Nucleotide Excision Repair in Mammalian Cells*

Richard D. Wood‡

From the Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, United Kingdom

A versatile strategy for repairing damaged DNA, termed nucleotide excision repair (NER), is found throughout the natural world in organisms ranging from mycoplasma to mammals. In humans, NER is a major defense against the carcinogenic effects of ultraviolet light from the sun. This repair pathway acts with varying efficiencies on a wide variety of DNA alterations and is especially important for bulky, helix-distorting lesions. The key event in NER is incision of the damaged strand on each side of a lesion in DNA, releasing the damage in a fragment that is ~24–32 nucleotides (nt) long in eukaryotes. Nucleotide excision repair defects of various types are found in individuals with the inherited syndromes xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (1). Individuals with XP are sun-sensitive and generally show a greatly increased incidence of UV-induced skin cancers. The disorder has seven genetic complementation groups, designated XP-A through XP-G, and the normal protein product XPA

Mechanism of Core NER Reaction in Eukaryotes

Many details of the NER reaction mechanism are being elucidated with purified proteins and reconstituted systems. At a minimum, the dual incision reaction of NER requires the factors XPA, RPA, XPC, TFIIH, XPG, and ERCC1-XPF in mammalian cells or the homologous components in yeast (3, 4). These are summarized in Table I. XPA and the single-stranded binding protein RPA associate with one another and are able to preferentially bind to damaged DNA. TFIIH includes the XBP and XPD DNA helicases among its subunits (5) and is involved in local opening of DNA around a site of damage. XPC, usually bound to a protein partner hHR23B, may also be involved in damage recognition and opening. The XPG and ERCC1-XPF factors are structure-specific DNA nucleases and are responsible for cleaving on the 3'- and 5'-sides of a lesion, respectively (Fig. 1). The excised fragment is replaced in a DNA repair synthesis reaction mediated by DNA polymerase δ or ε holoenzyme (6), and the process is completed by sealing the repair patch of about 30 nt with a DNA ligase.

NER Nucleases

The cDNA encoding XPF was recently isolated, completing the list of cloned XP core factors (7, 8). XPF forms a tight complex with the ERCC1 protein, directly homologous to the similar Rad1-Rad10 complex in the budding yeast Saccharomyces cerevisiae. Both the Rad1-Rad10 and ERCC1-XPF complexes have a structure-specific nuclease activity that cleaves near the border between single-stranded and duplex DNA when the single strand has a polarity 5' to 3' moving away from the border. Junctions between duplex and single-stranded DNA are cleaved with the opposite polarity by the XPG protein and its S. cerevisiae homolog Rad2.

The structure-specific cleavage properties of the NER nucleases have strongly suggested that they act on an opened, "bubble" intermediate during repair (Fig. 1D). Evidence for the existence of such an intermediate has been provided by probing for potassium permanganate sensitivity around a lesion during repair in vitro. ATP-dependent opening of a region of approximately 25 base pairs occurs around the damaged site before dual incision (9). Opening does not require the nuclease activity of XPG (9) but is dependent on the DNA helicase activity of TFIIH. XPG and Rad2 are members of a family of enzymes that includes the DNase IV/FEN1 group of structure-specific nucleases which function in DNA replication (10). Two conserved domains in all of these nucleases are related to sequences in a group of prokaryotic exonucleases, exemplified by the 5'-3' exonuclease domain of Escherichia coli DNA polymerase I. The solved structures of eubacterial enzymes in this family, such as bacteriophage T5 5'-3' exo/endonuclease (11), show that the conserved domains are folded together in the predicted active site and coordinate the binding of two Mg²⁺ ions. The T5 enzyme has a helical arch with a size and ionic environment appropriate for single-stranded DNA to thread through, starting at a free end. It will be interesting to learn how DNA is bound during repair by XPG or Rad2, which must load onto sites in DNA where there is no free 5’-end. The intervening region between the conserved domains in the various nucleases in this family probably mediates specific protein-protein interactions for replication and repair. The XPG and ERCC1-XPF nucleases require Mg²⁺ or Mn²⁺ but not ATP and on some model substrates do not require other protein factors for their structure-specific cleavage activity (7, 9). With other substrates, RPA protein can dramatically stimulate nuclease activity (12).

