses a limited life span and is a good model organism to study the role of mitochondrial antioxidant genes in cellular aging. Aging in yeast is determined microscopically by counting the number of daughter cells produced by each cell. In this study, using yeast deletion mutants, the role of mitochondrial antioxidant genes on life span was investigated.

MATERIALS AND METHODS

Yeast Strains and Media

Wild-type (WT) and 10 different deletion mutants(Δ*ccp1,* Δ*sod1,* Δ*glo4,* Δ*trr2,* Δ*trx3,* Δ*ccs1,* Δ*grx2,* Δ*sod2,* Δ*grx5,* Δ*prx1*) of *S. cerevisiae* were examined. Yeast strains were grown in rich YPD medium (1% yeast extract, 2% peptone, and 2% glucose) for both growth and aging assays.

Identification of Mitochondrial Antioxidant Genes

Identification of mitochondrial antioxidant genes was carried out by using Mitochondrial Proteome Database (MitoP2).[6]

Yeast Aging Assay

Yeast strains were grown on fresh media for 2 days before analysis. For each strain, 20 daughter cells (starter mothers) were collected and lined up by a

micromanipulator on agar plates. New buds (daughters) from these virgin cells were removed and discarded as they formed. This process continued until cells ceased dividing. Life span was determined as the total number of daughter cells that each mother cell generated.

RESULTS AND DISCUSSION

We first obtained a list of mitochondrial antioxidant proteins using MitoP2 program, which analyzes the mitochondrial targeting sequence in genes and

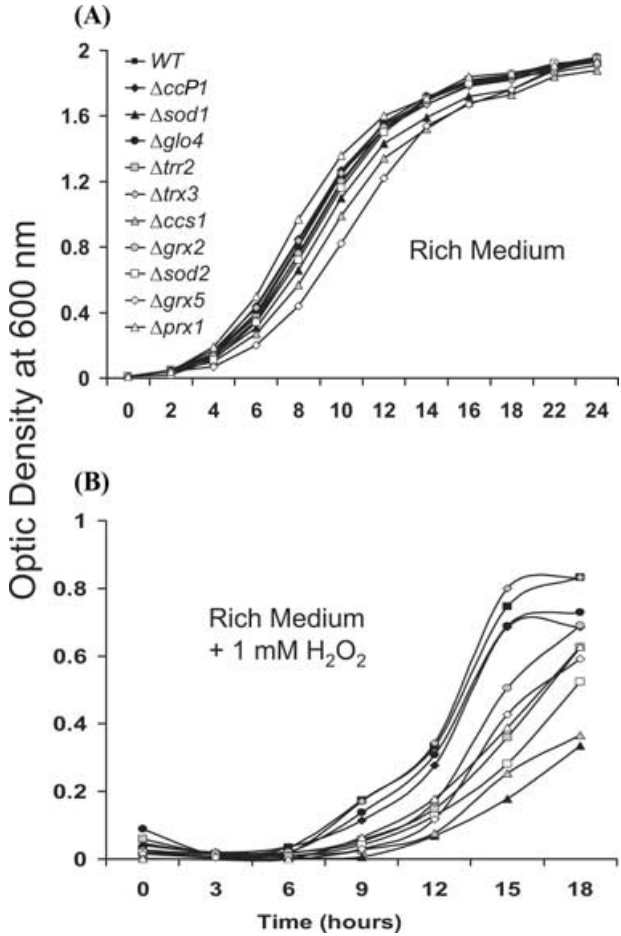

FIGURE 1. Overnight cultures were diluted to 0.05 OD_{600} in liquid and shaked at 30°C for the indicated time. Growth rate was monitored by OD_{600} measurements. (**A**) Growth in rich (YPD) media. (**B**) Growth in rich media containing 1 mM H_2O_2.

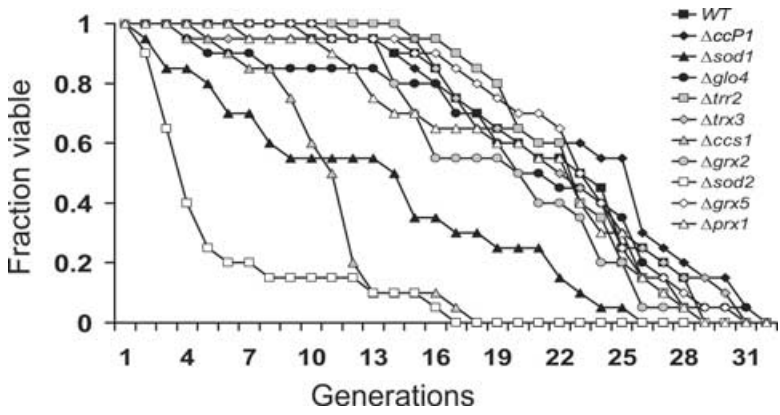

FIGURE 2. Replicative life span analyses of WT and mutant cells.

matches the results with published experimental data to predict localization of proteins with a high accuracy rate.[6] As shown in TABLE 1, 10 different proteins were found to be antioxidant and residing in mitochondria. To investigate whether these genes are important in cell growth, deletion mutants were grown in rich medium and growth rate was followed by OD_{600} measurements. As shown in FIGURE 1 A**,** none of the mutants showed a significant growth defect under normal conditions, but mutants lacking *GRX5, SOD2, SOD1, CCS1, PRX1, TRR2,* and *GRX2* genes grew slower when exposed to 1 mM of H_2O_2 suggesting that these mutants are sensitive to oxidative stress (FIG. 1 B).

To see the effect of deleting these genes on life span, replicative aging profile of each mutant was determined. Deletion of *SOD1*, *SOD2,* and *CCS1* genes caused a major drop in both average and maximum life spans (FIG. 2 and TABLE 2). The life span decrease was 40% for Δ*sod1* mutant, 72% for Δ*sod2* mutant, and 50% for Δ*ccs1* mutant. Absence of *GRX2*, *GLO4,* and *PRX1* genes also shortened life span (10%), but the extent of decrease was not as significant as deleting *SOD1* and *SOD2* or *CCS1* genes. Surprisingly, deletion of the other mitochondrial antioxidant genes did not lead to a decrease in life span. Role of superoxide dismutases in aging was described earlier[7,8] and our results are consistent with the previous findings.

TABLE 2. Maximum and average life spans of mutants

	WT	Δ*ccP1*	Δ*sod1*	Δ*glo4*	Δ*trr2*	Δ*trx3*	Δ*ccs1*	Δ*grx2*	Δ*sod2*	Δ*grx5*	Δ*prx1*
Max life span	29	32	26	32	29	31	18	29	17	31	29
Average life span	22	23	13	20	23	22	11	20	6	23	20

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