76 Identification of Eukaryotic Homologues of *Escherichia coli* MutY Protein

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7, 8-dihydro-8-oxoguanine (8-oxoG) is one of the major mutagenic base lesions caused by reactive oxygen species and ionizing radiation in DNA. 8-oxoG residues generate not only G:C-T:A but also G:C-C:G transversion mutations, because they can form a base pair with adenine and guanine as well as cytosine during DNA replication. In many prokaryotic and eukaryotic cells, excision activities of adenine and guanine mispaired with 8-oxoG are present to avoid the base substitutions. In this study, we attempted to identify a MutY homologue, an adenine DNA glycosylase activity in *Saccharomyces cerevisiae*. We screened the *S. cerevisiae* genomic cosmid library that suppress the spontaneous mutation frequency of *E. coli* CC104 *mutMmutY* double mutant. To these clones we extended the analysis of 8-oxoG-containing DNA cleavage assay and of borohydride-dependent trapping assay using crude extracts prepared from *S. cerevisiae*.

77 Identification of Repair Enzymes Recognizing 7,8-Dihydro-8-oxo-guanine Opposite Guanine or Adenine in *Escherichia coli*

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7,8-Dihydro-8-oxoguanine (8-ohG), produced by spontaneous oxidation, is the most important mutagenic lesion in DNA. This oxidized base induces GC-TA or AT-CG transversion mutation, because of mispairing with A during DNA repliucation. In *E. coli*, MutM, an 8-ohG-DNA glycosylase/AP lyase, removes 8-ohG preferably from 8-ohG:C pair in dsDNA, but cannot process 8-ohG of 8-ohG:A mispair. The A of 8-ohG:A mispair is repaired by *E. coli* MutY. Sodium borohydride-dependent cross-linking of 8-ohG-containing DNA oligonucleotide substrates with *E. coli* extract suggested that Nei (EndoVIII) and Nth (EndoIII) preferentially recognized 8-ohG of 8-ohG:A and 8-ohG:G mispairs. These enzymes process oxidized pyrimidines in DNA. The 8-ohG:A and 8-ohG:G mispair are arisen through mis-incorporation of 8-ohdGTP opposite A or G in the template during DNA replication. Like Ogg2 of *S. cerevisiae* (recently identified as an *E. coli* Nth homolog) or Ogg2 in human, the Nei and Nth may act in 8-ohG repair systems, preventing AT-CG (and probably GC-CG) transversions.

78 Purification and Characterization of 5-Formyluracil DNA Glycosylase from Human Cells in Culture

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5-Formyluracil is a major type of oxidatively modified thymine in DNA. The methyl group of thymine is vulnerable to hydroxyl radical attack and produces 5-hydroperoxymethyluracil, which is spontaneously decomposed to form 5-formyluracil and 5-hydroxymethyluracil. 5-Formyluracil is formed in yield comparable to thymine glycols and 8-hydroxyguanine by ionizing radiation and quinone-sensitized UV-A photo-oxidation. 5-Formyluracil is a potent mutagenic lesion leading T to G transversions. Our recent studies suggested that there is a DNA glycosylase activity to release 5-formyluracil from DNA as a free base in extracts prepared from rat and mouse tissues. Furthermore, we have detected the DNA glycosylase activity in extraxt from HeLa cells. In this study, we attempted to purify the 5-formyluracil DNA glycosylase of HeLa cells and determine the molecular characteritics and substrate specificity.