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Chemical Biology of Mammalian DNA Repair

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Abstract: Damage to the heterocyclic bases of DNA in the genome is mainly corrected by the base excision repair (BER) or nucleotide excision repair (NER) pathways. Base excision repair involves the sequential action of at least four enzymes and is initiated by DNA glycosylases. A lot of progress has recently been made toward elucidating of the molecular mechanisms by which DNA glycosylases recognize damaged bases in DNA and catalyze the cleavage of the N-glycosidic bond between the base and the sugar-phosphate backbone. This advance was brought about by a combination of chemical and biochemical approaches to generate stable complexes of DNA glycosylases bound to their substrates or substrate analogs and X-ray crystallography to determine the structure of these complexes at atomic resolution. Nucleotide excision repair requires the concerted action of 15–18 polypeptides to excise an oligonucleotide of about 30 bases in length containing the damaged residue. The structures of several DNA intermediates in the process are known and the reaction has been recently reconstituted with purified proteins. We know less about the details of how the proteins involved recognize and excise damaged DNA and how specific protein-protein interactions govern the overall process. It is expected that our understanding of nucleotide excision repair will be significantly advanced through the development of novel chemical and cell biological approaches in the near future.

Keywords: Chemical biology · Damage recognition · DNA repair · Enzyme inhibitors · X-ray crystallography

Introduction

DNA, the carrier of genetic information in all living organisms, is under constant threat from agents that cause damage to its structure, which can result in the loss of vital genetic information and lead to the development of cancer. To counteract the deleterious effect of such damage, all organisms have evolved a number of DNA repair pathways by which most types of damage to DNA can be corrected [1][2]. The importance of DNA repair is evident from a number of human genetic disorders that are caused by defects in certain DNA repair pathways. For example, individuals suffering from xeroderma pigmentosum (XP) have a defect in nucleotide excision repair and are unable to clear their genomes from damage caused by UV radiation from the sun, resulting in an up to 2000-fold increase in skin cancer [3]. Patients with hereditary nonpolyposis colon cancer (HNPCC) are unable to repair mismatched bases that arise as errors in replication [4]. Once viewed as independent house-keeping functions, it is now clear that DNA repair processes belong to an intricate network of tightly regulated cellular events, which includes replication, transcription, cell cycle control and cell division [5]. The understanding of DNA repair is also of great importance for anti-tumor therapy, since most anti-tumor drugs used clinically are cytotoxic DNA damaging agents [6]. Thus, whereas intact DNA repair is essential for healthy organisms, selective inhibition of DNA repair in tumor cells might greatly enhance the efficiency of anti-tumor therapy.

Here I will discuss some recent progress in our understanding of the molecular basis underlying two DNA repair pathways, base excision repair (BER) and nucleotide excision repair (NER) and highlight the role of chemical approaches in the elucidation of the mechanisms underlying DNA repair.

