

102 Purification and Characterization of *Schizosaccharomyces pombe* Nth Homologue

Shin-Ichiro YONEKURA¹, Qiu-Mei ZHANG¹, Hiroshi SUGIYAMA², Shuji YONEI¹ (¹Labo. Radiat. Biol. Grad. Sch. Sci. Kyoto Univ.; ²Labo. Chem. Grad. Sch. Sci. Kyoto Univ)

8-oxoguanine (8-oxoG) is an abundant and critical base modification. It is excised from the DNA by MutM in *E. coli* and Ogg1 in *S. cerevisiae* and mammalian cells. However, any structural and functional homologs of them are not yet identified in *Schizosaccharomyces pombe*. Recently, Nth-Spo, an ortholog of *E. coli* Nth, was cloned from the *S. pombe*. It removes oxidative pyrimidines from the DNA. *E. coli* Nth and Nei remove 8-oxoG from the 8-oxoG:G mispairs in DNA. We examined whether the Nth-Spo functions as a DNA glycosylase/AP lyase for 8-oxoG in DNA. The Nth-Spo was expressed in *E. coli* and partially purified using glutathione affinity column chromatography. The DNA glycosylase activity was assayed for 8-oxoG:A, 8-oxoG:G or 8-oxoG:C containing duplex oligonucleotides. The Nth-Spo efficiently removed 8-oxoG from 8-oxoG:A and 8-oxoG:G pairs from DNA. The activity to remove 8-oxoG from 8-oxoG:A and 8-oxoG:G mispairs was much higher than that from 8-oxoG:C. Furthermore, we report about the effects of Nth-Spo expression in *mutMmutY* and *nthnei* mutants of *E. coli*.

103 Roles of LexA and LexA2 in the DNA Damage Response Mechanism of *Deinococcus radiodurans*

Katsuya SATOH¹, Hirofumi OHBA^{1,2}, Masahiro KIKUCHI¹, Issay NARUMI¹ (¹JAERI Takasaki; ²United Grad. Sch. Agric. Sci., Tokyo Univ. Agric. Tech.)

It has been suggested that *Deinococcus radiodurans* possesses a DNA damage response mechanism. However, little is known about the molecular basis for the control of the inducible proteins. We found that, like in *E. coli*, RecA is the sole protein required for LexA cleavage. We also found that the co-protease activity rather than recombination activity of RecA contributes to the high proficient DNA repair in *D. radiodurans*. Interestingly, it has been shown that the *D. radiodurans* genome encodes different two LexA homologues (LexA and LexA2). We analyzed the function of these genes by generating gene disruption strains. The *lexA2* disruptant strain exhibited much higher resistance than the wild-type, suggesting that DNA repair genes are down-regulated by LexA2. Furthermore, to gain insight into contributions of LexA and LexA2 in the DNA damage response mechanism, we examined the gene dosage effects of *lexA* and *lexA2*.

104 DNA Damage-Induced Phosphorylation of Histone H2AX and Alteration of Chromatin Structure

Keiji SUZUKI¹, Seiji KODAMA¹, Masami WATANABE¹ (¹Div. Radiat. Biol. Grad. Sch. Biomed. Sci. Nagasaki Univ.)

Ionizing radiation induces DNA double-strand breaks, which are the major cause of the detrimental effects of radiation. They activate a checkpoint protein, ATM, and it phosphorylates a variety of proteins to exert its functions. Histone H2AX, a member of the histone H2A family, is phosphorylated immediately after irradiation, and phosphorylated H2AX forms discrete foci. Because the number of foci corresponds to the number of DNA double strand breaks, it has generally been thought that the phosphorylation of H2AX is caused by DNA double strand breaks. Present study reveals phosphorylated histone H2AX foci on mitotic cells irradiated with X-rays. As expected, phosphorylated H2AX foci were found at the ends of chromosome fragments in metaphase cells. In addition, the foci were detected on chromosomal bridges between two sister nuclei in anaphase. Furthermore, we found duplicated phosphorylated H2AX foci exactly at the same sites on both sister chromatids 20 hours after irradiation. These results indicate that a change in higher-order chromatin structure, caused by DNA double-strand breaks, is involved in the phosphorylation of histone H2AX.

105 Effect of DNA Ligase IV Deficiency on Muagenesis with Allelic Losses in FM3A cells

Kouichi TATSUMI¹, Eiko KUBO¹, Yuko HOKI¹, Tomoko ICHIKAWA¹, Akira FUJIMORI¹, Masahiko MORI¹, Ryoko ARAKI¹, Ikuko FURUNO¹, Koki SATO², Masumi ABE¹ (¹Nat. Inst. Radiol. Sci.; ²Kinki Univ.)

In mammalian cells there are two major pathways for spontaneously arising and ionizing radiation (IR) induced DNA double strand breaks: homologous recombination (HR) and non-homologous end-joining (NHEJ). While a deficiency in any step of either pathway could render cells hypersensitive to IR, the resultant effect on mutations with allelic losses has not been fully understood. We measured spontaneous and X-ray induced mutagenesis with LOH in DNA ligase IV deficient SX10 cells and its parental SR-1 cells both heterozygously inactivated at the *aprt* locus. In SX10 cells, spontaneous mutation frequency was clearly higher than in the parental SR-1 cells. The mutation frequencies were not significantly elevated after X-irradiation in SX10, whereas a dose dependent increase was noted for SR-1.

106 Effects of Point Mutations in Ku80 Proteins on Ku-dependent DNA Repair

Manabu KOIKE¹, Aki KOIKE¹ (¹Natl. Inst. Radiol. Sci.)

Ku, a heterodimer of Ku70 and Ku80, plays a key role in multiple nuclear processes, e.g. DNA repair, chromosome maintenance, and transcription regulation. We generated cell lines expressing the human Ku80 tagged with the green fluorescent protein