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DNA Excision Repair Pathways

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Most life forms have the ability to respond to alterations in genomic DNA that occur spontaneously or are caused by environmental agents. Generally, these responses take one of two forms. Cells can either repair the damage and restore the genome to its normal physical and functional state, or they can tolerate lesions in a way that reduces their lethal effects (Friedberg et al. 1995). This brief overview exclusively considers the former cellular response to DNA damage, which represents true DNA repair. However, the tolerance of base damage, typically by replicative bypass, sets the stage for permanent mutations in DNA. In fact, a major function of DNA repair is the prevention of mutations, which can have significant phenotypic consequences, including neoplastic transformation in mammalian cells (Friedberg et al. 1995).

The repair of altered bases in DNA is frequently classified into two major categories that have important mechanistic distinctions. A relatively limited group of lesions in DNA can be repaired in single-step reactions that directly reverse the damage. The light-dependent monomerization of cyclobutane pyrimidine dimers by DNA photolyase is a well-characterized example (Kim and Sancar 1993). DNA photolyases have been extensively characterized from many prokaryotes and fungi, and from vertebrates, including fish (Yasuhira and Yasui 1992) and marsupials (Yasui et al. 1994). However, this mode for the repair of the quantitatively major form of DNA damage induced by ultraviolet (UV) radiation seems to have been lost in placental mammals (Li et al. 1993).

DNA Replication in Eukaryotic Cells

The direct removal of small alkyl groups (such as methyl groups) specifically from the O^6 position of guanine and the O^4 position of thymine in DNA is another notable example of repair by the reversal of base damage. The enzyme that removes alkyl groups from these specific sites in guanine and thymine is designated O^6-methylguanine-DNA methyltransferase and is ubiquitous in nature.

A more general mode of DNA repair, which is the primary topic of this review, is the physical excision of damaged or inappropriate bases from the genome by multistep biochemical reactions. At present, three specific modes of such repair have been identified and characterized in eukaryotes. These are referred to as base excision repair, nucleotide excision repair, and mismatch repair.

BASE EXCISION REPAIR OF DNA IN EUKARYOTES

The term base excision repair (BER) was coined to emphasize that this DNA repair mechanism is characterized by the excision of nucleic acid base residues in the free form (Friedberg et al. 1995). In contrast, nucleotide excision repair (NER) removes damaged nucleotides as part of fragments which are about 30 nucleotides long. The primary and initiating event of BER is the hydrolysis of the N-glycosyl bond linking a nitrogenous base to the deoxyribose-phosphate chain, thereby releasing the free base (Fig. 1). The hydrolysis of N-glycosyl bonds in DNA is catalyzed by a class of enzymes called DNA glycosylases. Multiple DNA glycosylases have been identified in eukaryotic cells (Table 1). Each enzyme removes a limited spectrum of base alterations. Uracil-DNA glycosylase is particularly specific, as it catalyzes the excision exclusively of the base uracil (and 5-fluorouracil) from DNA. Uracil in DNA usually results from the spontaneous or chemically induced deamination of cytosine, although it can occasionally arise by incorporation from small intracellular pools of dUTP or from the deamination of dCTP (Friedberg et al. 1995). Since deamination of cytosine in DNA generates a U•G mispair, excision of uracil is important to avoid G•C→A•T transition mutations during subsequent semiconservative DNA replication. The enzyme from human cells is encoded by the *UNG* gene, which maps to chromosome 12 (Aasland et al. 1990). The amino acid sequence of human uracil-DNA glycosylase shares extensive amino acid sequence identity with the *ung* gene product from a variety of other organisms.

Another highly specific DNA glycosylase is the thymine mismatch-DNA glycosylase, which to date has been identified only in extracts of human cells (Neddermann and Jiricny 1993). This enzyme specifically

