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Functional analysis of an alkyltransferase-like protein by DNA microarray

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O6-methylguanine (O6-meG) is a potentially mutagenic and toxic lesion in DNA. O6-meG is caused by certain classes of carcinogenic and chemotherapeutic alkylating agents. Repair process of this lesion is thus notable in both the etiology and treatment of cancer. It is well known that the biological effects of such alkylating agents can be extensively prevented by the highly conserved DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT). AGT repairs O6-meG damage in DNA by the stoichiometric and auto-inactivating transfer of the methyl group to a cysteine residue in its active site (PCHRV). Sequence alignment analysis shows the existence of a group of alkyltransferase homologues in which the cysteine residue in the putative active site has been replaced with other residues. These proteins are defined as alkyltransferase-like (ATL) proteins. *Thermus thermophilus* HB8 has only ATL protein (TTHA1564) and lacks AGT. In organisms that have only ATL protein and lacks AGT, the repair pathway of alkylated lesion is unknown because ATL protein has no cysteine residue needed to transfer the methyl group of O6-meG. Therefore, we expect that a novel pathway exists for repair of alkylated lesion. It is suggested that TTHA1564 recognizes O6-meG in DNA and interacts with some proteins (Table 1). Based on these results, we made a hypothesis that ATL protein initially recognizes O6-meG in DNA as a damage sensor, and then recruits other proteins involved in repair of alkylated lesion. In order to verify this hypothesis, we performed DNA microarray to investigate gene expression pattern of wild-type and TTHA1564 disruptant in the presence of an alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine. In this poster, we discuss the result of DNA microarray analysis.

Table 1. **Summary of proteins interacted with TTHA1564.**

Gene number	Gene ID	Protein name	Molecular mass (kDa)	Peptides matched
TTHA1812	YP_145078	RNA polymerase β′ chain (RpoC)	170.7	29
TTHA1813	YP_145079	RNA polymerase β chain (RpoB)	125.2	20
TTHA1440	YP_144706	Excinuclease ABC subunit A (UvrA)	105.1	10
TTHA1355	YP_144621	DNA gyrase subunit A	89.1	8
TTHA1139	YP_144405	Polynucleotide phosphorylase	78.1	17
TTHA1097	YP_144363	DNA ligase [NAD^+]	76.9	9
TTHB068	YP_145307	Hypothetical protein	76.6	10
TTHA1634	YP_144900	Peptide ABC transporter, peptide-binding protein	70.4	9
TTHA1329	YP_144595	Glutamine synthetase	50.5	14
TTHA0196	YP_143462	Transporter, periplasmic component	40.3	5
TTHA1664	YP_144930	DNA-directed RNA polymerase α chain (RpoA)	35.0	6
TTHB045	YP_145284	Repeat motif-containing protein	31.9	5
TTHA0244	YP_143510	Single-stranded DNA-binding protein (SSB)	29.8	8
TTHA0081	YP_143347	Conserved hypothetical protein	24.3	4
TTHA1657	YP_144923	AT-rich DNA-binding protein	23.2	6
TTHA0175	YP_143441	Cold shock protein	8.2	4
Proteins below were not significant.				
TTHA0506	YP_143772	Malate synthase	58.7	7
TTHA1427	YP_144693	UvrD helicase	57.4	7
TTIIA1153	YP_144419	Mercuric reductase	48.5	4
TTHA0718	YP_143984	Uracil-DNA glycosylase A (UDGA)	23.0	5
TTHA1349	YP_144615	DNA-binding protein HU	10.4	3

These proteins were identified by peptide mass fingerprinting with MALDI-TOF MS.

Reference

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