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Functional analysis of an alkyltransferase-like protein that involved in a novel nucleotide excision repair system.

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A great amount of DNA damage arises from both endogenous and exogenous DNA-damaging agents, for example, alkylating agents. The major damage to DNA caused by alkylating agents involves the formation of O^6-alkylguanine. O^6-methylguanine (O^6-meG) is one such lesion with potent toxicity and potential for mutagenesis. Because O^6-meG forms hydrogen bonds with thymine in preference to cytosine, this lesion usually leads to GC-to-AT transition mutation.

Almost all species possess O^6-methylguanine-DNA methyltransferase (Ogt) to repair such damage. Ogt repairs O^6-meG lesions in DNA by stoichiometric transfer of the methyl group to a cysteine residue in its active site (PCHR). *Thermus thermophilus* HB8 has an Ogt homologue, TTHA1564, but in this case an alanine residue replaces the catalytic cysteine in the putative active site. To reveal the possible function of TTHA1564 in processing O^6-meG-containing DNA, we characterized the biochemical properties of TTHA1564. No methyltransferase activity for synthetic O^6-meG-containing DNA could be detected, indicating TTHA1564 is an alkyltransferase-like (ATL) protein. Nevertheless, gel shift assays showed that TTHA1564 can bind to DNA containing O^6-meG with higher affinity (9-fold) than normal (unmethylated) DNA. Experiments using a fluorescent oligonucleotide suggested that TTHA1564 recognizes O^6-meG in DNA with nucleotide flipping, the same mechanism as other Ogts. We then investigated whether TTHA1564 functions as a damage sensor. Pull-down assays identified 20 proteins, including a nucleotide excision repair (NER) protein UvrA and RNA polymerase, which interacts with TTHA1564. Interaction of TTHA1564 with UvrA was confirmed using a surface plasmon resonance assay. These results suggest the possible involvement of TTHA1564 in DNA repair pathways (Figure 1).

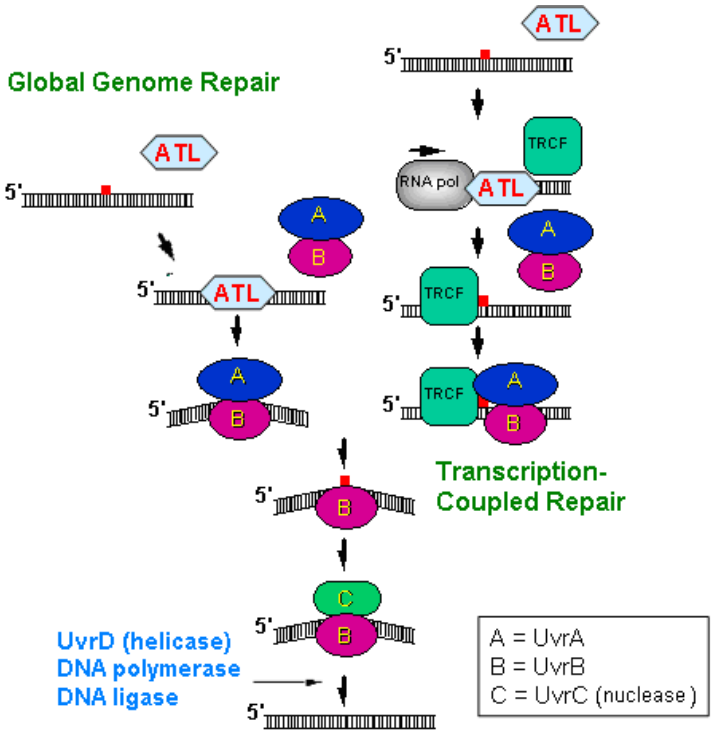

Figure 1: Possible participation of ATL in NER responses

Reference

[1] Morita R., Nakagawa N., Kuramitsu. S. and Masui R. (2008) *J. Biochem*, 144, 267-277