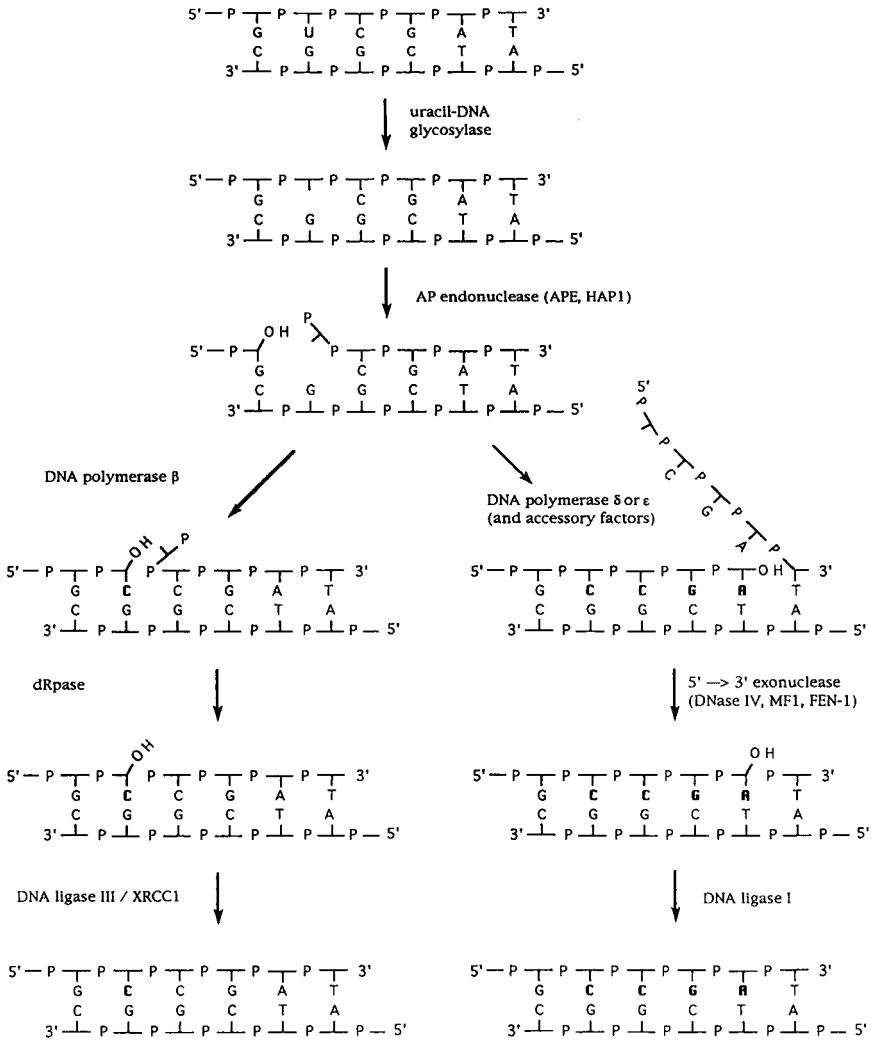

Figure 1 Base excision repair (BER) of DNA in eukaryotes. Repair of a uracil residue initiated by uracil-DNA glycosylase is shown as an example. A branched pathway of repair synthesis is depicted, resulting in either single nucleotide repair patches (*left*) or longer patches of variable size (*right*), depending on which enzymes are utilized. Enzymes implicated in the longer patch repair mode are tentatively assigned. (Adapted, with permission, from Lindahl et al. 1995.)

catalyzes the excision of T when it is mispaired with G, a mispairing that results from the deamination of 5-methylcytosine in DNA. Hence, this form of BER represents one of the several biochemical strategies that hu-

Table 1 DNA glycosylases for base excision repair in eukaryotes

DNA glycosylase	*E. coli* homolog	Substrates
Uracil-DNA glycosylase	*ung*	uracil or fluorouracil in DNA
3-MeA-DNA glycosylase	*alkA*	3-methyladenine hypoxanthine O^2-methylthymine (minor groove alterations)
FaPy-DNA glycosylase	*fpg* (*mutM*)	formamidopyrimidines 8-hydroxyguanine (oxidized and ring-opened purines)
Pyrimidine hydrate-DNA glycosylase	*nth* (*endoIII*)	thymine glycol cytosine hydrate urea (oxidized and ring-opened pyrimidines)
G:T mismatch glycosylase	–	thymine when paired to guanine, O^6-methyl-guanine, or 6-thioguanine

man cells possess for the repair of mismatched bases (see below).

The excision of bases from duplex DNA generates apurinic or apyrimidinic (AP) sites (Fig. 1). The repair of these sites of base loss utilizes a specific class of endonucleases designated AP endonucleases (Friedberg et al. 1995). Prokaryotes such as *Escherichia coli* have at least two such enzymes. However, studies of yeast and mammalian cells have thus far resulted in the purification and characterization of a single major endonuclease that catalyzes the incision of phosphodiester linkages exclusively 5′ to AP sites, generating 3′-OH and 5′-deoxyribose-phosphate residues (Fig. 1). This enzyme also has 3′-phosphatase activity, and, in some organisms, a weak 3′→5′ exonuclease activity. In mammalian cells, the gene that encodes this AP endonuclease is variously called *BAP1* (bovine AP endonuclease), *APEX* (AP endonuclease/exonuclease), *HAPI* (human AP endonuclease), or *APE* (AP endonuclease) (Demple et al. 1991; Robson et al. 1991, 1992; Seki et al. 1991). Intriguingly, the HAPI/APE protein was also independently isolated as REF-1, a redox factor that can regulate the Fos-Jun transcriptional activation proteins (Walker et al. 1993; Xanthoudakis et al. 1994). Thus, in addition to its role in BER, this protein may play a role in transducing signals associated with oxidative stress to a regulatory network which in-

volves genes associated with the metabolism of reactive oxygen species. Like human uracil-DNA glycosylase, the HAP1 protein shows extensive evolutionary conservation at the amino acid sequence level, and the protein can correct some of the mutant phenotypes of *E. coli* cells defective in Xth protein, the major AP endonuclease in that organism.