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Base Excision DNA Repair

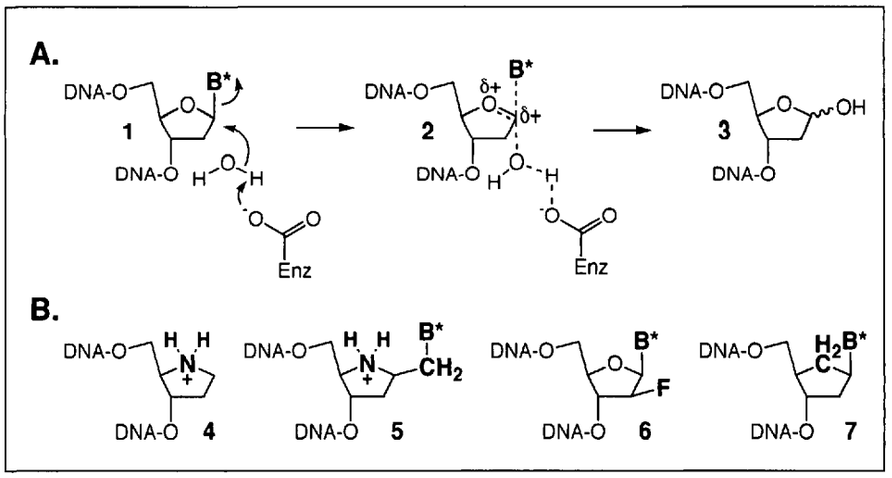
Damage to DNA bases resulting from alkylation, oxidation and deamination by agents of normal cellular metabolism is principally repaired by the base excision repair (BER) pathway [2][7][8]. BER is initiated by DNA glycosylases, which recognize damaged bases in DNA and remove them by cleaving the N-glycosidic bond between the base and the sugar–phosphate backbone (Scheme 1). Eight different human DNA glycosylases have been cloned and characterized to date and each one of these has a unique substrate specificity. DNA glycosylases can be divided into two mechanistic classes: 1) monofunctional DNA glycosylases, which cleave the glycosidic bond using a water molecule as a nucleophile and generate abasic sites as products (Scheme 2A). 2) Bifunctional DNA glycosylases/AP lyases, which use an amino group of the enzyme as nucleophile and additionally catalyze ß-elimination to cleave the C(3')–O bond of the abasic site. The abasic site generated by monofunctional glycosylases is most frequently processed *via* short patch BER (Scheme 1): An AP

endonuclease generates a single stranded break 5' to the abasic site, polymerase β then introduces the proper nucleotide and removes the abasic site and ligase III seals the nick. Other minor variants of BER for processing abasic sites and products generated by DNA glycosylases/AP lyases exist to complete repair and these have been reviewed recently [7][8].

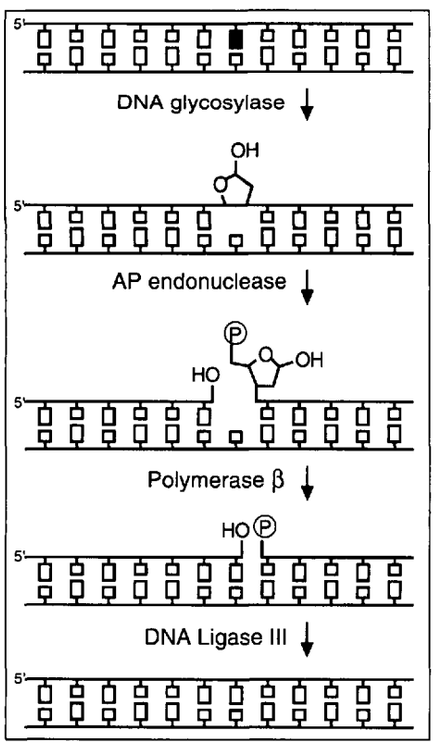
Mechanisms of Damage Recognition and Catalysis by DNA Glycosylases

DNA glycosylases are a particularly intriguing class of DNA repair enzymes. They are faced with the task of specifically recognizing damaged bases in DNA with only subtle alterations from their native counterparts, which are present in vast excess (up to ~ 10^9 fold) in the genome. In the past several years much progress has been made in understanding the chemical and structural basis by which DNA glycosylases recognize and excise damaged bases from DNA through studies involving organic chemistry, biochemistry and structural biology [7][9]. Two strategies have been employed to make the intrinsically short-lived complexes of DNA glycosylases bound to their substrates available for structural and biochemical analysis. One has relied on site-directed mutagenesis of enzyme active site residues to abrogate catalysis while preserving specific substrate binding. This approach has led to the solution of several crystal structures of mutant DNA glycosylases bound to their DNA substrates [10–13]. The design and synthesis of inhibitors of DNA glycosylases based on the mechanism of glycosidic bond cleavage has been used as an alternative approach to separate binding and catalysis (Scheme 2B) [14]. The model for the mechanism of the reaction catalyzed by DNA glycosylases is based on detailed studies of enzymes such as glycosidases and nucleoside hydrolase [15–17]. According to this model, the reaction is likely to proceed by a mixed S_N1/S_N2 mechanism with a transition state that is likely to resemble **2** (Scheme 2A) with substantial positive charge accumulated in the ribose ring, especially at C(1') and O(1'). Based on this transition state structure, two classes of inhibitors were designed. In the pyrrolidine derivatives **4** and **5** (Scheme 2B), the oxygen in the ribose ring was replaced by positively charged ammonium group to mimic the positive charge in the transition state [18–20]. The substrate analogs **6** and **7** contained a stabilized glycosidic bond through modification of the ribose sugar that made them resistant to processing by DNA glycosylases [21–23]. Both classes of inhibitors formed stable complexes with DNA glycosylases and the structures of some of these complexes were determined by X-ray crystallography [24–27]. The structural and biochemical studies of DNA glycosylase-DNA complexes have revealed many of the key features of substrate recognition and catalysis by these enzymes.