Although the 3'- and 5'-incisions are nearly simultaneous, the 3'-incision mediated by XPG is normally first (13, 14). For some lesions, the 3'-incision can be carried out by purified core incision factors in the absence of ERCC1-XPF (14), although repair of a 1,3-intrastrand d(GpTpG)-cisplatin cross-link in DNA required all components to be present to form either incision (15). After the 5'-incision, mediated by ERCC1-XPF nuclelease (7, 14), the damaged oligonucleotide is excised and seems to be bound to one or more components of the repair complex (14). In repair-proficient cell extracts some uncoupled 3'- or 5'-incisions can occur, indicating that the order of incisions is not always strict, although uncoupled 3'-incisions are more frequent than uncoupled 5'-incisions (7, 14). The precise positions of incisions for several different lesions have been mapped in the human system. Depending on the adduct, incisions are introduced 2–9 phosphodiester bonds away from the 3'-side of a lesion and 16–25 phosphodiester bonds away from...
the 5′-side. A unifying feature is that the modal length of incision products is 26–27 nt in each case (15).

**XPC-hHR23B Complex and Transcription-coupled Repair**

The XPC protein plays a role in NER that is just beginning to be revealed. This 106-kDa human protein copurifies with a tightly bound 43-kDa partner, hHR23B. XPC binds DNA with a preference for single-stranded (16, 17) or damaged DNA (16). In some reconstituted repair systems, recombinant XPC protein alone is sufficient, and adding hHR23B does not increase repair (16). In other fractionated and purified systems, only weak repair occurs with XPC alone, and repair is significantly stimulated by adding hHR23B (17). Intriguingly, the XPC subunit is dispensable for the repair of two different types of DNA structures in vitro, an observation that has implications for the mechanism of transcription-coupled repair in human cells. Transcription-coupled nucleotide excision repair is a specialized mode of NER that removes DNA adducts significantly faster from the transcribed strand of genes than from the non-transcribed strand or the bulk of DNA. In mammalian cells, transcription-coupled repair of pyrimidine dimers can take place in the absence of XPC (while requiring the other NER subunits including XPA and TFIIH) and is believed to be initiated when RNA polymerase is stalled at a lesion in DNA (18). A particular cholesterol moiety incorporated synthetically into a DNA backbone is thought to cause an unusual degree of helix distortion, and NER of this moiety does not require XPC (19). Similarly, a model substrate consisting of a pyrimidine helix distortion, and NER of this moiety does not require XPC into a DNA backbone is thought to cause an unusual degree of helix distortion (20). A particular cholesterol moiety incorporated synthetically initiated when RNA polymerase is stalled at a lesion in DNA resulting in an unusual degree of helix distortion (20). A particular cholesterol moiety incorporated synthetically initiated when RNA polymerase is stalled at a lesion in DNA.

**Accessory Factors and Cellular Interactions**

An XP group that remains to be fully explained at the molecular level is XP-E. XP-E patients are generally mildly affected and show 50% or more residual repair capacity. Cells from some but not all XP-E individuals are missing an activity that binds damaged DNA, as measured in electrophoretic mobility shift experiments. This activity is associated with two polypeptides of 127 and 48 kDa. Sequence changes in the 48-kDa subunit are present in cell lines that are missing the DNA damage-binding activity (20). This XP-E-associated complex is not required for the core NER system but may well play an accessory role.

Further accessory proteins that modulate NER activity are being uncovered. A factor designated IF7 was found to be needed for appreciable repair of UV-irradiated plasmid DNA (21) and stimulates incision at a cisplatin lesion in DNA by about 6-fold, but a gene encoding this stimulatory protein component has not been identified. The p53 protein or some of the downstream target genes controlled by p53 may also be accessory factors for NER. A study with human fibroblasts showed that cells homozygous for p53 mutations have about 50–60% of the normal rate of excision of pyrimidine dimers from the overall genome (22). Although p53 can bind to some components of the NER apparatus, including TFIIH (reviewed in Ref. 23), this binding may not have a direct effect on repair efficiency. Instead, protein products of genes that are transcriptionally regulated by p53, such as Gadd45 and p21cip1/waf1, may enhance NER. There is good evidence that in human cells, less repair of UV damage to DNA occurs in the absence of Gadd45 or p21cip1/waf1 induction (24–26). Post-translational modifications are also likely to regulate the activity of core factors. For example, the proper phosphorylation state of one or more components is necessary to observe significant NER in a cell-free system (27).

NER in eukaryotes might work either by sequential assembly of individual factors or by the action of a preformed “repairstosome” or by the interaction of intermediate subassemblies. Many interactions that would be consistent with any of these possibilities have been documented between NER proteins. An extreme view is that all the required repair factors can reassemble in the cell, and evidence has been provided for such assemblies in yeast (28) and mammalian cells (29). An intermediate hypothesis is that several subassemblies (for example Rad1-Rad10-Rad14 and TFIIH-Rad2 in yeast) exist in the cell, which are then sequentially recruited to a repair site (30). The details of observed interactions vary and depend on the exact purification and analysis procedures. In particular, moderate salt conditions disrupt some interactions that may be significant. A line of study that could help clarify this subject is a systematic and quantitative measurement of the binding constants between different NER proteins.

