WURJ Health and Natural Sciences Nucleotide Excision Repair in the Three Domains of Life

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Abstract

Nucleotide excision repair (NER) is a vital DNA repair pathway which acts on a wide range of helixdistorting lesions. The importance of this pathway is highlighted by its functional conservation throughout evolution and by several human diseases, such as xeroderma pigmentosum, which are caused by a defective NER pathway. This review summarizes the NER mechanisms present in all three domains of life: eukaryotes, bacteria, and archaea.

Introduction: Conservation of Function

ucleotide excision repair (NER) is conserved throughout the three domains of life and is the main pathway through which organisms remove bulky lesions from their DNA. NER was first discovered in the 1960s (1), and has since been demonstrated to act on a wide range of helix-distorting lesions (Table 1). For example, cyclobutane pyrimidine dimers and pyrimidine (6-4) photoproducts are DNA lesions that form upon exposure to UV radiation and are repaired by NER (2).

The basic NER mechanism is functionally conserved throughout evolution, although the specific proteins involved differ between prokaryotes and eukaryotes. It is more difficult to identify a prototypical NER pathway in archaea, since homologues of both bacterial and eukaryotic NER proteins are present in different archaeal species (3). The basic NER mechanism consists of recognizing the DNA lesion and locally unwinding the helix on both sides of the damaged DNA. This forms a pre-incision complex where the lesion is surrounded by single-stranded DNA (ssDNA) and is therefore vulnerable to excision by endonucleases. Other NER factors are then recruited to the pre-incision complex, leading to a dual incision of the DNA backbone

on both sides of the lesion. The damage-containing oligonucleotide is then removed and а new oligonucleotide is synthesized, completing the NER pathway. In humans, a defective NER pathway results in diseases such as xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD) (2). While a discussion of these diseases is outside the scope of this review, other articles (4, 5) provide extensive discussion of the biological importance of NER with respect to human disease. Complementation studies using cells from patients affected by these diseases have identified many of the proteins known to be involved in eukaryotic NER, such as the XP proteins which are mutated in xeroderma pigmentosum (2).

Table 1. DNA lesions repaired by bacterial nucleotide excision repair (NER). Eukaryotic and archaeal NER pathways also repair a similar range of lesions (2). Adapted from (6)

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Type of lesion	Lesion
Single base	Thymine glycol
modification	Dihydrothymine
	Benzo[α]pyrene adduct
	Anthramycin adduct
	O ⁴ -alkyl thymine
	O ⁶ -methyl guanine
	N ⁶ -methyl adenine
	Psoralen adduct
	Nitrogenous base removed (AP
	site)
Intra-DNA strand	<i>cis</i> -Platin adduct
cross-links	Pyrimidine dimer
	(6-4) photoproduct
Inter-DNA strand	cis-Platin adduct
cross-links	Nitrogen mustard adduct
Non-covalent	Psoralen bisadduct
modifications	Caffeine complex
	Ditercalinum complex

Eukaryotic NER: All the Bells and Whistles

Eukaryotic NER is divided into global genome NER (GGR) and transcription coupled NER (TCR). In GGR (Figure 1), DNA lesions are repaired irrespective of their location in the genome, whereas lesions on the transcribed strand of active genes are preferentially repaired during TCR (2). Both GGR and TCR pathways converge at the recruitment of transcription factor IIH (TFIIH) to the DNA lesion, but they differ in the mechanism of DNA lesion identification. In GGR, damaged DNA is recognized by the XPC-HR23B (UV excision repair protein Rad23 homologue B) heterodimer, which binds tightly to the damaged DNA, possibly by interacting with unpaired nitrogenous bases (7). In some cases, XPC-HR23B has a low affinity for the lesion and requires the presence of the damaged DNA-binding protein (DDB) in order to initiate NER (2). Bound XPC-HR23B then recruits TFIIH to the damaged DNA (2). In TCR, the recognition signal is a stalled RNA polymerase upstream (5') of the DNA lesion (2). It is hypothesized that CSA and CSB, proteins mutated in Cockayne syndrome, recruit TFIIH to the stalled RNA polymerase (2), replacing the role of XPC-HR23B in GGR. TCR provides cells with a means to quickly recover normal RNA synthesis and cellular function by preferentially repairing lesions which pose an immediate risk to the cell, as opposed to less threatening lesions within non-coding DNA or silent genes. Whether or not the stalled RNA polymerase is displaced or dissociates from DNA during TCR remains unknown (2).

