DNA Repair

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Once Overlooked, Now Made Visible: ATL Proteins and DNA Repair**

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The DNA of a cell is continuously exposed to numerous endogenous and exogenous factors. The resulting DNA damage can lead to mutations or cell death.[1] Some of the major DNA lesions are generated by the reaction of alkylating reagents with DNA bases. The alkylated reaction products can arise endogenously from cellular alkylating reagents as *S*-adenosylmethionine or from the influence of exogenous factors (e.g. environmental stress). In cancer therapy, alkylating agents are also used to damage the DNA of tumor cells, resulting in various alkylated bases. In addition to the N^7 position of guanine and adenine, the O^6 position of guanine and the O^4 position of thymine are susceptible to alkylation. It is known that O^6-methylguanine pairs with thymine during replication, resulting in a G·C to A·T transition mutation.[2]

Currently three different repair mechanisms are known for the removal of alkylated bases. Oxidative dealkylation by the oxygenase AlkB family directly removes the alkyl group at the base.[3] The alkyl group is oxidized and eliminated. Another mechanism is base-excision repair by glycosylases,[4] in which the damaged base is removed from the DNA strand. Most cells, however, repair O^6-alkylguanine damage mainly with O^6-**a**lkyl**g**uanine-DNA **t**ransferases (AGTs). These proteins demethylate O^6-methylguanine and O^4-methylthymine using an active cysteine residue.[5,6] AGTs transfer the alkyl group in an S_N2 active site reaction to this cysteine, which results in an irreversibly alkylated enzyme ("suicide" enzyme, Figure 1). Crystal structures exist of human AGT in complex with O^6-methylguanine containing double-stranded DNA.[7] AGT has a helix–turn–helix (HTH) motif, which binds to DNA through interactions with the minor groove. Protein binding expands the minor groove of the DNA, and an arginine finger intercalates into the helix. As with DNA photolyases,[8] the alkylated base flips out into the catalytic pocket of the protein (base flipping). In addition, this flipping of the lesion out of the DNA duplex is supported by a conserved tyrosine residue, which induces the rotation of the 3′-phosphate backbone of the alkylated base through charge repulsion and steric hindrance. Moreover, an arginine forms a hydrogen bond to the remaining unpaired cytosine in the DNA double helix. This interaction is essential in stabilizing the flipped-out nucleotides.

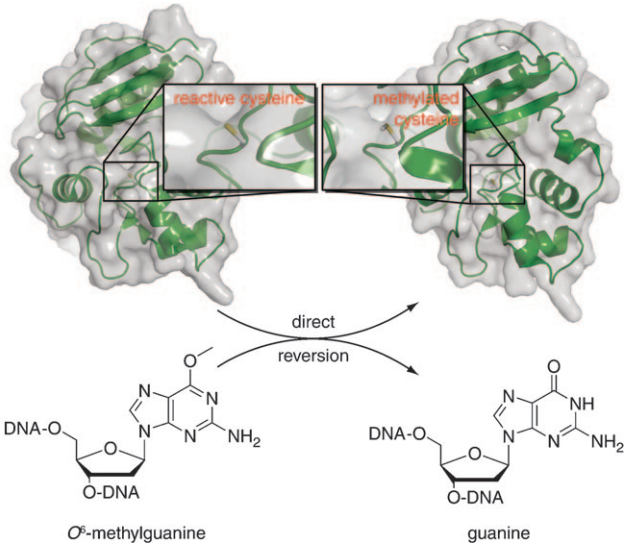

Figure 1. Representation of suicide repair by O^6-alkylguanine-DNA transferase (AGT).

The repair of alkylated bases by AGTs plays a major role in cancer therapy. Various chemotherapeutic agents such as Procarbazine, Dacarbazine, and Temozolomide, and the pharmaceutical group of nitrosourea compounds act as alkylating agents (Scheme 1). Repair of alkylated guanines in DNA by AGTs leads to the resistance of tumor cells against the alkylating drugs. Therefore, AGT inhibitors would be necessary to improve chemotherapy with these drugs in order to prevent the rapid development of resistance.[9,10]

In early 2003 Margison and co-workers discovered a new class of proteins from prokaryotes and lower eukaryotes that show sequence similarities to the AGTs in their DNA-binding domain. These proteins were termed **a**lkyl**t**ransferase-**l**ike proteins (ATLs).[11] Analogous to the actions of AGTs, here the damaged nucleotide is also recognized and flipped out of

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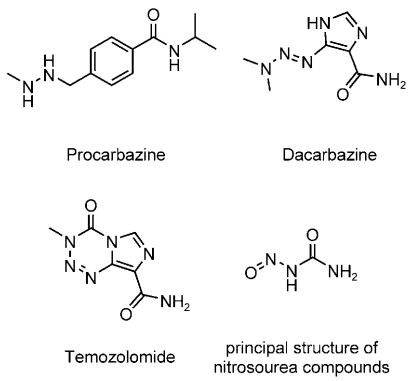

Scheme 1. Alkylating chemotherapeutic agents.

the DNA helix. The ATLs, however, differ from the AGTs in some characteristic properties:[12–16]

- Despite the significant sequence homology, a tryptophan or an alanine is usually found in the active center of these novel ATLs instead of the active cysteine.
- As this cysteine group is lacking, alkyl transferase activity is not observed, that is, no direct repair of the DNA lesion takes place. A mutation of tryptophan to cysteine does not restore the alkyltransferase activity, which shows that the ATL proteins cannot develop repair activity.
- Moreover, neither glycosylase nor endonuclease activity could be detected as an alternative repair mechanism.
- ATLs reversibly inhibit the activity of the AGT repair enzymes because they bind specifically to the alkyl DNA damage.
- ATL gene inactivation leads to an increased sensitivity to DNA-alkylating agents, that is, ATLs protect the organism against the biological consequences of DNA alkylation.