The densely packed chromosome structure in the nucleus introduces an additional level of complexity, in that chromatin disassembly and reassembly need to be coupled with DNA repair. A recent study analyzed nucleotide excision repair of 3′-side DNA damage (31). DNA helicase (3′ to 5′); also known as ERCC3 DNA helicase (5′ to 3′); also known as ERCC2 TFIIH subunit; also known as GTF2H1 TFIIH subunit; formerly known as p41 TFIIH subunit TFIIH subunit DNA endonuclease for 3′ side of damage; also known as ERCC5 Subunit of DNA endonuclease for 5′ side of damage; also known as ERCC4

| Human protein | Nearest *S. cerevisiae* homolog | Comments                                                                 |
|---------------|---------------------------------|--------------------------------------------------------------------------|
| XPA           | Rad14                           | Preferentially binds damaged and single-stranded DNA                       |
| RPA           | Rpa                             | 3-subunit single-stranded DNA binding protein; binds to XPA               |
| XPC           | Rad4                            | Preferentially binds damaged and single-stranded DNA; not needed for transcription-coupled repair or some DNA lesions   |
| hHR23B        | Rad23                           | Binds to XPC and stimulates activity                                     |
| TFIIH         |                                 |                                                                          |
| XPB           | Ssl2 (Rad25)                    |                                                                          |
| XPD           | Rad3                            |                                                                          |
| p52           | Tbl1                            |                                                                          |
| p52           | Tbl2                            |                                                                          |
| p44           | Ssl1                            |                                                                          |
| p34           | Tbl4                            |                                                                          |
| XPG           | Rad2                            |                                                                          |
| ERCC1         | Rad10                           | Subunit of DNA endonuclease for 3′ side of damage; also known as ERCC5   |
| XPF           | Rad1                            | Subunit of DNA endonuclease for 5′ side of damage; also known as ERCC4   |
DNA in a cell-free system capable of chromatin assembly (31). Chromatin formation occurred concomitantly with repair DNA synthesis and required the chromatin assembly factor CAF-1. Yeast lacking CAF-1 are viable but UV-sensitive (32). Like DNA replication and transcription, NER might be preferentially localized at a limited number of foci in the nucleus. Using immunofluorescence techniques, the XPG protein was observed to change position in the nucleus after UV irradiation of cells, suggesting that NER may occur at specific sites (33).

**Cockayne Syndrome and Transcription-coupled Repair**

Cockayne syndrome is an NER-related disorder of particular current interest. Like XP patients, individuals with CS are also sun-sensitive but show a distinctive array of severe developmental and neurological abnormalities. Classical CS is caused by mutations in the CSA or CSB genes. The disease also occurs simultaneously with XP in rare patients belonging to XP groups B, D, and G. Remarkably, features of CS can therefore be caused by particular mutations in at least five genes (1).

Cells in the CS-A and CS-B groups do not preferentially remove DNA damage such as UV-induced pyrimidine dimers from the transcribed strand of active RNA polymerase II-transcribed genes (34, 35). However, the clinical features of CS are not simply explained by a defect in transcription-coupled repair of pyrimidine dimers, since individuals with complete defects in NER (e.g., most XP-A and some XP-G patients) do not have CS symptoms. One possibility is that like XPB and XPD, CSA and CSB are somehow involved in basal transcription and that particular mutant forms of these proteins lead to subtle transcription defects that can account for the developmental abnormalities of CS (34, 35). The CS proteins have properties consistent with this view; the 44-kDa CSA contains WD repeat motifs, and the 168-kDa CSB has motifs common with the Swi/Snf family (34, 35). Such an explanation still leaves the dilemma of the occurrence of CS in some XP-G patients, even though XPG has no obvious role in transcription. There are indications, however, that XPG may interact with some subunits of TFIIH (36). Moreover, with one purification protocol, active yeast TFIIH copurified with overexpressed Rad2, the S. cerevisiae homolog of XPG (30). It is possible that lack of a proper XPG-TFIIH association can adversely affect transcription, giving rise to the CS symptoms in some XP-G patients. Interestingly, it has been found that CS patients from XP group G have mutations that would produce severely truncated XPG protein, whereas XP-G patients without CS produce full-length XPG protein with mutations that reduce or eliminate the nuclease function (37). The absence of full-length XPG might affect transcription of some genes.