All DNA glycosylases flip the target nucleotide out of the double helix and into an active site pocket of the enzyme where catalysis takes place (Fig. 1A). Nucleotide flipping is an ingenious way for these enzymes to make the anomeric center, which is buried deep in the minor groove in B-form DNA, accessible to nucleophilic attack. Once located in the active site of the enzyme the damaged bases are bound in specific pockets through π-stacking, hydrophobic and hydrogen bonding interactions. Some DNA glycosylases, for example uracil DNA glycosylase (UDG), have a very narrow substrate specificity and recognize their sub-



Scheme 2. Reaction mechanism and inhibitors of DNA glycosylases: **A.** Mechanism of glycosidic bond cleavage by DNA glycosylases: A water molecule in the active site is deprotonated by a carboxylic acid side chain of the enzyme and positioned to displace the damaged base through nucleophilic attack at the anomeric position. In the transition state (**2**), substantial positive charge accumulates on the ribose sugar ring, notably at C(1') and O(1'). Expulsion of the base leads to an abasic site as the reaction product. **B.** Synthetic inhibitors of DNA glycosylases: Pyrrolidine **4** was designed to mimic the positive charge of the transition state of the glycosylase reaction. The base in the homopyrrolidine analog **5** is linked through a methylene group to the anomeric position; direct attachment of the base to the pyrrolidine at the C(l') position would result in an unstable aminal linkage. The fluorine group in **6** electronically destabilizes the positive charge in the transition state. The carbocyclic nucleoside **7** lacks the endocyclic oxygen that stabilizes the positive charge of the transition state. Molecules **4–7** are bound, but not processed by DNA glycosylases. For a more complete listing and discussion of DNA glycosylase inhibitors see [7][14].



Scheme 1. The base excision DNA repair pathway: A base modified through alkylation, oxidation or deamination (depicted in black) is recognized and excised by a DNA glycosylase, generating an abasic site. The abasic site is processed to a single-stranded break by an AP endonuclease, which cuts the phosphodiester bond 5' to the abasic site. The repair synthesis of one nucleotide and the excision of the abasic site through scission of the C(3')–O bond *via* β-elimination is catalyzed by polymerase β and the nick is sealed by DNA ligase III.

strate (uracil) with exquisite specificity. Consequently, UDG has a highly specific base-binding pocket with a network of hydrogen bonding and steric interactions that rigorously excludes binding of all other bases [11]. Other enzymes, like AAG have a much broader and more relaxed substrate specificity (AAG excises 3-methyladenine, 7-methylguanine, 1,N^6-ethenoadenine and hypoxanthine from DNA, among others), which is reflected in a less specific binding pocket (see Fig. 1B for the details of base binding by AAG) [13][24][28]. What we have learned so far about the mechanism of glycosidic bond cleavage is in general agreement with the originally proposed model. In the crystal structure of AAG a water molecule poised to be the nucleophile in the reaction is clearly visible between an aspartate residue of the enzyme and the ammonium group of the pyrrolidine inhibitor or the anomeric center of the substrate nucleotide (Fig. 1B). It has become clear that different structures of the same enzyme bound to various ligands are needed to understand the intricacies of enzymatic mechanisms, and certain glycosylase structures have provided unexpected surprises (see for example [25][27]). More detailed mechanistic studies using spectroscopic techniques and the measurement of kinetic isotope effects have been carried out for uracil DNA glycosylase [29–31] that have revealed further details of the glycosylase catalyzed reactions. The combination of structural and solution-based studies should lead to a more complete description of the mechanisms underlying excision of damaged bases.