Tainer and co-workers now investigated the mechanism of damage detection by ATLs and their connection to the general nucleotide excision repair (NER), which removes typically bulky adducts from the genome of organisms.[17]

To obtain information about the structure and mode of action of the ATLs they crystallized Atl1 from *Schizosaccharomyces pombe* in complex with different oligonucleotides. Both O^6-methylguanine (O^6-mG) and the toxicologically relevant adduct O^6-4-(3-pyridyl)-4-oxobutylguanine (O^6-pobG) were used as DNA lesions. Tainer and co-workers found that the DNA binding domain of Atl1 shares great structural similarities with human AGT. In the active center the residues responsible for DNA binding and nucleotide flipping are conserved. However, Atl1 lacks the reactive cysteine for the direct reversion of alkyl DNA damage. As expected, the reactive cysteine in Atl1 is replaced by a tryptophan, which stabilizes the flipped-out base through hydrophobic interactions. In complex with Atl1 the O^6-mG or O^6-pobG lesion is rotated into the active center with the typical sequence motif PWHRV. An arginine forms a hydrogen bond with the unpaired cytosine and additionally stabilizes the flipped-out alkyl guanine. The Atl1 binding pocket for alkylated nucleotides is approximately three times larger than that of AGT. This explains the diversity of damage detected by Atl1, for example, O^6-benzyl-, O^6-(4-bromothenyl)-, or O^6-hydroxyethylguanine. The promiscuity of the binding pocket is amazing. It is interesting that there is not sufficient space in the active site pocket of *E. coli* AGTs (Ada and Ogt) for the flipped-out O^6-pobG. This suggests that ATLs are required for the repair of bulky adducts in organisms such as *E. coli*. The DNA is bound by Atl1, in analogy to its binding with AGT, through a helix–turn–helix motif. Tainer and co-workers also verified more DNA–protein contacts, which explains the strong affinity for alkyl-damaged bases. In addition, the DNA is bent by approximately 45°. In particular the N-terminal helix supports the strong bending of DNA by Atl1.

Flipping of damaged bases is a process widely used by many nucleotide-modifying enzymes (e.g. DNA-repair enzymes or RNA-modifying enzymes). All of these enzymes associate a catalytic function with the flipping process, with the consequence of modifying or eliminating the flipped-out base. Despite forming flipped-out bases, Atl1 shows no enzymatic activity for cleavage. This is remarkable: why should a protein flip out a damaged base if no chemical processes follow? This lack of a catalytic function suggests that the flipping of the nucleotide may act as switch to activate other repair systems. For example O^6-meG does not cause a transcription stop. Therefore, Atl1 could act as recognition protein that binds to this lesion, and thereby blocks transcription and allows repair (Figure 2). Tainer and co-workers demonstrated a direct correlation between ATLs and the NER system in interaction studies with various NER proteins. NER is a general repair process in which a damaged DNA segment is completely cut out and the resulting single-strand gap is filled with the correct nucleotides. *E. coli* Atl, for example, interacts with *E. coli* UvrA and UvrC, proteins involved in the NER mechanism. Biochemical interactions

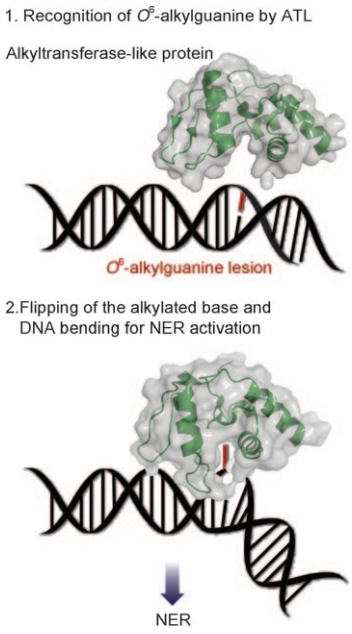

Figure 2. Recognition of O^6-alkylguanine lesion by ATLs. The resulting DNA–protein complex activates nucleotide excision repair (NER) by strong bending of the DNA and flipping of the alkylated base.

between *S. pombe* Atl1 and *S. pombe* Rad13 have also been clearly demonstrated. Presumably, ATL is actually a detection unit for lesions and introduces these lesions to the repair by NER proteins. This is, in addition to the already known repair mechanisms, a new way to repair alkyl-damaged bases.

But there is still no evidence that ATLs also play a role in general damage detection in higher organisms, although they were able to detect the first ATLs in a multicellular organism (the sea anemone *Nematostella vectensis*). ATLs in humans have not been found yet. Anyhow, the role of ATL proteins is becoming clear. It seems that many minor damages are overlooked during replication or transcription which leads to mutations.[18] ATL proteins bind to these damaged bases and flip them out. This process makes the otherwise "invisible" damages visible to repair systems such as NER.

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