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The partial reconstitution of DNA mismatch repair in *Thermus thermophilus* HB8

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Many DNA damages are not only generated by exogenous chemical or physical agents but also occur during normal DNA metabolism such as DNA replication, recombination, and repair. Instead of the high fidelity of the DNA polymerase mainly involved in DNA replication, misincorporation of nucleotides by DNA polymerases is unavoidable error to result in base-base mismatches. DNA mismatch repair (MMR) is a highly conserved biological pathway to maintain the genomic stability. In MMR pathway, many proteins are reported to participate in each step: MutS and MutL recognize a mismatched base and distinguish between templating strand and newly synthesized one, helicase (UvrD) unwinds the DNA strand containing a mismatched base, single-stranded DNA (ssDNA) specific exonucleases degrade unwound ssDNA, DNA polymerase synthesizes correct strand (Fig. 1). We have studied MMR proteins from *Thermus thermophilus* HB8, which can live at the highest temperature (> 75˚C) and has the smallest genome size (about 2,000 gene) in the organisms established the methods of genetic engineering. Because MMR pathway of *T. thermophilus* HB8 is eukaryote type (in other words, the type does not have MutL nickase), the study about MMR in *T. thermophilus* HB8 could contribute to give the archetype of MMR in human and other eukaryote. We focused on the strand removal step in MMR pathway and reported a novel ssDNA specific exonuclease involved in this step [1]. In this study, we reported the MMR proteins participating in the strand removal step in detail and the partial reconstitution of MMR pathway.

We overexpressed and purified MMR proteins to homogeneity. To investigate the effect of MMR proteins on the recognition of mismatched base by MutS, we performed the gel mobility shift assay using the 120 mer double-stranded DNA whose both 3' ends were blocked by biotin-streptavidin complex. The result showed that MutS binds on DNA more strongly with clamp loaders and β clamp than without the proteins. Furthermore, we showed that the activity efficiency of UvrD from *T. thermophilus* was different from that of UvrDs have reported. We will introduce the results in detail at this symposium.

[1] Atsuhiro S., Ryoji M., Noriko N., Yoshio T., Kwang K., Seiki K., and Kenji F. (2010) *Nucl. Acids Res.*, (In press)

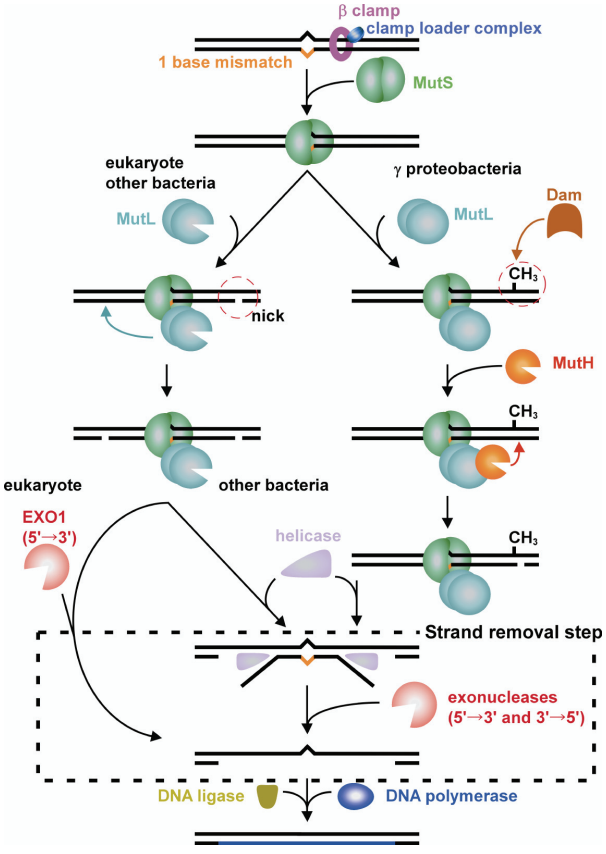

Figure 1. MMR pathways