DNA REPAIR 6 (2007) 1222–1228

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/dnarepair

Alkyltransferase-like proteins

Geoffrey P. Margison[a,*], *Amna Butt*[a], *Steven J. Pearson*[a], *Stephen Wharton*[a], *Amanda J. Watson*[a], *Andrew Marriott*[a], *Cátia M.P.F. Caetano*[a], *Jeffrey J. Hollins*[a], *Natalia Rukazenkova*[a], *Ghazala Begum*[a], *Mauro F. Santibáñez-Koref*[b]

[a] *Cancer Research-UK Carcinogenesis Group, Paterson Institute for Cancer Research, University of Manchester, Manchester M20 4BX, United Kingdom*
[b] *Institute of Human Genetics, University of Newcastle, Newcastle-upon-Tyne, United Kingdom*

ARTICLE INFO

Article history:
Published on line 17 May 2007

Keywords:
O^6-Alkylguanine
Alkylating agent
DNA alkylation
AGT
MNNG
Epistasis

ABSTRACT

Recent *in silico* analysis has revealed the presence of a group of proteins in pro and lower eukaryotes, but not in Man, that show extensive amino acid sequence similarity to known O^6-alkylguanine-DNA alkyltransferases, but where the cysteine at the putative active site is replaced by another residue, usually tryptophan. Here we review recent work on these proteins, which we designate as alkyltransferase-like (ATL) proteins, and consider their mechanism of action and role in protecting the host organisms against the biological effects of O^6-alkylating agents, and their evolution.

ATL proteins from *Escherichia coli* (eAtl, transcribed from the *ybaz* open reading frame) and *Schizosaccharomyces pombe* (Atl1) are able to bind to a range of O^6-alkylguanine residues in DNA and to reversibly inhibit the action of the human alkyltransferase (MGMT) upon these substrates. Isolated proteins were not able to remove the methyl group in O^6-methylguanine-containing DNA or oligonucleotides, neither did they display glycosylase or endonuclease activity. *S. pombe* does not contain a functional alkyltransferase and *atl1* inactivation sensitises this organism to a variety of alkylating agents, suggesting that Atl1 acts by binding to O^6-alkylguanine lesions and signalling them for processing by other DNA repair pathways. Currently we cannot exclude the possibility that ATL proteins arose through independent mutation of the alkyltransferase gene in different organisms. However, analyses of the proteins from *E. coli* and *S. pombe*, are consistent with a common function.

1. Introduction

The alkylating agents are a large family of structurally diverse compounds that elicit a wide range of biological effects in living organisms. Alkylating agents interact with cellular macromolecules to form covalent addition products ("adducts"). For DNA, it is well established that damage can occur at all of the available nitrogen and oxygen atoms in DNA bases, and also at the phosphodiester linkages, resulting in more than a dozen different adducts. These are generated in widely varying amounts depending on the reactivity of the interacting species. The biological effect of alkylating agents, which include mutagenesis (leading to both point mutations and recombinational events) and toxicity [1–3] have been attributed to specific adducts. Indeed, alkyl adducts in DNA have widely different biological and genotoxic properties and potencies. For many types of lesions, DNA repair processes have evolved, presumably to protect organisms against

* *Corresponding author*. Tel.: +44 161 446 3183; fax: +44 161 446 8306.
E-mail address: gmargison@picr.man.ac.uk (G.P. Margison).
1568-7864/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2007.03.014

the potentially adverse biological effects (see recent reviews [4,5]) and, perhaps not surprisingly, such repair processes are highly conserved.

The most abundant DNA lesion, N7-methylguanine (N7-meG) is not directly mutagenic or cytotoxic [6] whereas the less abundant N3-methyladenine lesions are cytotoxic [7]. O^6-meG lesions are mutagenic: DNA polymerases usually read them as adenine residues and they typically cause GC–AT transition mutations following two rounds of DNA replication [2]. O^6-meG is also recombinogenic and can cause cell death and both of these effects are mediated by the postreplication mismatch repair (MMR) system [2]. Cells lacking functional MMR are relatively resistant to the toxic and recombinogenic effects of O^6-alkylating agents but hyper-sensitive to their mutagenic effects [8].

In pro and eukaryotes the first line of defence against the adverse effects of O^6-alkylating agents is provided by alkyltransferases. These were first recognized in *Escherichia coli* as part of the adaptive response to low doses of the methylating agent MNNG [9]. Subsequent studies elaborated the mechanism of this effect and the involvement of two different alkyltransferase functions of a single protein, Ada. Later, a second alkyltransferase, Ogt, with extensive sequence homology to the carboxy-terminal region of the ada protein, and with overlapping specificities was identified. Alkyltransferase activities were subsequently reported in other prokaryotes and mammalia.