An alternative hypothesis is that CS is instead due to a deficiency in transcription-coupled repair of some types of endogenous oxidative damage (37, 38). Some lesions induced in DNA by active oxygen, such as thymine glycols, are subject to preferential removal from the transcribed strand of active genes. Unlike pyrimidine dimer removal, this strand-specific repair of oxidative damage is apparently independent of XPA, XPF, and the nuclease function of XPG but depends on CSA and CSB. The repair is, however, disrupted in those XP-G cells with severely truncating mutations (38). This suggests that XPG may have a second function in addition to its role in NER, aiding the transcription-coupled repair of some forms of oxidative damage. It might, for example, interact with a transcription complex blocked at a thymine glycol and accelerate thymine glycol-DNA glycosylase-mediated base excision repair at such a site.

It has been further suggested that some lesions in DNA block transcription and are not well removed by normal NER but may only be efficiently dealt with by a CSA- and CSB-dependent repair mechanism. The CSA and CSB proteins might also help RNA polymerase to occasionally bypass damaged sites on a template without repair or play a role in releasing transcription complexes blocked at damage (35, 39). If blocking lesions (for example, certain types of oxidative damage) accumulated with age, these adducts could trap transcription components on the DNA and gradually cripple transcription in CS cells (35). This might help account for the severe neurological symptoms and short lifespan of human CS patients.

How do the CSA and CSB proteins function in transcription-coupled repair? One possibility is that they are “coupling factors,” mediating an interaction between a stalled RNA polymerase and the repair proteins and thereby helping to attract DNA repair to a damaged site on a transcribed strand. Such a
mechanism appears to operate during transcription-coupled repair of pyrimidine dimers in E. coli, where the Mfd protein acts as a coupling factor that promotes the interaction of RNA polymerase with the NER factor UvrA (40). An indication that the CS proteins actually do interact directly or indirectly with RNA polymerase II is the observation that UV light induces modification of the catalytic subunit of human RNA polymerase II by ubiquitination and that this modification is defective in CS-A and CS-B cells (41). It is not yet known if the ubiquitination affects transcription activity or the ability to repair damaged templates.

Connections between Mismatch Repair and Nucleotide Excision Repair

Single-base mismatches and short unpaired loops can arise during DNA replication. To prevent mutations, human cells use a repertoire of homologues of the E. coli mismatch repair proteins MutS and MutL to recognize and initiate correction of mismatches (42). Unexpectedly, disruptions of the DNA mismatch repair genes mutS and mutL were found to reduce transcription-coupled NER of the lactose operon in E. coli (43), and human cells with mutations in particular mismatch repair genes were likewise found to have a deficiency in transcription-coupled repair of UV-induced pyrimidine dimers (44). S. cerevisiae mutants defective in the homologous mismatch repair genes are not, however, defective in transcription-coupled repair of UV-induced DNA damage (45).

The mechanism of any influence of mismatch repair proteins on NER is unknown, but it has been observed that the mismatch binding protein hMsh2 and a functional complex denoted hMutSα (a heterodimer of hMsh2 and hMsh6) can bind to DNA base damage to some extent. This has been demonstrated for pyrimidine dimers and for a 1,2-intrastrand d(GpG)-cisplatin cross-link (46, 47). The binding of hMutSα can be considerably enhanced when the adducts are paired to non-complementary bases during DNA replication. To prevent mutations, human cells require to establish a functional connection in mammalian cells. Studies of physical interactions between mismatch repair proteins and NER proteins should be informative in this regard.

Acknowledgments—I thank the members of my laboratory and Deborah Barnes, Stephanie Kong, and Alan van Goor for helpful comments. Charlie Haden and Robert Plant provided useful assistance in preparing the manuscript.

REFERENCES
1. Lehmann, A. R. (1995) Trends Biochem. Sci. 20, 492–495
2. Lehmann, A. R., Bootsma, D., Clarkson, S. G., Cleaver, J. E., McAlpine, P. J., Tanaka, K., Thompson, L. H., and Wood, R. D. (1994) Mutat. Res. 315, 41–42
3. Wood, R. D. (1996) Annu. Rev. Biochem. 65, 135–167
4. Sancar, A. (1996) Annu. Rev. Biochem. 65, 43–81

Because of space limitations, a number of the citations here are to other specialized review articles.
Nucleotide Excision Repair in Mammalian Cells
Richard D. Wood

J. Biol. Chem. 1997, 272:23465-23468.
doi: 10.1074/jbc.272.38.23465

Access the most updated version of this article at http://www.jbc.org/content/272/38/23465

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 50 references, 27 of which can be accessed free at http://www.jbc.org/content/272/38/23465.full.html#ref-list-1