TFIIH is a multi-subunit complex which includes subunits XPB and XPD, among others (2). The XPB and XPD subunits of TFIIH are 3'-to-5' and 5'-to-3' DNA helicases (8) which are hypothesized to act either on opposite or identical sides of the pre-incision complex. These helicases unwind the double-stranded DNA so that the lesion is now surrounded by cleavable ssDNA, and also allow other NER factors to bind to the open preincision complex. Recent studies suggest that only the ATPase activity of XPB, which functions as a conformational switch, is required to open the DNA bubble (9), whereas the helicase activity of XPD is responsible for most of the DNA unwinding (10). Endonucleases XPF, which associates with the excision to form an XPF-ERCC1 repair protein ERCC1 heterodimer, and XPG are both structure-specific endonucleases that incise the DNA backbone on opposite sides of the lesion (2).

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In GGR and TCR, the recruitment of TFIIH to the site of DNA damage is not mediated by a simple, passive interaction, but involves active ATP hydrolysis by XPB (11). Once TFIIH is recruited to the DNA lesion, a sub-complex of TFIIH, trichothiodystrophy group A (TTDA)-p52, is activated and promotes the ATPase activity of XPB. This ATPase activity is required for stable binding of TFIIH to the pre-incision complex (12), which in turn stabilizes and positions XPD (12). The helicase activity of XPD is then activated by the p44 subunit of TFIIH (12), and it unwinds the DNA helix around the lesion.



Figure 1. Global genome nucleotide excision repair in eukaryotes. UV radiation from the sun can cause DNA damage (A). XPC-HR23B then recognizes the DNA damage (B) and recruits the multi-subunit transcription factor IIH (TFIIH) (C). The XPB and XPD helicase subunits of TFIIH then unwind the DNA helix (D) to allow XPA and replication protein A (RPA) to verify the presence of damaged DNA (E). This forms the preincision complex. Endonucleases XPF-ERCC1 and XPG then incise the damaged DNA strand (F). The pre-incision complex and damage-containing oligonucleotide then dissociate (G) and the repair process is completed by DNA polymerase and ligase (not shown).

Other eukaryotic proteins involved in NER, such as XPA and the ssDNA-binding protein replication protein A (RPA), have also demonstrated a binding preference for sites of damaged DNA (13). This finding suggests that several factors could be involved in DNA damage recognition and verification during eukaryotic NER. Moreover, it would be logical for XPA and RPA to play a role in damage verification, since they bind to the open pre-incision complex just in advance of the endonucleases XPG and XPF-ERCC1 (12), which then make irreversible incisions in the DNA backbone. Through binding to the undamaged strand, RPA protects the undamaged ssDNA from cleavage by nucleases and recruits replication factors for DNA repair synthesis (2). RPA also demonstrates polarity when it binds to ssDNA at the lesion site (2), which further supports its potential role in the assembly of NER factors such as XPG and XPF-ERCC1.

Once the pre-incision complex is formed and all potential DNA damage verification checkpoints have been passed, XPG hydrolyzes a phosphodiester bond in the DNA backbone of the damaged strand two to eight nucleotides downstream (3') of the DNA lesion (2). Once XPG has made this incision, XPF-ERCC1 then incises the damaged DNA strand approximately 15 to 24 nucleotides upstream of the DNA lesion (2). This dual incision results in the removal of a single-stranded oligonucleotide, of approximately 24 to 32 nucleotides, which contains the DNA lesion.

DNA polymerases, such as DNA polymerase δ and ϵ , perform DNA repair synthesis (2) using the undamaged strand as a template to replace the excised oligonucleotide. Here, RPA is involved in assembling processivity factors required for efficient DNA repair synthesis, such as proliferating cell nuclear antigen (PCNA) and replication factor C (RFC) (2). DNA ligase completes the NER process by forming a phosphodiester bond between the 3' end of the newly synthesized DNA oligonucleotide and the 5' end of the original DNA sequence.

