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DNA repair without flipping out

Time-resolved molecular snapshots of the bacterial enzyme AlkD reveal an unprecedented mechanism for the recognition and removal of damaged bases in DNA, with implications for cell biology and cancer therapy.

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Many people imagine DNA as a highly stable blueprint that allows offspring to inherit the traits of their parents. However, DNA is a dynamic molecule that is not only read, replicated and modified by various cellular factors, but is also constantly being damaged. DNA repair mechanisms are crucial for maintaining sufficient sequence fidelity for cell survival and disease prevention. In October 2015, Tomas Lindahl, Paul Modrich and Aziz Sancar shared the Nobel Prize in Chemistry for their descriptions of DNA repair processes, which all involve enzymatic opening of the DNA double helix and unpairing of one or more DNA bases. In a paper online in *Nature*, Mullins *et al.*[1] describe a new class of DNA base-repair enzyme that unexpectedly leaves the double helix largely intact during the first step of damage recognition and removal.

Every human cell contains around 3 billion nucleotides — each consisting of one of four bases, along with a sugar and a phosphate molecule — that join together to form DNA. This structure was assumed to be unusually stable until Lindahl challenged this idea when he found that DNA was subject to decay (see Lindahl's review[2]). DNA can be damaged by environmental effects, such as ionizing radiation, ultraviolet light and chemicals, and by internal sources, such as oxygen radicals, alkylating agents or even hydrolysis by water. Lindahl also discovered DNA glycosylase enzymes (which remove damaged DNA bases, referred to as lesions) and was the first to describe protein interactions with DNA for base repair[2].

Forms of base damage include deamination (loss of an amine group), oxidation, the attachment of alkyl or larger groups, and spontaneous detachment from the DNA backbone, which leaves behind toxic abasic sites termed apurinic or apyrimidinic sites[2]. All such lesions are predominantly repaired by the base-excision repair pathway[3,4], in which a specific glycosylase binds to and bends DNA, opening the minor groove of the helix to remove the lesion, followed by replacement of the nucleotide by DNA-repair proteins.

Given the variety of lesions and the fact that bases are largely buried in the double-helix structure, how are damaged bases specifically recognized and removed? Crystal structures have revealed that the whole nucleotide is 'flipped out' of the DNA helix and sequestered in the active site of the enzyme for repair[5,6] (Fig. 1a). During this process, an amino acid from the enzyme moves into the base stack to stabilize the base-flipped DNA intermediate[3,6]. The geometric and chemical complementarity between the enzyme's active site and the damaged base provides specificity for the reaction, and the positioning leaves the glycosidic bond that links the damaged base to its sugar group accessible to cleavage by the enzyme.

Mullins *et al.* present a mechanism for base recognition and removal that forgoes base flipping and amino-acid intercalation. The authors studied the alkylpurine DNA glycosylase (AlkD), which binds to adenine and guanine bases that have been modified by alkylation in the bacterium *Bacillus cereus*. These bases are prone to spontaneous detachment, leaving abasic sites, and their presence may inhibit DNA replication[7]. The interactions of enzymes with alkylated bases is of great interest — control of DNA alkylation is central to the epigenetic marks that enable cells to differentiate into distinct tissues and to have distinct protein-expression profiles. And unrepaired alkylation damage can lead to mismatched bases and even cell death without successful mismatch repair, which was the focus of Paul Modrich's Nobel-prizewinning work. Furthermore, some cancer therapies are designed to induce alkylation damage with the aim of overwhelming aberrant repair mechanisms in tumour cells[8]. Indeed, future therapies may target specific repair responses as a 'weak spot' for many cancer cells[9].

Previous X-ray crystal structures have shown AlkD to have a different architecture from other glycosylases, being composed of repetitive structures (HEAT repeats) that cradle DNA in a concave channel[10]. Mullins *et al.* now provide structures of the enzyme 'flash-cooled' at different times as it excises alkylated bases. These structures show that, although the DNA does bend and has slight minor-groove widening, the distortion is mild in comparison with DNA bound to other glycosylases, and the modified nucleotide is not flipped out and sequestered in an active-site pocket (Fig. 1b).