The completion of BER requires the removal of the 5′-terminal deoxyribose-phosphate residue generated by the AP endonuclease, followed by repair synthesis and DNA ligation. A 47-kD enzyme activity designated DNA deoxyribophosphodiesterase (dRpase) has been identified in human cells and can remove such sugar-phosphate residues from duplex DNA (Price and Lindahl 1991). If this enzyme is indeed utilized in vivo, a single nucleotide gap would be generated that can be filled in by a DNA polymerase (Fig. 1). In vitro, such repair synthesis is efficiently catalyzed by DNA polymerase-β (Dianov et al. 1992). It has been suggested that because of its limited fidelity, polymerase-β may be utilized in this particular very short patch mode of repair synthesis during BER and not in repair synthesis associated with longer repair patches.

BER is sometimes associated with the generation of longer repair patches. There are experimental indications that DNA polymerases δ and ε are primarily involved in this second BER synthesis mode in both yeast and human cells. These longer repair tracts are thought to result from a nick translation reaction accompanied by strand displacement in the 5′→3′ direction, thereby generating a flap type of structure (Fig. 1). Removal of the overhanging 5′-terminal single-stranded region of DNA is believed to be effected by a 5′ single-strand/duplex junction-specific nuclease originally designated DNase IV (Lindahl et al. 1969; Robins et al. 1994) and more recently as FEN-1 (Harrington and Lieber 1994). This nuclease, which is conserved in the yeasts *Saccharomyces cerevisiae* as Rad27 protein and in *Schizosaccharomyces pombe* as rad2 protein (Murray et al. 1994), has also been implicated in lagging-strand DNA synthesis during semiconservative replication (Ishimi et al. 1988; Turchi et al. 1994; Waga et al. 1994). Inspection of the predicted amino acid sequences of DNase IV, Rad27, *S. pombe* rad2 protein, and various prokaryotic DNA polymerases endowed with 5′→3′ exonuclease activity (such as *E. coli* DNA polymerase I), shows limited regions of amino acid sequence homology (Table 2). Interestingly, this homology is shared with junction-specific nucleases that are involved in NER in *S. cerevisiae* and in mammalian cells (Table 2; see later discussion).

The final biochemical event during BER is DNA ligation. Mammalian cells (and possibly the yeast *S. cerevisiae*) contain several DNA ligases. These are fully discussed in chapter 20. A recently recognized

Table 2 Amino acid sequence homology between various nuclease families

E. coli pol I	(101)	-MGLPLL--SGV**EAD**D-IG-LAR-A---G
Bacillus caldotenax pol I	(97)	-Y-IP-Y----Y**EAD**D-IG-LA--A---G
Streptococcus pneumoniae pol I	(102)	-MGI--Y--A-Y**EAD**D-IG-L-K-A---G
Thermus flavus pol I	(104)	LLGL--L--PGF**EAD**D-LA-LAK-A---G
T4 rnh (orfA)	(119)	YMPY-VM----Y**EAD**D-IAVL-K---L-G
T5 D15	(116)	---FP-F---GV**EAD**D--AYI-K----L-
T7 6	(121)	---F-CI--P-L**EGD**D-MGVIA--P--FG
S. cerevisiae Rad27	(145)	LMGIPYI--AP-**EAE**AQCA-LAK-GKVYA
S. pombe Rad2	(147)	LMGIPFV--APC**EAE**AQCA-LARSGKVYA
Mus musculus FEN-1	(145)	LMGIPYL--AP-**EAE**A-CA-LAKAGKVYA
Homo sapiens DNase IV	(147)	LMGIPYL--AP-**EAE**A-CA-L-KAGKVYA
S. cerevisiae Rad2	(781)	-FGIPYI--APM**EAE**AQCA-L-----V-G
S. pombe Rad13	(767)	LFGLPYI--AP-**EAE**AQCS-L-----V-G
Xenopus laevis XPG	(811)	LFGIPYI--APM**EAE**AQCAIL--T----G
H. sapiens XPG	(778)	LFGIPYI--APM**EAE**AQCAIL--T----G

The various nucleases shown are grouped according to known functions. The top group are DNA polymerases with associated nuclease activities. The next group are members of the eukaryotic DNase IV family, followed by the *S. cerevisiae* Rad2 nuclease family. The numbers in parentheses refer to amino acid position. Only identical or related amino acids are shown. The dashes indicate nonconserved positions. The EA(G)E(D) motif that is conserved in all 15 sequences is shown in bold. (Adapted, with thanks, from Stuart G. Clarkson.)