ATP-dependent chromatin remodelling complexes are also involved in eukaryotic NER (14), where chromatin structure impedes the access of repair proteins to damaged DNA (15). For example, the switch/sucrose non-fermentable chromatin remodelling complex (SWI/SNF) in yeast has been reported to promote NER of (6-4) photoproducts *in vitro* (16). SWI/SNF also associates with the yeast homologue of XPC-HR23B, Rad4-Rad23, which suggests another role for chromatin remodelling complexes in recruiting NER factors (14).

Circadian rhythms have also been reported to influence NER. A study by Kang et al. (2009) demonstrates that NER in the mouse brain is regulated by a circadian rhythm, with maximum NER activity in the afternoon-to-evening hours and minimum activity in the midnight-to-morning hours. XPA was the only NER factor that was found to oscillate on a circadian pattern (17). Since NER is the only mammalian pathway that removes bulky DNA lesions, such as those produced by some chemotherapeutic drugs, Rabik and Dolan (2007) suggest that XPA could potentially be used as a marker for the temporal optimization of chemotherapy treatment.

Bacterial NER: No Gimmicks

In bacteria, complementation studies of NERdeficient cells revealed that three genes are involved in NER: uvrA, uvrB, and uvrC (19). This system is commonly referred to as the UvrABC system, and is much simpler than eukaryotic NER. DNA damage recognition and incision steps of mammalian NER require 15 to 18 proteins, whereas *Escherichia coli* cells only require three proteins to accomplish a similar effect (20). These Uvr proteins are only found in prokaryotes and a lack of sequence homology with eukaryotic XP proteins (21) indicates convergent evolution of NER between bacteria and eukaryotes.

In bacterial NER, two molecules of UvrA and one of UvrB form a heterotrimer which recognizes damaged DNA (22). UvrA binds to the DNA first and mediates the binding of UvrB to the undamaged strand (23). Once UvrB has bound to the DNA, it is hypothesized that UvrA hydrolyzes an ATP molecule which causes a conformational change that promotes the dissociation of UvrA from the DNA (21). This forms a stable UvrB-DNA pre-incision complex (24). UvrC is then recruited to the pre-incision complex and incises the damaged DNA strand twice: first it incises four to five nucleotides downstream of the lesion and then again eight nucleotides upstream of the lesion (25). Although UvrC catalyzes both incisions, each incision is executed by a distinct catalytic site (26). After incision (Figure 2), UvrC dissociates and UvrD (a helicase) separates the damagecontaining oligonucleotide. of approximately 12 nucleotides in length, from the undamaged DNA strand (21). DNA polymerase I then synthesizes a new oligonucleotide to fill the gap, and also displaces UvrB from the DNA (27). As in eukaryotes, DNA ligase completes the repair process.

Another area of similarity between bacterial and eukaryotic NER is the presence of GGR and TCR. In bacterial TCR, a stalled RNA polymerase recruits the transcription repair coupling factor (TRCF) which in turn recruits UvrA to the site of DNA damage (28). In both TCR and GGR, UvrA loads UvrB onto the damaged DNA strand and then dissociates to leave a stable UvrB-DNA pre-incision complex (21). In this sense, UvrB acts as a DNA damage verification factor. While UvrB does contain helicase domains, ATP hydrolysis by UvrB is only associated with limited DNA unwinding (29). UvrB's helicase action is therefore more accurately described as 'helix destabilization' (30), and may fulfill a role similar to that of the weak eukaryotic helicase XPB. UvrB therefore locally distorts the DNA at lesion sites, which promotes the binding and endonuclease activity of UvrC (21). This distortion is accomplished by a flexible β-hairpin which is used by UvrB to stably bind to DNA at the pre-incision complex (21). Solved structures of helicases that are homologous to UvrB suggest that hydrophobic residues at the tip of UvrB's β-hairpin insert between the damaged and undamaged DNA strands, and securely bind to another domain on the same UvrB molecule (6). DNase I

homologous to UvrB suggest that hydrophobic residues at the tip of UvrB's β -hairpin insert between the damaged and undamaged DNA strands, and securely bind to another domain on the same UvrB molecule (6). DNase I footprinting experiments also suggest that UvrB grasps the undamaged DNA strand in this manner (31). Furthermore, this model is consistent with the finding that the stable UvrB-DNA pre-incision complex does not form spontaneously, since a conformational change in UvrB would be required to separate the hydrophobic β -hairpin tip from its binding domain on UvrB (21). UvrA is hypothesized to cause this conformational change (21).



