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DNA Repair: Unconventional Lesions Require Unconventional Repair

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All cells have evolved a complex network of signaling and repair pathways to identify damaged DNA and rapidly correct it. Small alkylpurine lesions are common modifications that can be generated upon exposure to environmental chemicals, radiation, or genotoxic drugs. These DNA adducts are typically removed by the base excision repair (BER) pathway. On the other hand, bulky DNA adducts, such as pyrimidine dimers, are resolved by the nucleotide excision repair (NER) pathway. Tomas Lindahl, Paul Modrich, and Aziz Sancar shared the 2015 Nobel Prize in Chemistry for their fundamental studies of DNA repair, including BER and NER.

Lindahl's 1974 discovery of the first DNA glycosylase (Ung) in *Escherichia coli* marked the birth of our understanding of BER. DNA glycosylases initiate BER by catalyzing the hydrolysis of the *N*-glycosidic bond of a damaged or mismatched base. The generally accepted mechanism of DNA glycosylase activity is termed the base-flipping pathway, wherein the damaged base is flipped out of the duplex with concomitant insertion of an amino acid residue from the glycosylase to occupy the vacant site.[1] The flipping moves the damaged base into a nucleobase binding site of the glycosylase. This binding allows for detection of purine damage and initiation of BER by cleavage of the glycosidic bond. The requirement for nucleotide binding in the base-flipping mechanism places a ceiling on the size of the lesions that can be corrected by BER. Bulky adducts that are too large to enter the nucleobase binding pocket cannot be repaired.

A recent study by Eichman and co-workers added another exciting dimension to our understanding of BER.[2] In this work, the authors demonstrated that the prokaryotic DNA glycosylase alkylpurine glycosylase D (AlkD) excises cationic N3 and N7 alkylpurine lesions by a discrete mechanism that does not involve base-flipping. Instead, AlkD gains access to the glycosidic bond of the modified base by prying open the DNA duplex, rather than flipping the base out of the stack. This interaction is driven exclusively by electrostatic interactions between the enzyme and the phosphodeoxyribose backbone. Because this mechanism does not require accommodation of the alkylated base in a nucleotide binding site, it has the capacity to ameliorate larger alkylation lesions that cannot be processed by canonical BER.

In a recent *Nature Chemical Biology* paper, Eichman and co-workers build on this work and explore the AlkD-mediated removal of DNA adducts produced by the bacterial natural product yatakamycin [YTM (Figure 1)].[3] YTM and related spirocyclopropylcyclohexadienone natural products (e.g., CC-1065 and duocarmycin) are potent anticancer and antimicrobial agents. Many possess half-maximal inhibitory potencies (IC_{50}s) in the nanomolar to picomolar range against human cancer cell lines. Earlier studies established that YTM alkylates DNA by addition of a nucleotide to the cyclopropane.[4] This more recent study by Eichman and co-workers provides a biochemical basis for the remarkable potency of YTM that is likely extensible to other members of this natural product family. This work provides important and generalizable lessons in the study of small molecule genotoxins.

In their work, Eichman and co-workers demonstrate that the extreme toxicity of YTM derives in part from a tremendous increase in duplex stability following DNA alkylation. The authors show that the adenosine adducts of YTM (YTMA−DNA) are exceedingly stable, an effect attributed to 24 CH−π interactions between the natural product and the alkylated duplex. For example, the melting point of a GC-rich YTMA−DNA dodecamer is 36 °C higher than that of the corresponding unalkylated duplex. This value is remarkably large for a small molecule−DNA adduct. Although these adducts derive from formation of a bond to a single nucleotide, the authors point out that the extreme stability imparted to the duplex suggests they are more appropriately conceptualized as noncovalent interstrand cross-links. In accord with this dramatic increase in stability, the half-life for spontaneous depurination of YTMA−DNA was estimated to be 1.2 years. This increase in duplex stability impairs NER, which requires duplex destabilization by ligand binding, thereby requiring alternative mechanisms for resolution.

The authors proceed to show that AlkD rapidly hydrolyses YTMA−DNA under single-turnover conditions (e.g., an excess of AlkD relative to YTMA−DNA). Under these conditions, the glycosidic bond of the alkylated base is hydrolyzed within 30 s. This determination creates a paradox: if AlkD is so effective at resolving YTM lesions, why is the metabolite so toxic? The answer is non-intuitive. The authors demonstrate that following the first turnover of AlkD, the resulting AP−DNA and YTMAde residues form a stable ternary complex with AlkD, impeding dissociation of the glycosylase and further processing of other lesions. Thus, from one perspective, in the context of YTM lesion repair, AlkD can be regarded as a suicide enzyme that is deactivated after a single turnover. Suicide enzymes are known to participate in DNA repair, but their mechanisms are distinct. For example, *O*-6-methylguanine DNA methyltransferase (MGMT) is irreversibly deactivated following removal of a single *O*-6 guanine methyl residue. However, in the case of MGMT, an active site cysteine residue is covalently modified (methylated), while the "suicidal nature" of AlkD derives from formation of a noncovalent, yet very stable, ternary complex. In support of this, the authors show that the AlkD ortholog YtkR2, which is present in the YTM-producing strain and believed to provide resistance to YTM toxicity,[5] shares only 4 of 12