aspect of DNA metabolism that can affect the kinetics of BER is the potential competition for strand breaks in DNA between the enzymatic machinery required for the completion of this repair process and the enzyme poly(ADP-ribose) polymerase. This enzyme normally has a high affinity for strand breaks in DNA. However, in the presence of NAD^+, the bound enzyme undergoes extensive autoribosylation, which results in decreased binding affinity for DNA and its eventual dissociation (Molinete et al. 1993; Satoh et al. 1993). It has been suggested that the binding of non-ribosylated poly(ADP ribose) polymerase to strand breaks introduced by the sequential action of a DNA glycosylase and AP endonuclease during BER may signal a slowing or cessation of DNA replication while BER takes place. Alternatively, or additionally, the binding of poly(ADP-ribose) polymerase to DNA may serve to reduce the potential for the initiation of recombination at sites of strand breakage (Lindahl et al. 1995).

NUCLEOTIDE EXCISION REPAIR OF DNA IN EUKARYOTES

NER is characterized by the excision of damaged bases in oligonucleotide fragments. In contrast to the limited substrate specificity of most DNA glycosylases, NER operates on a large spectrum of base damage, particularly that produced by environmental mutagenic and carcinogenic agents which produce bulky, helix-distorting perturbations in DNA structure. In human cells, NER is the principal mechanism by which base damage produced by UV radiation is removed from DNA. Individuals with the rare inherited disorder xeroderma pigmentosum (XP) have defects in NER genes (Table 3), generally leading to a greatly increased risk of sunlight-induced skin cancer (Cleaver and Kraemer 1989).

There is now compelling evidence that in both prokaryotes and eukaryotes, the oligonucleotides excised during NER are generated by dual incisions which flank sites of base damage. Unlike BER, which is believed to require no more than 5 proteins to complete the entire process, there is good evidence that in eukaryotes the events that precede repair synthesis and DNA ligation during NER require the participation of between 15 and 20 gene products (Table 3). This degree of biochemical complexity is reminiscent of that associated with the initiation of basal transcription by RNA polymerase II. Indeed, it has been suggested that the generation of a transcription "bubble" as part of the process of promoter clearance during RNA polymerase II transcription, and the generation of a bubble that defines the single-strand/duplex DNA junctions required for bimodal incision during NER (Fig. 2), may be mechanistically related (Hoeijmakers and Bootsma 1994). This suggestion is to a large extent prompted by the recent discovery that both in the yeast *S. cerevisiae* and in human cells the processes of NER and RNA polymerase II-mediated transcription share multiple proteins in common (Table 3) (Bootsma and Hoeijmakers 1993; Chalut et al. 1994; Drapkin and Reinberg 1994; Friedberg et al. 1994).

Among the many proteins required for the initiation of RNA polymerase II transcription in *S. cerevisiae* (all of which are encoded by essential genes) are a complex of six polypeptides designated core TFIIH (Table 3) (Svejstrup et al. 1995). This core complex is believed to assemble with three other proteins (TFIIK) endowed with kinase activity for the carboxy-terminal domain of the largest subunit of RNA polymerase II, to yield a holoTFIIH supercomplex, the form of TFIIH that is functional in transcription initiation (Svejstrup et al. 1995). Four of the six subunits of core TFIIH have been directly shown to be indispensable for NER in this yeast (Table 3) (Svejstrup et al. 1995). In crude extracts of *S. cerevisiae* that are competent for RNA polymerase II basal transcription,

Table 3 Eukaryotic nucleotide excision repair genes and proteins

Human gene	Human map position	*S. cerevisiae* homolog	*S. pombe* homolog	M_r (human gene product unless indicated otherwise)	Comments
XPA	9q34.1	*RAD14*		31 kD (40/42 kD on gels)	binds damaged DNA
XPB/ERCC3	2q21	*SSL2* (*RAD25*)	*ercc3*$^{sp+}$	89 kD (89 kD on gels)	3′→5′ DNA helicase; in TFIIH
XPC	3p25	*RAD4*		106 kD (125 kD on gels)	binds ssDNA
HHR23B	3p25	*RAD23*		43 kD (58 kD on gels)	associated with XPC
XPD/ERCC2	19q13.2	*RAD3*	*rad15*$^{+}$	87 kD (80 kD on gels)	5′→3′ DNA helicase; in TFIIH
XPG/ERCC5	13q33	*RAD2*	*rad13*$^{+}$	133 kD (180–200 kD on gels)	CS in some affected individuals; DNA nuclease
XPF/ERCC4? (ERCC4)	16p13.13	*RAD1*	*rad16*$^{+}$	126 (*S. cerevisiae*)	component of DNA nuclease
ERCC1	19q13.2	*RAD10*	*swi10*$^{+}$	31 (human) (39 kD on gels)	component of DNA nuclease
p44	5q13	*SSL1*		44 kD (yeast 50 kD)	in TFIIH
p62	11p14-15.1	*TFB1*		70–73 kD in yeast	in TFIIH
p52	6p21.3-22.2	*TFB2*		55 kD in yeast	in TFIIH
CSA/ERCC8	5	*RAD28*		44 kD	WD-repeat protein
CSB/ERCC6	10q11.2	*RAD26*		168 kD	DNA helicase? transcription coupling?
RPAp70	17p13	*RFA1*		68 kD (70 kD on gels)	binding to
RPAp32	1p35-36.1	*RFA2*		29 kD (34 kD on gels)	single-stranded
RPAp14	7p21-22	*RFA3*		13.6 kD	DNA
LIG1	19q13.2-3	*CDC9*	*cdc17*$^{+}$	102 kD (120 kD on gels)	DNA ligase I
RFC1		*CDC44*		140 kD	RF-C large subunit
PCNA	20	*POL30*	*pcn1*$^{+}$	29 kD (36 kD on gels)	toroidal sliding clamp

