67 Mutational Analysis of the *Escherichia coli* OxyR protein, the positive regulator for a hydrogen peroxide-inducible regulon

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The *oxyR* gene of *Escherichia coli* encodes a transcriptional activator protein for a regulon that is induced by oxidative stress. The OxyR protein is a bifunctional protein, acting as both a sensor of oxidative stress and a transcription activator of genes that respond to the stress. To locate the functional regions in OxyR important for stress sensing and transcription activation, we isolated *oxyR* mutants defective in transcription activation by random mutagenesis of the *oxyR* gene followed by *in vivo* screening for hydrogen peroxide sensitivity. We found three clusters of mutations in the coding region of the *oxyR* gene by DNA sequencing. The roles of these regions in stress sensing and transcription activation will be discussed.

68 Radiation-induced long life-time radical which causes genetical effects

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Many researcher studying in area of radiation biology have been believed that active short life-time radicals such as OH and H radicals, play an important role to express biological effects of radiations in cells, such as cell killing and mutation induction. However, we recently found a new type of radicals with long life-time in cells (T1/2>20hr) using ESR method and it may be more important in mutation induction than the active short-live radicals. When cells were treated with radical scavengers such as DMSO and vitamin C just before irradiation, short life-time radicals were scavenged well. However, if cells were treated with scavengers after irradiation, vitamin C scavenge the long life-time radicals, but DMSO did not. In addition, vitamin C treatment after irradiation drastically reduced mutation frequency at HPRT locus in human cells, but DMSO treatment did not. These results suggested that mutation is probably caused by the presence of radicals with a long lifetime in the cells, rather than short life-time radicals. Vitamin C reacts with long life-time radicals efficiently, resulting in the decrease of the mutation induction.

69 Induction of mutation in *supF* gene in *Escherhia coli* by treatment with riboflavin plus visible light.

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Photosensitized formation of 8-hydoroxyguanine(oh^{8}Gua) and the induction of mutation in *supF* gene in *Escherichia coli* gene were examined. Plasmid pUB3 derived from pZ189 was irradiated with visible light in the presence of riboflavin. Mutagenesis was determined by DNA sequencing. The mutations detected so far were exclusively the single base substitutions occurring at G:C pairs. Among them, 13.6% were G:C to A:T transition. 40.9% and 45.5% were G:C to T:A and G:C to C:G transversions respectively. The premutagenic lesion of G:C to T:A transversion may be oh^{8}Gua. G:C to C:G occurred at a similar extent to T:A change by unkown mechanism.