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## THE MECHANISM OF GENOME INSTABILITY INDUCED BY OXIDATIVE STRESS

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Genome stability is of crucial importance in all living organisms for the maintenance of cellular metabolism. The catastrophe of sustaining system of the genome stability may lead to cellular death or the induction of cancer in the case of humans. A typical example of the catastrophe of such system is the inactivation of mismatch repair (MMR), which results in high spontaneous mutation rates and increased cancer susceptibility. In fact, genome instability and deficit in MMR are properties of many tumor cells. MMR recognizes mismatched base pairs, such as G:T mismatch, generated during DNA replication and removes DNA stretch including the incorrect bases in non-template DNA strand for re-synthesis of DNA in the gap by DNA polymerases. MMR substantially reduces the spontaneous mutation rates and plays an important role in the genome maintenance. However, the job assignment of MMR may be much severer than previously thoughts. Recently, we (ISS, Italy) have found that the expression of human MTH1 that degrades 8-OH-dGTP significantly reduces the mutator phenotypes of MMR deficient cells and suggested that MMR somehow eliminates oxidized bases in DNA incorporated from nucleotide pool (dNTP pool) (Current Biol., 12, 912, 2002). The presence of MTH1 reduces the mutations in all classes including frameshifts and those that may be induced by 2-OH-dATP (Mol. Cell. Biol., 24, 465-474, 2004). These oxidized dNTPs are induced by reactive oxygen species (ROS) generated during normal aerobic metabolism. The group at the NIHS (Japan) has been working on a novel class of DNA polymerase named Y-family. This class of polymerases is robust in bypassing DNA lesions with or without base incorporation errors and thus plays an important role in genome stability (tolerance to DNA damage) and instability (induction of mutations). An interesting feature of this class of polymerase is the distinct specificity incorporating oxidized dNTPs (EMBO Reports, 4, 269-273, 2003). Y-family DNA polymerases almost exclusively incorporates 8-OH-dGTP opposite template adenine (A) and 2-OH-dATP opposite template guanine (G) and thymine (T). Incorporation of 8-OH-dGTP opposite template A may result in the induction of A:T to C:G transversions, which are more than 1,000 times higher in *E. coli* cells deficient in MutT, a bacterial counterpart of human MTH1. 2-OH-dATP induces G:C to T:A transversions, which can be induced when it is incorporated opposite template G. In contrast, Klenow enzyme (A family) and *Sso* DNA pol B1 (B family) from *Sulfolobus solfataricus* evenly incorporate 8-OH-dGTP opposite template C and A, and incorporate 2-OH-dATP opposite template T. The goal of collaborative work is to clarify the roles of MMR and Y-family DNA polymerases in the genome stability and instability via elimination and incorporation of oxidized dNTPs into DNA. The Japanese partners will conduct in vitro forward mutation assays using gapped M13mp2 DNA and determine the mutation spectra generated by the incorporation of oxidized dNTPs (8-OH-dGTP and 2-OH-dATP) by Y-family DNA polymerases (pol □ and □). The spectra will be compared with those generated in the ISS using MMR deficient mammalian cells. The similarity and difference of the spectra may shed light on the possible involvement of Y-family DNA polymerases in the genome instability via oxidation of nucleotide pool. In the ISS the expression of Y-family DNA polymerases will be shut down using siRNA technique and the effects on the mutator phenotypes in MMR deficient cells will be examined.