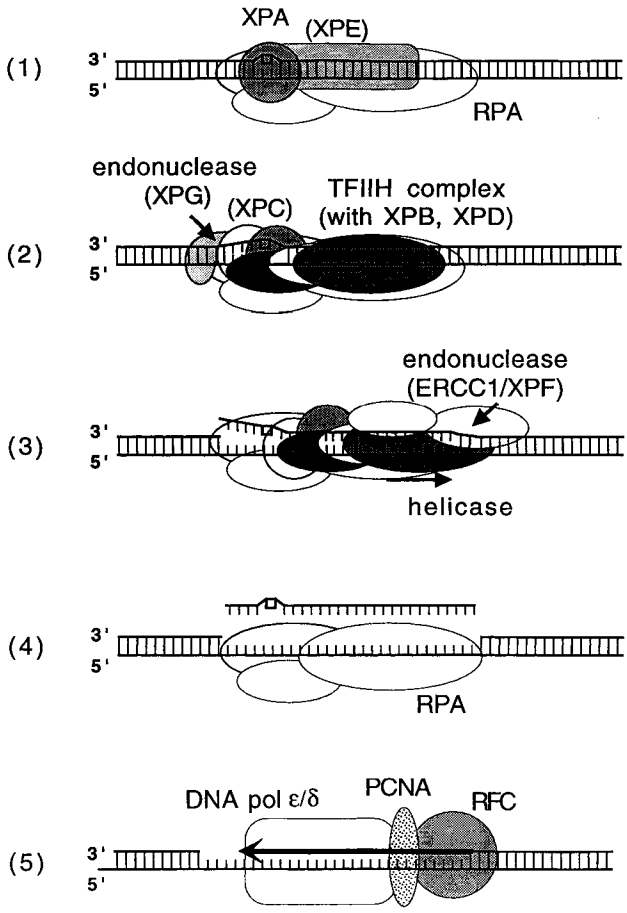

Figure 2 Nucleotide excision repair (NER) in mammalian cells. The steps shown are (1) DNA damage recognition; (2) incision on the 3′ side of the damage; (3) the generation of a further opened structure by the helicase function(s) of TFIIH and incision on the 5′ side of the damage; (4) release of a damage-containing oligonucleotide; (5) repair synthesis.

a different supercomplex can be identified that includes all six subunits of core TFIIH plus at least five other proteins which are indispensable for NER but are not known to be required for transcription, namely, Rad1, Rad2, Rad4, Rad10, and Rad14. This supercomplex comprising at least these 11 proteins is referred to as the nucleotide excision repairosome (Svejstrup et al. 1995). There are indications of comparable protein complexes for transcription and NER in human cells (Roy et al. 1994).

The observation that core TFIIH is common to both repair and tran-

scription may help explain the fact that in both yeast and mammalian cells NER takes place significantly faster in the template strand of transcriptionally active genes than in the nontranscribed strand (Bohr 1992; Hanawalt 1992). However, it remains to be determined whether the particular form of TFIIH that participates in transcriptionally coupled NER is the same as that loaded onto the DNA during transcription initiation as part of the TFIIH holocomplex. It is intuitively compelling to consider that when the TFIIH holocomplex is loaded onto promoter sites, the NER proteins are retained in the transcription elongation complex. Thus, if transcription is arrested at sites of base damage in the template strand, the core TFIIH complex would constitute a strategically positioned nucleation site for the assembly of a functionally active repairosome. However, there is as yet no direct experimental evidence for this mechanism. An alternative possibility is that TFIIH does not participate in the process of transcription elongation. There is indeed some evidence for this view (Drapkin and Reinberg 1994; Goodrich and Tjian 1994). In this event, repair proteins would be recruited to sites of arrested transcription at base damage by a mechanism(s) that is yet to be specifically determined. Additionally, in mammalian cells, most of the genome is transcriptionally silent, so NER frequently occurs in the absence of a coupling to transcription. Yet components of TFIIH are still required, and it remains to be discovered precisely how these are delivered to damaged sites in transcriptionally silent regions of the genome.