One of the most intriguing aspects of damage recognition by DNA glycosylases has so far remained unsolved: How do DNA glycosylases recognize damaged bases in duplex DNA before they are flipped out into the active site pocket of the enzyme? Is every single base flipped out in a processive manner or are specific features of damaged base pairs in double stranded DNA recognized? Advanced physical techniques such as single molecule fluorescence or atomic force microscopy might provide opportunities to address this question.

Nucleotide Excision Repair

Nucleotide excision repair (NER) is the main pathway by which various bulky lesions, including those formed by UV light and various environmental mutagens or chemotherapeutic agents such as benzpyrenes, aflatoxin or cisplatin, are repaired. Defects in NER are associated with the human disorder xeraderma pigmentosum (XP) and its variant forms, which are characterized by a complex phenotype that includes a marked sensitivity to UV light and predisposition to skin cancer [3]. The steps involved in NER are the recognition of damaged residues in DNA, dual incision of the damaged strand around the lesion to excise an oligonucleotide of 24-32 nucleotides in length, and DNA synthesis and ligation to fill the gap (Fig. 2) [32]. The recognition and excision of damage requires 15 to 18 polypeptides and another dozen or so replication factors are needed for repair

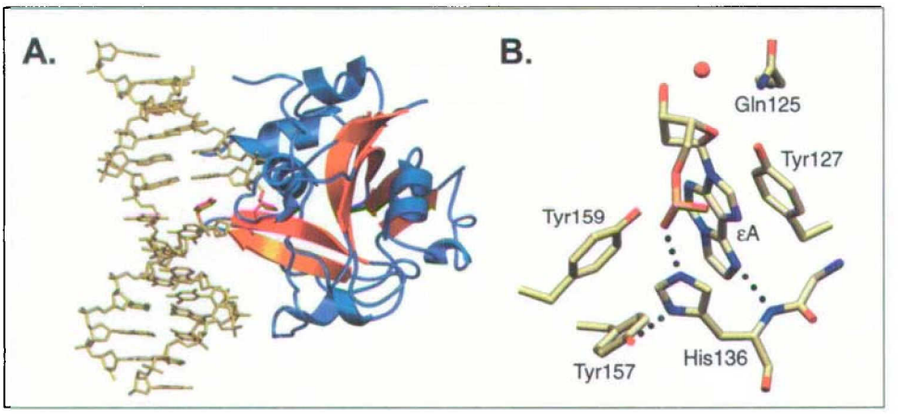


Fig. 1. Structure of the Human AAG bound to DNA. **A.** Overview of the structure of AAG bound to a pyrrolidine inhibitor (shown in pink). AAG binds in the minor groove of DNA and expels the pyrrolidine into the enzyme active site. Tyrosine 162 (orange) intercalates in the space left by the flipped out pyrrolidine residue. Protein contacts to the phosphates and sugars of the DNA backbone widen the minor groove and bend the DNA away from the protein. Reprinted with permission of Elsevier Science © 1998 [24]. **B.** Binding of an εA residue in the AAG Glu125Gln mutant active site pocket. The main chain amide of His136 forms a hydrogen bond with N6 of εA, which could be formed with an unmodified adenine. This hydrogen bond may be responsible for the selective binding of εA over A. Tyrosine 127 and histidine 136, which is positioned by tyrosine 157, bind the base through π-stacking interactions. A water molecule (shown in red) is located between Gln125 (Glu in wild type AAG) and the anomeric position of the target nucleoside. This water is ideally positioned for nucleophilic attack and is conserved in the AAG-pyrrolidine structure. Reprinted with permission of Elsevier Science © 2000 [28].

