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REPAIR OF METHYL LESIONS IN DNA AND RNA BY OXIDATIVE DEMETHYLATION

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Abstract—It was established several decades ago that it is crucial for all organisms to repair their DNA to maintain genome integrity and numerous proteins are dedicated to this purpose. However, it is becoming increasingly clear that it is also important to prevent and repair lesions in the macromolecules encoded by the DNA, i.e. RNA and protein.

Many neurological disorders such as Alzheimer's disease and Parkinson's disease are associated with the aggregation of defective, misfolded proteins, and several mechanisms exist to prevent such aggregation, both through direct protein repair and through the elimination and repair of faulty or damaged RNAs. A few years ago, it was discovered that the *E. coli* AlkB protein represented an iron and 2-oxoglutarate dependent oxygenase capable of repairing methyl lesions in DNA by a novel mechanism, termed oxidative demethylation. Furthermore, it was found that both human and bacterial AlkB proteins were able to demethylate lesions also in RNA, thus representing the first example of RNA repair. In the present review, recent findings on the AlkB mechanism, as well as on RNA damage in general, will be discussed.

Key words: AlkB proteins, iron, 2-oxoglutarate, oxygenase, macromolecular damage.

All cellular macromolecules constantly run the risk of becoming damaged by various agents of exogenous or endogenous origin. Basically, such damage can be dealt actively with in two ways; either the damage is repaired or, alternatively, the damaged molecule is subjected to a specific degradation process. In the case of DNA, degradation is evidently not a real alternative, since, in most situations, no backup copy of this molecule exists. Thus, DNA repair is highly prioritized in the cell and numerous mechanisms exist to repair a wide range of different DNA lesions. However, when DNA damage is so extensive that it cannot be repaired prior to cell division, apoptosis (programmed cell death) is induced. DNA is then fragmented and the constituents of the cell disposed of in a controlled manner, thus minimizing damage to the organism as such.

In contrast, RNA and protein molecules can easily be replenished by new synthesis based on the information in DNA, and degradation of faulty and damaged molecules occurs continuously within the cell. However, repair mechanisms exist also for the removal of damage from these macromolecules, and a relevant question is the following: What determines whether degradation or repair is used as the strategy for removing a specific lesion? First, these two strategies may not be mutually exclusive; some lesions may be targets for both mechanisms. Second, it may also be a matter of "cellular economy"; if the damage is frequent enough it may be preferable to invest in a specialized repair system. Third, chemistry may also play a significant role; some lesions may not be easily reversed, and it may be impossible to evolve a repair function based on the repertoire of enzymes already present in the cell.

The main focus of this review will be the AlkB mechanism. This repair function was initially found to be involved in DNA repair, but has later been shown to represent the first mechanism for reversing RNA damage. Furthermore, several mammalian AlkB homologues of unknown function are likely to represent completely novel cellular functions.

THE AlkB MECHANISM FOR DNA REPAIR: AN UPDATE AND FURTHER PERSPECTIVES

Repair of alkylation damage in DNA

Methylations and other alkylations of the base moieties pose a serious threat to the integrity of the genetic information stored in DNA. Substantial amounts of alkylating agents are generated both in the environment and inside cells, and the majority of N- and O-atoms of the nucleobases are subject to attack by such agents, leading to the formation of harmful lesions (Singer and Grunberger, 1983). A subset of these lesions abrogates the ability of the bases to participate in Watson-Crick base pairing, and thus block replication and transcription; processes that depend on appropriate pairing between a DNA template and an incoming nucleotide. Such blockage will lead to cell death, and these lesions are therefore referred to as cytotoxic. Other lesions may cause mutagenesis by allowing the formation of non-cognate base pairs, and are designated premutagenic lesions.

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Abbreviations: εA, 1,N^6-ethenoadenine; ABH, mammalian AlkB homologue; BER, base excision repair; εC, 3,N^4-ethenocytosine; ds, double-stranded; Fe^{2+}, ferrous iron; hABH, human AlkB homologue; JHDM, JmjC domain-containing histone demethylase; LSD1, lysine specific demethylase 1; mABH, mouse AlkB homologue; MMS, methyl methanesulfonate; NMD, nonsense-mediated decay; ss, single-stranded; 1-meA, 1-methyladenine; 1-meG, 1-methylguanine; 2-OG, 2-oxoglutarate; 3-etC, 3-ethylcytosine; 3-meC, 3-methylcytosine; 3-meT, 3-methylthymine.

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Due to the harmful consequences of alkylation damage in DNA, cells harbor repair systems for handling all significant methyl lesions on the DNA bases. These repair mechanisms were initially discovered in the bacterium *Escherichia coli*, where mutants with increased sensitivity toward alkylating agents were identified, and the affected genes were shown to encode repair proteins. In *E. coli*, the adaptive response to alkylation damage involves the induction of three different repair proteins, AlkA, Ada, and AlkB, (Fig. 1) upon cellular exposure to alkylating agents (reviewed by Sedgwick and Lindahl, 2002). AlkA is an alkylbase DNA glycosylase, i.e. it catalyzes the excision of certain methylated bases, e.g. 3-methyladenine, from DNA (Evensen and Seeberg, 1982; Karran et al., 1982). Ada is a transcription factor governing the adaptive response, but it also is a DNA alkyltransferase repairing O-methylated bases, such as O^6-methylguanine, by a suicide reaction where the deleterious methyl group is transferred to a Cys residue within the transferase itself (Karran et al., 1979). Thus, an entire Ada protein is killed for the removal of a single methyl lesion. Finally, AlkB was recently shown to be an oxidative demethylase capable of reversing methyl lesions not repaired by the two other mechanisms (see below). Functional homologues of all these three proteins are present in a number of different organisms, including humans, demonstrating that repairing DNA alkylations is an important and fundamental cellular function.