Biochemical functions have been identified for several NER proteins (Table 3). The bimodal incision mechanism involves single-strand/duplex junction-specific nucleases. In *S. cerevisiae* the nuclease that is believed to incise DNA 5′ to sites of base damage is carried in the Rad1/Rad10 protein complex (Tomkinson et al. 1993). The mammalian homologs of Rad1/Rad10 are ERCC4 and ERCC1, respectively (Table 3). In vitro, the yeast Rad1/Rad10 endonuclease (and presumably the ERCC1/XPF complex in mammalian cells) cuts splayed-arm substrates specifically at 3′ single-strand/duplex junctions where the single strand has a 3′ end (Bardwell et al. 1994). Reciprocally, purified human XPG protein cuts splayed-arm substrates specifically at single-strand/duplex junctions where the single strand has a 5′ end, and additionally cuts bubble substrates with a consistent polarity (O'Donovan et al. 1994). Thus, XPG protein is believed to incise DNA on the 3′ side of damage during NER. Presumably the homologous Rad2 protein acts similarly (Harrington and Lieber 1994). Rad2 protein (Habraken et al. 1993), Rad1/Rad10 complex (Tomkinson et al. 1993; Sung et al. 1993), and XPG protein (O'Donovan et al. 1994; Habraken et al. 1994) also can cut bac-

teriophage M13 DNA, presumably at single-strand/duplex junctions at hairpin loops in such DNA. Thus, it seems reasonable to conclude that bimodal damage-specific incision during NER in eukaryotes is achieved by the concomitant or sequential actions of the Rad1/Rad10 and Rad2 nucleases in yeast, and by the ERCC1/XPF and XPG nucleases in mammalian cells (Fig. 2). There are indications that the XPC protein may also participate in the incision process (Shivji et al. 1994).

Studies on NER suggest that the excised oligonucleotide fragments have a precise size of approximately 30 ± 2 nucleotides in human (and presumably in yeast) cells. Based on the established specificity of the endonucleases just discussed for single-strand/duplex junctions, we are led to the model that an open structure of about 30 nucleotides is somehow generated in damaged DNA during NER in eukaryotes. Regardless of precisely how the TFIIH core complex is loaded onto DNA, the known biochemical properties of two components of this complex may help explain how such an open structure might arise. Both the yeast Rad3 (human XPD) and Ssl2 (human XPB) proteins are DNA helicases with opposite directionality. It is therefore possible that one or both of these helicases unwind limited regions of duplex DNA on either side of a damaged site during NER (Fig. 2). There is indeed extensive evidence that the 5′→3′ helicase function of Rad3 protein is specifically required for NER but not for transcription. The 3′→5′ helicase function of yeast Ssl2 (XPB) protein is essential for the viability of yeast cells and is therefore presumably indispensable for transcription as well.

It is not yet established how endonucleolytic cleavage is directed specifically to the DNA strand containing a lesion. The yeast Rad14 and homologous human XPA proteins are DNA-binding proteins with preferential affinity for certain types of DNA damage (Robins et al. 1991; Guzder et al. 1993; Jones and Wood 1993; Asahina et al. 1994). Presumably, these proteins play some role in the recognition of base damage. The XPE protein may also participate in this process (Chu and Chang 1988; Hirschfeld et al. 1990; Keeney et al. 1993; Takao et al. 1993; Payne and Chu 1994). It has also been shown that the helicase function of purified Rad3 (human XPD) protein is arrested by the presence of many types of base damage, specifically in the strand on which the protein translocates (Naegeli et al. 1992; Sung et al. 1994). Hence, Rad3 (XPD) protein may also be an important player in damage recognition, both in the nontranscribed bulk of the genome and in transcribed regions of DNA. Specific protein-protein interactions, such as found between XPA and ERCC1, may help direct endonucleolytic cleavage to the correct strand (Li et al. 1994; Park and Sancar 1994).

In addition to the proteins discussed above, there are indications that other polypeptides are involved in NER. In yeast, these include the *RAD7*, *RAD16*, and *RAD23* gene products, but the precise functional role(s) of these proteins remains to be determined. Recent studies using a cell-free system for NER in yeast indicate an absolute requirement for Rad7 protein (Z. Wang and E.C. Friedberg, unpubl.). The single-stranded DNA-binding replication protein A (RP-A) is necessary for NER supported by mammalian cell extracts in vitro, where it participates during DNA repair synthesis. Additionally, RP-A is required for damage-specific incision (Coverley et al. 1992; Shivji et al. 1992). The homolog of this heterotrimeric protein in *S. cerevisiae* is encoded by the *RFA* genes, and yeast strains carrying mutations in the *RFA1* gene are abnormally sensitive to UV radiation, suggesting a role for RP-A in NER in vivo (Longhese et al. 1994).