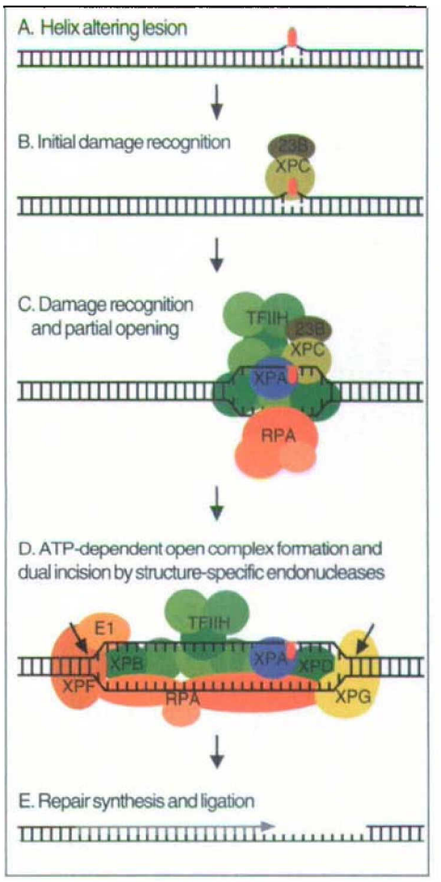


Fig. 2. Model for the nucleotide excision repair reaction: A. A DNA lesion such as pyrimidine dimer (shown as an orange oval) causes some distortion in the DNA double helix. B. XPC/hHR23B may be the first protein to recognize the damage in DNA; other models with RPA/XPA as the first damage recognition protein have also been proposed. C. Further damage recognition and initial bubble formation is then accomplished by the concerted action of XPC/hHR23B, XPA, RPA and TFIIH. D. The two helicases of TFIIH, XPB and XPD further open the bubble in an ATP-dependent manner. The two endonucleases XPG and ERCC1/XPF are then positioned to make the incisions 3' and 5' the lesion. E. The damaged oligonucleotide is released, the gap filled by the replication factors RFC, PCNA, polymerase δ/ε and RPA, and the nick is sealed by DNA ligase I.

synthesis. NER has been reconstituted *in vitro* with purified proteins and has thus become amenable to detailed mechanistic investigation [33][34]. Although the sequence of steps in NER and the corresponding DNA intermediates are known, we are only beginning to understand the mechanisms of the individual steps and the protein–protein interactions that coordinate the process.

Damage Recognition and Excision in NER

The mechanism of damage recognition in NER is a much-debated problem that has not yet been solved [35]. The efficiency by which various DNA lesions are repaired in human cells varies over several orders of magnitude [36]. There is a general correlation between the efficiency with which a given lesion is repaired and the extent of distortion of the DNA caused by the lesion. Distortion of the DNA backbone alone, however, is not sufficient for recognition since mismatches and bubbles formed by non-damaged bases are not substrates for NER. Based on these observations a bipartite model for recognition has been proposed, which involves recognition of distorted DNA followed by localization of the chemically damaged base [37]. Damage recognition involves at least four factors consisting of a total of 12–15 polypeptides, namely the XPC-hHRad 23B heterodimer, XPA, the RPA trimer and the 6–9 subunits of TFIIH (Fig. 2C) [32][35]. Two different views are currently prevalent in the literature about the identity of the initial damage recognition protein. Some authors favor the XPC-hRad23B dimer as the initial damage sensor [38–40]. XPC shows a strong preference (although not very high affinity) for damaged over undamaged DNA and has been shown to be required for the earliest steps of DNA unwinding. Furthermore, *in vitro* competition experiments have indicated that XPC acts before all other proteins in the pathway. Other authors have argued that XPA is the initial damage recognition protein [41][42]. XPA shows a preference for binding damaged DNA, which is synergistically enhanced by the RPA protein, particularly for oligonucleotides with single-stranded character containing damaged residues. A fifth factor, the XPE heterodimer also shows strong preference for binding UV damaged over non-damaged DNA. The role of XPE has, however, remained somewhat enigmatic. In contrast to the other factors described above, XPE is not required for excision of damage from DNA in a reconstituted *in vitro* system and stimulates the repair reaction only twofold [35]. Nonetheless, it has been shown that XPE is required for the repair of certain lesions *in vivo* and one hypothesis is that XPE plays a role in the recognition of DNA in the context of chromatin [43]. The issue of which protein is the initial damage sensor remains to be resolved and may well turn out to depend on the lesion to be repaired. Our group is developing NER substrate analogs for photocrosslinking and fluorescence studies to address this question.

