

4-6 Elucidation of the Gene Activation Mechanism and Application to Visualization of DNA Strand Breaks — DNA Repair Promoting Protein PprA —

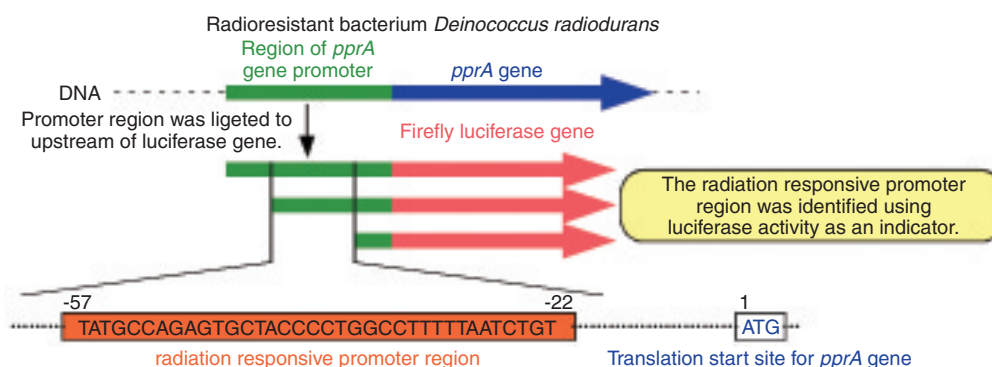


Fig.4-14 Identification of the radiation responsive promoter region of *pprA* gene

The promoter region of *pprA* gene was ligated upstream of firefly luciferase gene. The luciferase activity was monitored as an index of gene promoter activity. This result revealed that the radiation responsive promoter region was located between positions -57 and -22 from the start codon of the *pprA* gene.

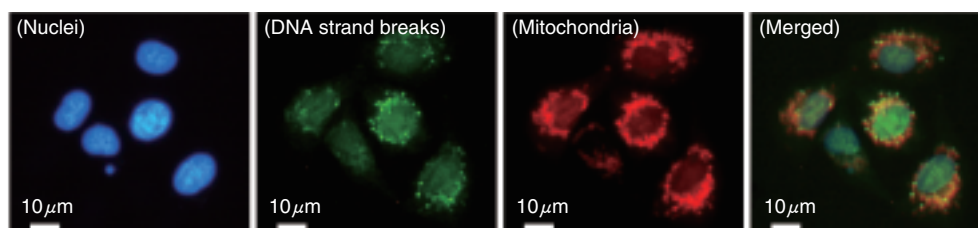


Fig.4-15 DNA strand breaks in mitochondria of mammalian cells following γ ray irradiation

Radiation-induced DNA strand breaks in mitochondria of mammalian cells were visualized by employing PprA protein using an immunofluorescence technique. Colocalization of fluorescence breaks and mitochondria was observed in the image merging the two (right panel). This image suggests that DNA strand breaks existing in mitochondria were effectively detected by utilizing DNA strand break binding ability of PprA protein.

Deinococcus radiodurans (*D. radiodurans*) possesses extraordinary resistance to ionizing radiation, which has been attributed to its highly proficient DNA repair capacity. We have identified the novel gene (*pprA* gene) from this bacterium, and revealed that the gene product (PprA protein) preferentially binds to DNA strand breaks and possesses DNA repair promoting activity. However, the radiation responsive DNA region (promoter) which is essential for the *pprA* gene expression has not been located. Moreover, though visualization of DNA damage utilizing the ability of PprA protein is promising, this detection technology has not been established.

To identify the radiation responsive promoter region, DNA fragments which may include the *pprA* gene promoter were ligated upstream of firefly luciferase gene. The luciferase activity was then monitored following γ irradiation as an index of gene promoter activity. It was revealed that the radiation responsive promoter region was located between positions -57 and -22 upstream from the *pprA* gene (Fig.4-14). Identification of the radiation responsive promoter is very useful finding to delineate the DNA repair network

mechanism of *D. radiodurans*.

In mammalian cells that are sensitive to ionizing radiation, several DNA strand breaks result in cell death. Therefore, it is important to assess intracellular distribution and generative frequency of DNA strand breaks directly for the evaluation of radiation effect in mammalian cells. Certain DNA strand break visualization methods using proteins in mammalian cells associated with their own DNA repair have been developed. However, these methods have disadvantage that is difficult to detect initial DNA damage immediately following γ irradiation. To overcome this disadvantage, we developed a new detection method to visualize the initial DNA damage by utilizing the *D. radiodurans* PprA protein, which possesses an ability to bind to DNA strand breaks specifically. In this method, initial DNA damage can be visualized using fluorescence-labeled anti-PprA antibody bound to PprA protein (Fig.4-15). This detection method of DNA strand breaks with increased sensitivity will be useful in evaluating radiation effect in mammalian cells, and widely applicable to genotoxic tests in environmental and pharmaceutical fields.

Reference

- Ohba, H. et al., The Radiation Responsive Promoter of the *Deinococcus radiodurans pprA* Gene, *Gene*, vol.363, no.9, 2005, p.133-141.
- Satoh, K. et al., Method for Detecting DNA Strand Breaks in Mammalian Cells using the *Deinococcus radiodurans* PprA Protein, *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, vol.596, no.1-2, 2006, p.36-42.