The repair synthesis step of NER in mammalian cells requires the DNA polymerase accessory factor proliferating cell nuclear antigen (PCNA) in vitro (Shivji et al. 1992), and there is also evidence that PCNA is involved in vivo (Celis and Madsen 1986; Toschi and Bravo 1988; Miura et al. 1992; Hall et al. 1993; Jackson et al. 1994). These observations, together with experiments with chemical inhibitors, implicate the PCNA-dependent DNA polymerases δ or ε in the repair synthesis step. Perhaps either enzyme works in this function, but DNA polymerase-ε appears to be the most suitable candidate in vivo (Nishida et al. 1988; Syväoja et al. 1990). Recent studies have shown that DNA polymerase-ε is also functionally well suited for NER in vitro, and in the presence of RP-A, replication protein C (RP-C) is also required (R.D. Wood et al., unpubl.). RP-C functions to load PCNA onto a DNA template in order to initiate DNA synthesis (Podust et al. 1994). The repair synthesis patch in vitro (Hansson et al. 1989; Shivji et al. 1992) and in vivo (Cleaver et al. 1991) is about 30 nucleotides long, reflecting precise filling of the gap created by the excision of oligonucleotides.

MISMATCH REPAIR IN EUKARYOTES

Mismatches can arise in DNA by two primary mechanisms. Replicative DNA polymerases do not copy templates with complete accuracy, so mismatches can arise because of replicative errors. Additionally, heteroduplexes formed as recombination intermediates between two homologous pieces of DNA (such as two alleles of a gene) can contain mismatches arising from polymorphisms. The former mechanism is the most important source of mismatches in somatic cells.

General (so-called long-patch) mismatch repair is best understood in *E. coli*, where the core enzymes of the system are the products of the *mutH*, *mutL*, and *mutS* genes (Modrich 1991; Friedberg et al. 1995). Mismatch repair can only protect cells from permanent mutations if the parental strand (containing the correct information) can be accurately distinguished from the daughter strand. In *E. coli*, the strand discrimination signal is provided by adenine methylation in GATC sequences; newly replicated DNA is not yet methylated on the daughter strand (Modrich 1991; Friedberg et al. 1995). The MutH protein binds to DNA at hemimethylated GATC sequences and effects incision on the unmethylated strand. MutS protein recognizes and binds to the mismatch, and the intervening region (often hundreds of nucleotides long) is excised and recopied by a DNA polymerase (Modrich 1991; Friedberg et al. 1995). The MutL protein mediates communication between the distantly bound MutH and MutS products, bringing them together by looping out the intervening region of DNA (Fig. 3) (Modrich 1991).

Long-patch mismatch excision repair is a highly conserved process that appears to work in a similar way in eukaryotes as in *E. coli*, except that strand discrimination does not appear to be methyl-directed in eukaryotes (Friedberg et al. 1995). Thus, there may be no eukaryotic MutH homolog. Instead, the strand-discrimination signal is thought to be provided by single-stranded nicks or gaps in newly replicated DNA, which have not yet been joined by a DNA ligase. A protein that recognizes these nicks or gaps would replace the function of MutH. However, several structural homologs of MutL and MutS have been isolated from yeast and from mammalian cells. Of the two known MutS homologs, one (hMSH2) is a nuclear protein and the other (hMSH1) is mitochondrial. Interestingly, there are at least three MutL homologs in humans (hMLH1, hPMS1, and hPMS2). hMSH2 has been demonstrated to be a DNA mismatch-binding protein in vitro (Palombo et al. 1994). It seems probable that the MutS and MutL proteins form complexes with one another in different combinations to facilitate recognition of a wide range of different types of mismatches, ranging from common single base-pair mismatches such as G•T, through loop-outs of one, two, or more nucleotides.