The AlkB function: history and discovery

Although it was reported more than two decades ago that mutations in the *alkB* locus of *E. coli* caused increased cellular sensitivity toward certain methylating agents (Kataoka et al., 1983), the actual biological function of the AlkB protein was unraveled only recently (Falnes et al., 2002; Trewick et al., 2002). So, why did it take so long to identify this function? First, unlike most other DNA repair proteins, AlkB was found to depend on specific cofactors to exert its action. Clues about this were provided when a bioinformatics study placed AlkB in the superfamily of 2-oxoglutarate (2-OG) and iron (Fe^{2+}) dependent oxygenases, and the proteins in the AlkB family were predicted to contain conserved amino acid residues involved in binding the two cofactors 2-OG (a.k.a. α-ketoglutarate) and Fe^{2+} (Aravind and Koonin, 2001). Second, while most DNA lesions are introduced at similar frequencies in dou-

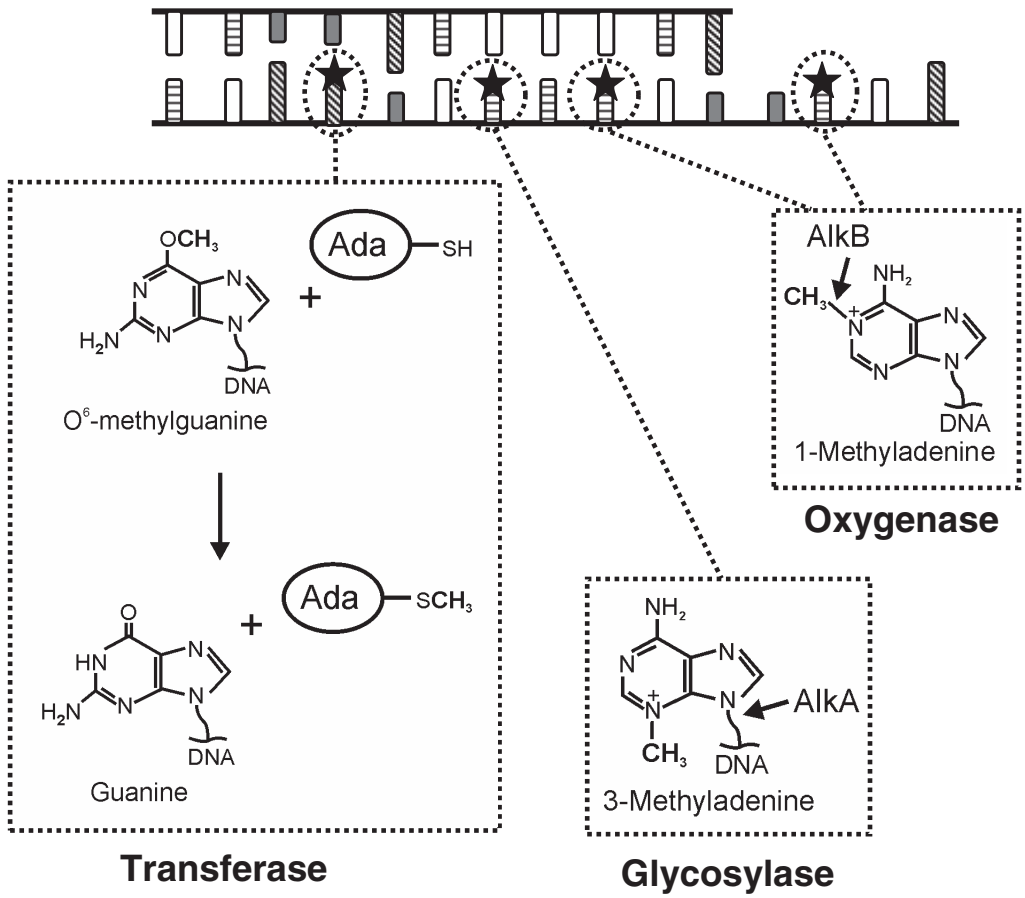

Fig. 1. Mechanisms for removing alkylation damage, exemplified by *E. coli* proteins. Ada and other DNA alkyl transferases exert their action by transferring the deleterious alkyl group in O-alkylated bases, e.g. O^6-methylguanine, to a Cys residue in the transferase, thereby reversing the lesion. Alkylbase DNA glycosylases, such as AlkA, cleave off the damaged base, thus leaving an abasic site to be repaired by other proteins. AlkB proteins are oxygenases which mediate direct demethylation of lesions that are preferentially introduced in ssDNA, but that can be removed from both ssDNA and dsDNA.