The biological impact of alkyltransferases is best revealed in studies involving overexpression, usually in alkyltransferase-deficient systems, or attenuation of expression, by gene deletion or the use of specific inactivating drugs. These almost invariably show a decrease or increase, respectively, in the genotoxic effects including point mutations, recombination and toxicity, of agents that alkylate the O^6-position of G in DNA.

The most conserved regions of the alkyltransferase proteins are shown in the upper part of Fig. 1. They include the active site region encompassing the catalytic cysteine residue and the residues involved in alkyl group transfer, including an arginine that flips the damaged guanine residue out of the major groove and into the active site pocket [10].

2. Alkyltransferase-like proteins

The functional activity of alkyltransferases is often demonstrated and quantitated by the transfer of methyl groups from O^6-methylguanine in radiolabelled methylated DNA or oligonucleotides to the active site cysteine residue in an autoinactivating stoichiometric reaction. However, analysis of sequence databases reveals proteins, which we have collectively designated as ATL proteins, in which the putative active site cysteine residue is replaced by another residues [11–13]. In the Conserved Domain Database [14] these proteins share a domain designated as COG 3695 (predicted methylated DNA-protein cysteine methyltransferase) although this group also includes some proteins with cysteine at the active site. So far, in most sequences the active site cysteine is substituted by tryptophan, but as can be seen in Fig. 1, other residues can be encountered at this location and other residues in the PCHR motif can also be substituted.

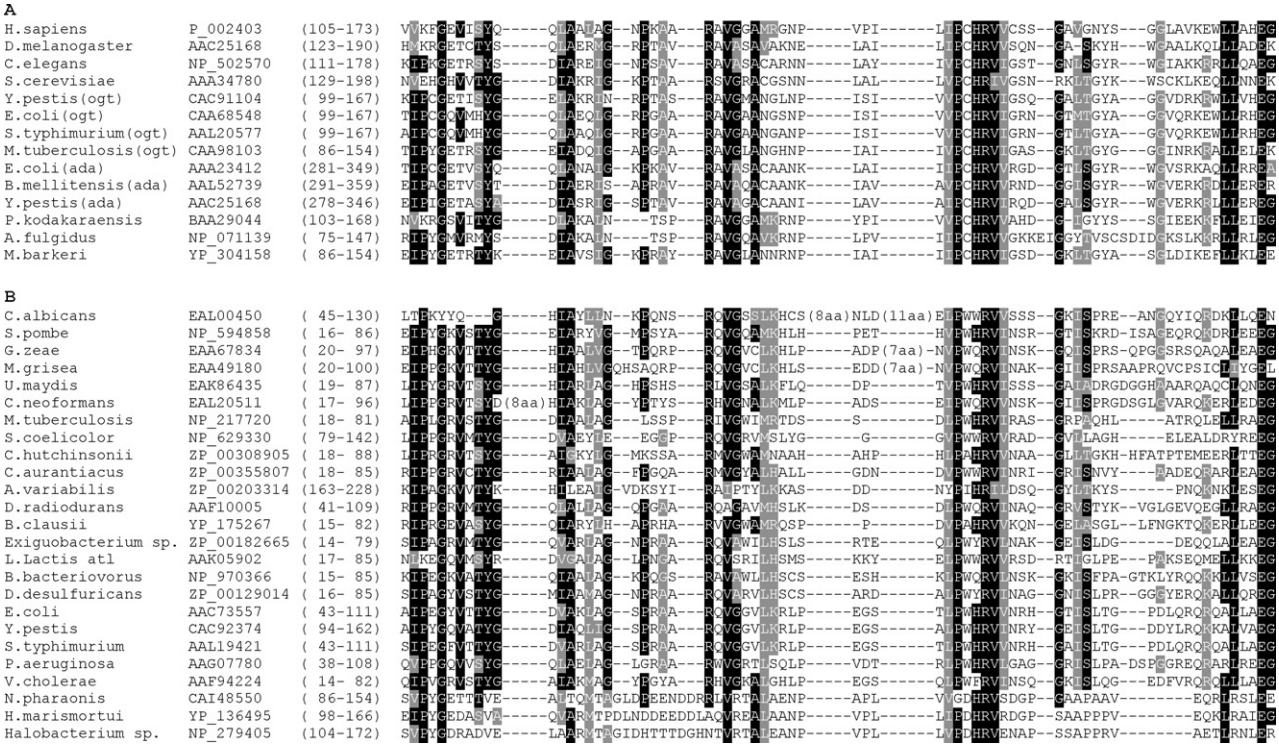

Fig. 1 – Alignment of selected MGMT and ATL sequences of the regions surrounding the putative PCHR motif or its homologues. Alignments were contructed using MUSCLE [19] and visualised using BOXSHADE (http://www.ch.embnet.org/software/BOX_form.html).