Inactivation of mismatch repair genes in humans is clearly implicated in the pathogenesis of hereditary non-polyposis colon cancer (HNPCC). Individuals with this condition inherit an inactivated allele of a mismatch repair gene, and colorectal carcinomas in these individuals (as well as sporadic tumors from non-HNPCC patients) have two inactivated alleles. The mismatch repair defect increases the spontaneous mutation rate in

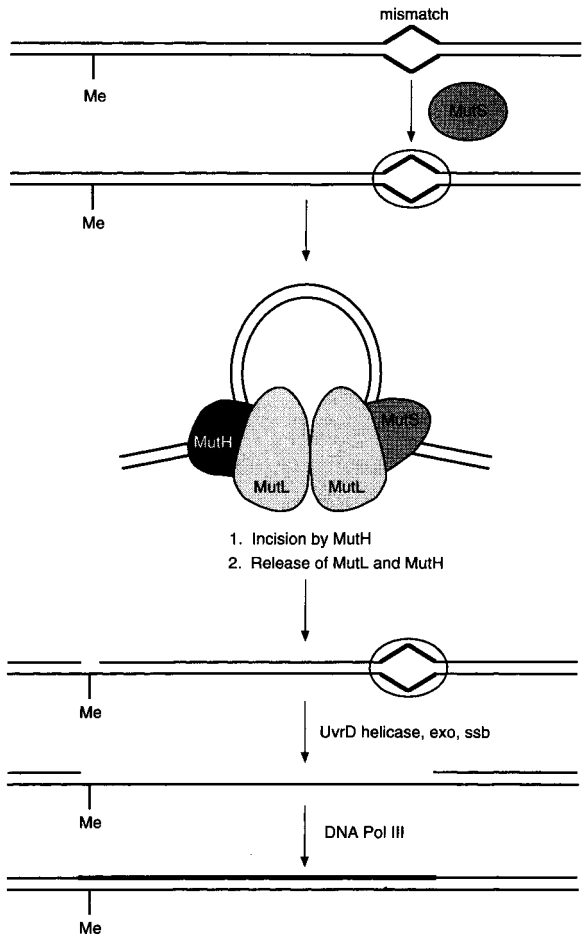

Figure 3 General (long-patch) mismatch repair of DNA in *E. coli*. The eukaryotic process is believed to share many of the features shown here. See text for details.

the cells. This hypermutable state is thought to be an early event in the progression of tumors to malignancy, as it greatly accelerates the acquisition of mutations in other tumor suppressor genes and oncogenes. Thus far, HNPCC families have been found with mutations in the hMSH2, hMLH1, hPMS1, and hPMS2 genes (Fishel et al. 1993; Leach et al. 1993; Bronner et al. 1994; Nicolaides et al. 1994; Papadopoulos et al. 1994). A notable characteristic of colorectal carcinoma cell lines is their high rate of polymorphism in microsatellite repeat sequences. During DNA replication, di- or trinucleotide repeat units in such sequences can

be accidentally lost or gained by replication fork slippage. In the absence of mismatch repair, the lengths of the microsatellite sequences change more rapidly than in normal cells, reflecting the hypermutable state (Aaltonen et al. 1993).

An interesting feature of mismatch repair-defective cells is an association with tolerance to simple DNA N-nitroso-methylating agents such as MNNG and MNU (Karran and Bignami 1994). This tolerance results because the most toxic DNA adduct produced by such agents, O^6-methylguanine, can pair with thymine and the resulting G^{O6Me}•T base pair is recognized as a mismatch. However, G•T mismatches are nearly always repaired by removal of the T residue, so futile cycles of mismatch repair are initiated. The major alternative pathway for the removal of O^6-methylguanine is by the O^6-methylguanine-DNA methyltransferase referred to earlier, but many cells, particularly those that are transformed, spontaneously lose expression of this enzyme. In such cases, the repeated excision cycles of futile mismatch repair eventually lead to lethal strand breaks (Karran and Bignami 1994). Thus, there may be selective pressure for loss of mismatch repair in cells that are frequently exposed to methylating agents. Colon cells are exposed to bile acids that can be converted into methylating compounds (Karran and Bignami 1994). Cells with defective O^6-methylguanine-DNA methyltransferase and defective mismatch binding proteins become more resistant to DNA-methylating agents, but are hypermutable and show microsatellite instability (Branch et al. 1993; Kat et al. 1993; Aquilina et al. 1994).

Mismatch repair can be studied in vitro and the entire repair process can be carried out in mammalian cell extracts, so details of the biochemistry of this excision repair mode are emerging rapidly.

CONCLUDING REMARKS

The general topic of excision repair of DNA in eukaryotes has undergone many exciting developments in recent years. The biochemistry of BER is essentially fully defined in vitro and is consonant with the genetics of this process. It has additionally been firmly established that NER is a complex biochemical process involving a large number of gene products. The findings in a number of laboratories that some components of the NER machinery are also components of the RNA polymerase II basal transcription apparatus have added new and exciting dimensions to this DNA repair mode, including new insights into the possible molecular pathogenesis of human hereditary diseases associated with defective NER (Friedberg et al. 1994; Vermeulen et al. 1994). Equally dramatic

strides have been made in deciphering the mechanism of strand-directed mismatch repair in eukaryotes. The association of defective NER and mismatch repair with a variety of human cancers provides convincing support for the somatic mutation hypothesis of neoplastic transformation and the crucial role of DNA repair in protecting against this consequence of DNA damage.

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