

## Evolution of antioxidant enzymes in cyanobacteria and its relationships to the rise of atmospheric oxygen

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The early Paleoproterozoic is a remarkable period in the geological history, marked by the rise of atmospheric O<sub>2</sub> (the Great Oxidation Event, the GOE) and the evolution of life. Fossil records suggest that the GOE coincided with the evolution of eukaryotes and/or large multicellular-like organisms. In addition, phylogenetic analyses show that the evolution of biologically important metabolisms, such as sterol synthesis and aerobic respiration, occurred during the GOE. However, at the same time, the rise in the environmental O<sub>2</sub> level must have been a significant stress to organisms, as it causes the increase in toxic Reactive Oxygen Species (ROSs) within cells.

In order to investigate the evolutionary response of life to the increase in ROS toxicity during the GOE, we conducted phylogenetic analyses on the evolution of antioxidant enzymes SODs (MnSOD, FeSOD, NiSOD and CuZnSOD) and catalases (MnCat, KatG and KatE). In this study, we focused on the antioxidant enzymes in cyanobacteria, the oxygen-producing photosynthetic bacteria that have co-evolved with the atmospheric O<sub>2</sub>. To comprehensively understand the evolution of the enzymes, we performed analyses in two steps. First, we investigated the occurrences and phylogeny of antioxidant enzymes in cyanobacteria. Second, we estimated the changes in the gene expression levels of the enzymes by estimating ancestral promoter sequences.

The results indicate that the common ancestor of cyanobacteria would have already possessed MnSOD, FeSOD and MnCat. Although the origin of NiSOD, KatG and KatE were less certain, our results suggest that they have been acquired before 2.5~2.45Ga. This implies that cyanobacteria acquired major SODs and catalases before the rise of O<sub>2</sub>. The results of ancestral promoter analyses show that the gene expression levels of cyanobacterial FeSOD and KatG have increased from low to high level around the GOE (2.5-2.0 Ga), suggesting that the adaptive evolution of cyanobacterial anti-oxidant systems occurred in response to the rise of atmospheric O<sub>2</sub>. In addition, interestingly, our results show that MnSOD, NiSOD and MnCat have been highly expressed before the emergence of cyanobacteria. This may be indicative of locally oxygenated conditions in the Archean caused by abiotic production of O<sub>2</sub>.

Keywords: the Great Oxidation Event, cyanobacteria, antioxidant enzymes