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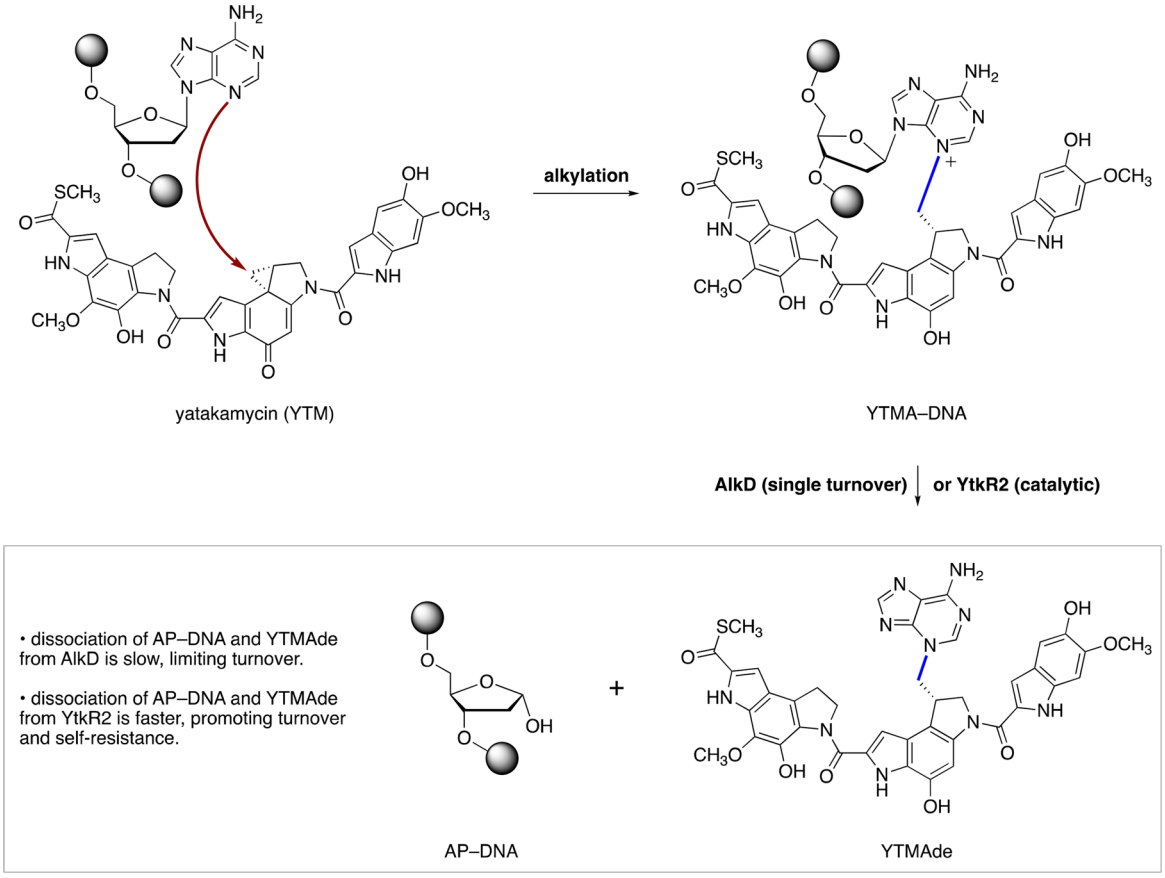

Figure 1. Yatakamycin (YTM) alkylates adenine by addition to the cyclopropane ring and aromatization. The resulting adducts (YTMA−DNA) are repaired by the bacterial DNA glycosylase AlkD or, in the YTM-producing organism, the DNA glycosylase YtkR2. Dissociation of the hydrolysis products, apurinic DNA (AP−DNA) and yatakamycin−adenine (YTMAde), from AlkD is slow, limiting turnover and leading to YTM toxicity. By comparison, AP−DNA and YTMAde dissociate more readily from YtkR2, promoting turnover and resistance to YTM self-toxicity.

YTMAde binding residues. This decrease in binding affinity may promote dissociation of the hydrolyzed product and turnover of YtkR2 in the producing strain, thereby providing a mechanism for self-resistance.

This study, in conjunction with the earlier report identifying the non-base-flipping mechanism of AlkD, suggests that additional noncanonical glycosylases may have evolved to resolve specific and unusual DNA lesions. More broadly, this work supports the idea that, in addition to characterizing the nature of the lesion produced by genotoxic small molecules, an understanding of the precise mechanism of their repair is essential to fully describe the basis of their cytotoxicity. Additionally, as many DNA repair pathways are mutated in cancer, understanding the repair mechanism associated with a given anticancer agent can point toward tumor types that would be expected to be sensitized to that agent. Despite intensive research aimed at targeting DNA repair pathways directly for cancer treatment, the functional roles of the large majority of proteins involved in these pathways remain unknown or poorly defined. Further studies of the type carried out by Eichman and co-workers are certain to lead to a higher-resolution understanding of DNA repair, to the identification of novel, specific, and evolved mechanisms for ameliorating genotoxic events, and to new therapeutic strategies for chemotherapeutic treatment.

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Notes

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