Following damage recognition, ATP-dependent opening of the DNA helix takes place through the action of the helicase activities of the XPB and XPD subunits of TFIIH. An open intermediate of about 25–30 base pairs in length is then formed (Fig. 2D), to which all the factors required for excision of the damaged oligonucleotide except XPC are bound [44]. It is thought that RPA is bound to the non-damaged strand and plays a role in positioning the two structure-specific endonucleases to ERCC1/XPF and XPG to make incisions 5' and 3' to the lesion [45]. An interesting property of the two endonucleases is that the positions at which they make their incisions vary depending on the nature of the lesion that is repaired. Cleavage occurs 16 to 25 phosphodiester bonds 5' to the lesion by ERCC1/XPF and 2 to 9 phosphodiester 3' to the lesion by XPG. This indicates that the specificity for phosphodiester bond cleavage is dictated by positioning of the endonucleases by the NER machinery. The mechanism by which these structure specific endonucleases catalyze their reactions and how their activity is stimulated by other NER factors is another area of investigation in our laboratory.

Progress Toward Studying the NER Reaction in Living Cells

The successful elucidation of a metabolic pathway requires both genetic studies (*in vivo*) to establish the involvement of a gene in a certain pathway and biochemical studies (*in vitro*) to assign function to the proteins encoded by each gene of the pathway. The investigations of the NER pathway have been especially successful in this regard. Studies of cell lines derived from XP patients and UV-sensitive chinese hamster ovary (CHO) cell lines have established a genetic framework for NER, and the studies of the excision and repair synthesis steps of NER in whole cell extracts and with purified proteins have provided the biochemical model of the pathway. There has always been a gap between the two approaches, providing physiologically sound but not very detailed data from genetic studies or detailed, but physiologically difficult to prove data from biochemical studies. This gap is now beginning to disappear as first steps are being made toward characterizing enzymatic reactions in living cells. It is now possible to observe the dynamic behavior of NER (and other) proteins fused to the green-fluorescent protein (GFP) in living cells [46]. GFP is a naturally occurring fluorescent protein isolated from jellyfish that can be expressed as a fusion protein with any partner protein using straightforward molecular biology techniques [47]. Using fluorescent microscopes, GFP or GFP-tagged proteins can be observed in living cells. Importantly, the attachment of the 27 kDa GFP to the N- or C-terminus of any protein does not significantly alter their activity *in vivo* in the vast majority of cases. Various mutant forms of GFP are now available with different absorption wavelengths and techniques like fluorescence energy transfer (FRET) and spot bleaching have been used to gain information about the function and localization of proteins in living cells. In the case of NER, several proteins involved in NER have been tagged with GFP and their mobility and in response to UV exposure have been investigated [46]. These studies have supported a model in which the NER proteins diffuse freely through the nucleus, become immobilized at sites of DNA damage and get released again after the damage has been repaired. While this technology has enabled the observation of NER proteins in living cells, the generation of fluorescent NER substrates and their introduction into cells is less straightforward and is currently underway in our group. The ability to observe both NER proteins and substrates in living cells will be an important step toward achieving the goal of describing the NER reaction *in vivo*.

Conclusions

The design and synthesis of specific ligands for DNA glycosylases has been crucial in elucidating the mechanisms of damage recognition and excision by this important class of DNA repair enzymes. For the near future the extension of

chemical biological approaches to study more complex DNA repair processes can be anticipated, which I illustrated here with the example of nucleotide excision repair. Other important DNA repair pathways such as mismatch repair and homologous and non-homologous recombination will also be amenable to similar approaches. Such studies will provide insight into the chemical basis of metabolism in mammalian cells and will be essential for understanding the molecular basis of human disease caused by DNA repair deficiencies. Furthermore, these insights should lead to the improvement of cancer chemotherapy, since most antitumor agents used clinically exert their function by damaging DNA.

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