Response of *Schizosaccharomyces pombe* against Oxidative Stress

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Schizosaccharomyces pombe is an Ascomycete fungi, dividing by binary fission. It contains about 14 Mbp of genomic information on 3 chromosomes. This yeast provides a good model system to study cell division and stress response, due to its close similarity to human system. Previous study revealed that *S. pombe* cells adapt to oxidative stress by inducing several antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione reductase. Genes encoding catalase (*ctt1*$^+$), cytoplasmic SOD containing Cu and Zn (*sod1*$^+$), mitochondria SOD containing Mn (*sod2*$^+$), and glutathione reductase (*pgr1*$^+$) have been isolated and examined for their regulation and role in the physiology and stress response of this organism. We examined their induction behavior, the signal transduction pathway for the gene expression, the phenotype of the disruption mutants. Through these studies, we expected to understand the role of these oxidative defense systems in stress response as well as cell proliferation, and tried to find any link between these two seemingly separate phenomena.

Regulation and the Role of Glutathione Reductase

The *pgr1*+ gene encoding glutathione reductase (GR) has been isolated from *S. pombe*. The level of *pgr1*$^+$ transcripts increased by treatment with oxidants such as menadione, dumene hydroperoxide, and diamide. It also increased by treatment with high osmolarity, heat shock, or at the stationary growth phase. The deletion of the *pap1*$^+$ gene encoding an AP-1 homolog in *S. pombe* caused reduction in the *pgr1*$^+$ gene expression. Furthermore, *Dpap1* cells lost the inducibility of *pgr1*$^+$ gene expression by the above stresses, implying that Pap1 is involved in general stress-inducible gene expression. When the *pgr*1$^+$ gene was disrupted, the haploid spores were not viable. Repression of *nmt1* promoter-driven *pgr1*$^+$ expression by thiamine caused cessation of growth, which was rescued by the episomal *pgr1*$^+$ gene. These results indicate that GR activity, which efficiently reduces GSSG, is essentially required for the growth of *S. pombe*, unlike in *Saccharomyces cerevisiae* or *Escherichia coli.*

Regulation and the Role of CuZnSOD

S. pombe contains two superoxide dimutases (SODs), one in the cytosol, and the other in mitochondria. Cu,Zn-containing superoxide dismutase (CuZnSOD) removes superoxide anion in the cytosol of eukaryotes. The amount of *sod1*$^+$ mRNA decreased in the stationary phase, consistent with the change in enzyme activity. The transcript increased by treatment with H_2O_2, menadione (MD), copper, NaCl, and heat, suggesting that CuZnSOD is a general stress-induced protein. Induction by H_2O_2 was rapid and transient, being dependent on Wis1-Spc1-Atf1 pathway of signal transduction. Induction by MD, salt, or heat, however, was either slow or sustained longer, being independent of Wis1 pathway. In *wis1D* or *spc1D* mutant, the amount of *sod1*$^+$ transcripts increased in the stationary

phase, suggesting that Wis1 and Spc1 functions in down-regulating *sod1*$^+$ gene in the stationary phase. Tetrad analysis following *sod1*$^+$ gene disruption revealed that the *sod1D* cells were not viable even on rich media. Repression of *sod1*$^+$ gene expression by thiamine through *nmt1* promoter resulted in growth arrest, consistent with the above observation. Sod1-depleted cells were arrested at G2 phase with 2C DNA content, suggesting that CuZnSOD plays a critical role in proper cell proliferation of *S. pombe*. The current and previous observations that the viability of *S. pombe* cells, unlike *S. cerevisiae*, sensitively depends on the action of oxidative defense enzymes in the cytosol, such as CuZnSOD and glutathione reductase, suggest that *S. pombe* can serve as a good model system to study the effect of oxidative stress on cell proliferation.

Regulation and the Role of MnSOD

The *sod2*$^+$ gene encoding putative mitochondrial superoxide dismutase containing manganese (MnSOD) has been isolated. Purification and analysis of the *sod2*$^+$ gene product revealed that it contained only manganese as a cofactor, thus verified to be a genuine MnSOD. It was localized in mitochondria as expected. Its N-terminal amino acid sequence indicated that the mitochondrial targeting sequence of 21 amino acids was removed. The native form consisted of two identical subunits. The *sod2*$^+$ expression was induced by external stresses, such as treatments with superoxide generators, high osmolarity, and heat. The induction by these stress treatments depended on Wis1-Spc1 MAPK signal transduction pathway, being independent of transcription factors Atf1 or Pap1. The *sod2* disruption rendered cells sensitive to various superoxide-generators, heat, and high osmolarity, suggesting that the mitochondrial MnSOD acts as a general defense agent against multiple stresses.

Stationary phase-specific transcriptional regulation of the *sod2*$^+$ gene encoding manganese superoxide dismutase (MnSOD) was examined. In glucose medium, *sod2*$^+$ mRNA, expressed at a low level in exponentially growing cells, increased dramatically as cells entered the stationary phase. This was dependent on Wis1-Spc1 MAPK pathway but Atf1 or Pap1 was not involved. Addition of glucose or cAMP to stationary phase cell caused the reduction of *sod2*$^+$ expression, suggesting that depletion of these components constitutes stationary condition. However, depletion of glucose in exponentially growing cells induce *sod2*$^+$ expression via transcription factor Atf1, indicating that there still exist other factors in the stationary phase-induction of *sod2*$^+$ independent of glucose depletion. cAMP was shown to act via cAMP-dependent protein kinase (PKA), whereas glucose can repress *sod2*$^+$ expression independent of cAMP.