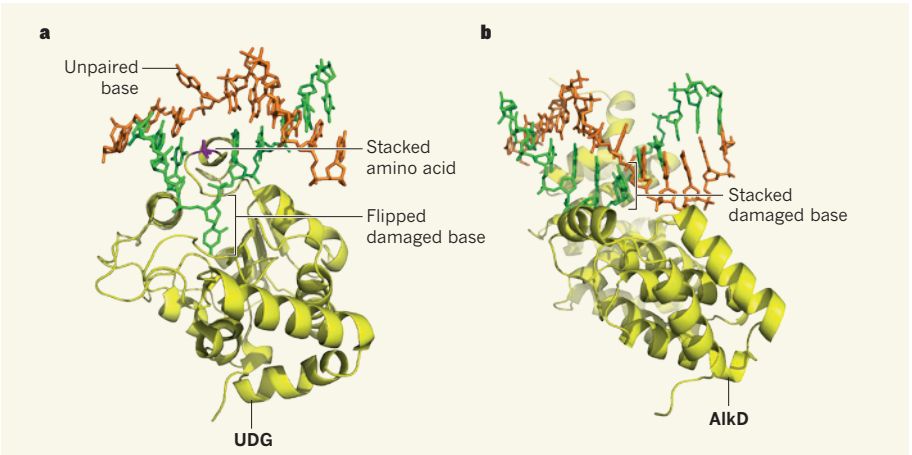

Figure 1 | Two mechanisms for base excision. Damaged DNA bases represent potential loss of fidelity and toxicity to a cell. **a**, A well-described mechanism for the excision of damaged bases is that enacted by glycosylase enzymes such as uracil DNA glycosylase (UDG)[2,3]. This process involves substantial conformational change in the DNA, which elicits unpairing of DNA bases such that the damaged base is 'flipped out' of the DNA double helix (DNA strands are shown in green and orange). The glycosylase enzyme then inserts one of its own amino acids into the helix to stabilize the remaining base stack and prevent the flipped base from returning before the enzyme's catalytic process has removed it. **b**, Mullins *et al.*[1] show that the glycosylase AlkD achieves excision of damaged bases without base flipping and without gross rearrangements to the DNA. How the excised base, which remains buried in the DNA helix, exits the DNA after cleavage remains unclear.

Instead, the authors show that much of the AlkD–DNA interaction is provided by multiple hydrogen bonds between the charged protein and the DNA phosphate backbone that flanks the lesion. Furthermore, two tryptophan amino-acid residues in the enzyme contribute to both binding to the lesion in the minor groove and enzyme catalysis, in what are called CH–π interactions. Such interactions are known from other protein–ligand recognition processes, but this may be the first example of CH–π interactions that directly function in DNA-repair catalysis. Thus, Mullins and colleagues' findings reveal new chemistry as well as an unprecedented damage-recognition mechanism.

For clinical applications directed at the DNA-damage response, we now face the possibility that humans and pathogens have enzymes that sense and remove alkylated DNA lesions without binding pockets, and thus require a different mode of targeting from other known glycosylase reactions. In fact, some bacteria produce an alkylating agent, yatakemycin, as an antibiotic, and protect themselves from their own alkylating toxin using a glycosylase similar to AlkD[11]. Mullins *et al.* found that the large yatakemycin molecule fits into a solvent-filled gap between AlkD and DNA and could also be excised from this position by AlkD. This finding highlights the remarkable properties of repair without base flipping — the ability to recognize and remove small base modifications in DNA and to have activity against large base adducts on the outside of DNA. Furthermore, the mechanism requires little conformational change in either the enzyme or the DNA; because conformational changes use energy and are often the rate-limiting step of enzymatic reactions, AlkD may act more rapidly than other glycosylases.

Mullins and colleagues' report expands our knowledge of recognition and excision between proteins and alkylated bases. However, we are left with questions about how well the AlkD mechanism works and which cells might use similar protein interactions with alkylated DNA. The damage-recognition and -removal mechanism of AlkD seems less specific than the flipping mechanism of other glycosylases, although the AlkD–DNA interface provides some specificity by restricting AlkD to removing inherently labile, positively charged lesions. In humans, base flipping by glycosylases helps to coordinate 'hand-off' to an endonuclease enzyme that cuts the DNA backbone at abasic sites[12]; it will be interesting to see whether AlkD can perform a similar hand-off. AlkD may also have roles in the removal of bulky lesions, which was the focus of Aziz Sancar's Nobel-prizewinning work. Thus, despite the earlier breakthrough research on DNA repair highlighted by this year's Nobel prize, Mullins and colleagues' findings on AlkD show that we still have much to learn. ■

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