Finding peroxiredoxin’s reducing system in yeast

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In a 1994 paper, Sue Goo Rhee, Ho Zoon Chae, and Sang Jin Chung showed for the first time that a thiol-specific antioxidant from yeast could catalyze hydrogen peroxide reduction using thioredoxin as its reductant (1). The thiol-specific antioxidant described in the paper became the founding member of a family of peroxidases later named peroxiredoxin, which protects cells by removing low levels of peroxides formed as byproducts of cellular metabolism.

Rhee had started out as a postdoc in the Laboratory of Biochemistry at the National Heart, Lung, and Blood Institute, led at the time by Earl Stadtman, and had become a tenured scientist in 1979. Stadtman and Rhee had worked together on glutamine synthetase, an enzyme that controls nitrogen levels in cells. “When I became tenured, then Stadtman pushed me to work on something else,” Rhee says. “He himself was slowly switching to aging research.” Rhee eventually settled on the field of cell signaling. “But at the same time, I still had an interest in glutamine synthetase in different organisms—particularly in eukaryotic cells,” Rhee says.

Rhee and his postdoc Kanghwa Kim tried to purify yeast glutamine synthetase to homogeneity in a dithiothreitol (DTT)-containing buffer. Each time, however, it would become degraded and inactivated the next day.

Rhee was curious as to why the enzyme became inactivated. Inspired by his mentor’s switch to aging research, he started to wonder whether the glutamine synthetase inactivation had to do with free radicals. When an electron is donated to oxygen, the oxygen becomes a superoxide anion that is readily converted to hydrogen peroxide and can then be further converted to a hydroxyl radical or other free radical. The radical sets off a chain reaction that can harm macromolecules such as DNA, proteins, and lipids. Rhee wondered whether the DTT was reacting with oxygen to produce free radicals.

Rhee asked Kim to flush a tube of the purified enzyme with nitrogen to see whether it would be inactivated in the absence of oxygen. “The next day, we found that the enzyme was protected and there was no loss in the enzyme activity, so we were very happy,” Rhee says. They also noticed that glutamine synthetase from crude yeast extracts under the same conditions was not degraded and inactivated the next day. Rhee and Chae inherited the project, with help in purifying the enzyme from Sang Jin Chung. The researchers set out to examine the 25-kDa protein’s functional groups. At the time, all antioxidant proteins were thought to have a cofactor such as a heme, flavin, manganese, molybdenum, nickel, or selenium group at its active center. This protein had no such group, but rather had a cysteine group at its active center. When Rhee and Chae mutated the cysteine residue, the protein lost its antioxidant activity.

To determine where the reducing electrons were coming from, they added back column chromatography fractions to see which could support the catalytic cycle. “For a while we got completely lost,” Rhee says. They initially thought that a thiol might serve as the electron donor; in fact, it was a system of two proteins: thioredoxin and thioredoxin reductase.

In the Journal of Biological Chemistry in 1994, they reported a yeast protein that had hydrogen peroxide or a lipid peroxide as its substrate, a thioredoxin as an electron donor, and a cysteine group at its active center. The electrons came from NADPH through thioredoxin reductase and thioredoxin and were eventually used to eliminate hydrogen peroxide. “This was the eye-opener: the thioredoxin system serving as the reductant for hydrogen peroxide reduction,” says Leslie Poole, a professor of biochemistry at Wake Forest University School of Medicine. Heme-peroxidases were well-known, but the concept of a thiol-dependent peroxidase was new. The researchers referred to this 25-kDa protein as thioredoxin-dependent peroxide reductase.

At first, Rhee had trouble convincing his colleagues that the cysteine residue could be involved in enzymatic redox reactions. After all, most proteins contain a cysteine residue. How could this specific protein be so sensitive to hydrogen peroxide? “It’s an important question,” Rhee says. “Even if you see very pure bands in the gel, it’s difficult to eliminate the possibility of contamination by a very efficient enzyme like catalase.”

Using the protein’s crystal structure, Rhee and his group later showed that the cysteine is surrounded by positively charged amino acids, which interact with the cysteine through hydrogen bonding to create a thiolate anion that is more sensitive to oxidation.

Rhee and Chae collaborated with researchers, including Poole, from several different groups to report on their findings on this and other peroxide reduction systems in a range of organisms in a 1994 paper in the Proceedings of the National Academy of Sciences (3); the cohort eventually christened the new group peroxiredoxins, since not every member of this family uses thioredoxin as an electron donor.

Soon after, in 1995, a group led by Toren Finkel, also at NIH, revealed the role of hydrogen peroxide as a second messenger in cell signaling (4). Rhee and his group later published on the importance of hydrogen peroxide to growth signaling. They observed that when hydrogen peroxide is produced as a signaling molecule, peroxiredoxin is phosphorylated and inactivated.

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Ruma Banerjee at the University of Michigan Medical School nominated this paper as a Classic.

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locally to prevent it from destroying the signaling molecule. Thus, peroxiredoxin is regulated in response to cell signaling.

Today, Rhee is looking into whether peroxiredoxin may participate in a circadian clock. Oxygen consumption and thus superoxide and hydrogen peroxide generation increase during the day and decrease at night. Rhee believes this metabolism may shed light on organ-specific metabolic phenomena. “Peroxiredoxin is not just a simple antioxidant. It is the coordinator for oxygen-dependent metabolism,” Rhee says. “Many signaling pathways can be coordinated by peroxiredoxin.”

Over the years, Poole says, Rhee “has continued to discover important proteins that people didn’t know about, and also important signaling-related processes.”

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