Figure 2. Bacterial UvrABC excision and repair. After UvrC has cleaved the DNA backbone of the damaged strand twice (A) a helicase, UvrD (not shown), removes the damage-containing oligonucleotide (B). In this figure, the DNA damage is depicted as a black triangle. DNA polymerase I then synthesizes a new oligonucleotide and

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displaces UvrB. The new oligonucleotide is sealed into place by DNA ligase (C).

Archaeal NER: Clues to NER Evolution

In the domain of archaea, identifying a common NER pathway, or rather pathways, is even more complicated. Genomic sequencing has revealed that most archaea possess homologues of eukaryotic NER proteins such as the helicases XPB and XPD (32), however, a few species of archaea have NER systems that are similar to the bacterial UvrABC system. Of all the genomes published, only archaeal mesophilic such Methanobacterium methanogens. as thermoautotrophicum, and halophiles, such as the Halobacterium species, possess UvrABC orthologues (2). To further complicate matters, some archaea, such as Methanosarcina mazei, have a mixture of bacterial and eukaryotic NER orthologues (3). A possible explanation for these observations is that archaeal NER was originally eukaryotic in character, but has since been replaced in some species by the bacterial UvrABC system through lateral gene transfer (3). This hypothesis, however, does not explain the apparent lack of archaeal homologues of the eukaryotic NER damage-recognition proteins such as XPA, XPC, and XPE (2). One possible explanation for these observations is that NER in eukaryotes might have experienced significant evolution such that it now includes acquired proteins and a more complicated damage-recognition system than was present in the last common ancestor of archaea and eukaryotes (2).

The thermophile Sulfolobus solfataricus is an example of an archaea which possesses a NER system similar to eukaryotic NER. Sulfolobus solfataricus contains helicases XPB and XPD, which fulfill similar roles in archaeal NER as eukaryotic XPB and XPD (33). For example, it is hypothesized that archaeal XPB binds first to the undamaged strand at sites of DNA damage such that its weak helicase activity locally destabilizes the DNA helix. It is possible that a ssDNA binding protein is involved in recruiting XPB to damaged DNA (34). This helix destabilization then allows XPD to bind and further unwind the duplex DNA to form the pre-incision complex (33). Once the pre-incision complex is formed, archaeal XPF incises the damaged DNA strand upstream of the DNA lesion, fulfilling a role which is analogous to that of eukaryotic XPF (33). Although an archaeal XPG endonuclease has yet to be discovered, recent studies have discovered a protein, Bax1, which binds to archaeal



XPB and is thought to replace eukaryotic XPG. Specifically, data from Rouillon and White (2010; 33) suggest that Bax1 cleaves the DNA backbone during archaeal NER at a similar position as is cleaved by eukaryotic XPG (Figure 3).



Figure 3. Archaeal XPB and Bax1 are analogous to eukaryotic XPB and XPG. Bax1 binds to XPB and incises the DNA backbone downstream of the lesion (black triangle) after XPB has locally destabilized the DNA helix. XPD may also be involved in unwinding the DNA helix at the site of the lesion, potentially allowing XPF to incise the DNA backbone upstream of the lesion.

Implications: From Archaea to Humans

NER is a fundamental pathway in the maintenance of genetic stability, and helps to sustain a balance between life and evolution through random mutation (2). The importance of this pathway is underlined by the presence of a conserved NER mechanism in all domains of life. Research concerning mammalian NER is of great interest to the scientific community since some human diseases, such as xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy, involve defective NER pathways. On the contrary, cancer cells use NER to resist the effects of some chemotherapeutic drugs. Therefore research pertaining to eukaryotic NER pathways, or their homologous archaeal equivalents, could provide important new therapeutic solutions for certain debilitating diseases and cancers.

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