

Chapter 17

Base Excision Repair and Nucleotide Excision Repair

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1. GENERAL OVERVIEW AND HISTORICAL PERSPECTIVES OF TWO DNA EXCISION-REPAIR PATHWAYS, BER AND NER

Base excision repair (BER) and nucleotide excision repair (NER) are two major DNA excision-repair pathways. They are conserved among eukaryotes from yeast to mammals, and prototype-repair systems exist in prokaryotes including *Escherichia coli*. It is well established that deficiencies in BER and NER can lead to mutations and cell death after exposure of cells to exogenous and endogenous forms of DNA-damaging agents. Biochemical, cell biological, and genetic studies unequivocally support the notion that BER and NER are pivotal for cells to survive exposure to different forms of DNA damage. If left unrepaired, mutations and cell death are unavoidable, and diseases arise in multicellular eukaryotes. Therefore, BER and NER have been intensively studied in molecular toxicology.

BER is capable of repairing small base damage, apurinic/apyrimidinic sites (AP-site, lacking a base), and DNA single-strand breaks [1–3]. NER, on the other hand, repairs relatively large (“bulky”) adducts of DNA. These include

photoproducts formed by ultraviolet irradiation and a multitude of base modifications produced by exposure to chemical carcinogens such as benzo(a)pyrenes and other aromatic hydrocarbons, aflatoxins, 2-acetylaminofluorenes, and chemotherapeutic agents such as platinum [4–8].

Four processes occur during both BER and NER: (1) recognition of damaged DNA, (2) excision of the damage, (3) DNA synthesis to fill the nucleotide(s) gap, and (4) the sealing of nicks (3'-OH and 5'-P pairs without gaps) in DNA. This simplified description will be expanded upon in greater detail later. Many BER and NER genes were identified and cloned by the mid-1990s, and we saw significant progress in understanding these core BER and NER reactions by using purified DNA-repair enzymes.

However, multicellular organisms conduct DNA repair in the context of the entire organism. Consequently, it is important to investigate and understand how DNA-repair proteins communicate with factors controlling cell-cycle checkpoints and apoptosis, and discern whether damage introduction and repair are influenced by other cellular processes such as transcription and DNA replication. These signaling activities are often referred to DDR (DNA-damage response), and they are currently extremely active topics of research. The sequencing of mammalian genomes and the development of new genomic approaches have required refinement of earlier studies of DNA repair to consider the consequences of the highly complex and dynamic DDR networks, to reveal the full scale of the cellular mechanisms needed to recover from DNA damage. We describe the basic mechanisms of BER and NER, and discuss recent advances in DDR that may functionally unite components of the BER and NER pathways.

2. MAMMALIAN BER

2.1 History and Overview of BER

Many BER proteins are relatively small, ranging from 20 to 60 kDa, and many enzymatic activities can be detected in biochemical assays without forming multi-subunit structures. This is in sharp contrast to many components of the NER pathway. In the late 1960s, enzymes functioning in BER were purified and characterized in studies using *E. coli*. An endonuclease that can recognize and cleave AP sites was biochemically isolated in the late 1960s and characterized in the 1970s. Also a uracil DNA glycosylase that recognizes and removes uracil in DNA to generate AP site was characterized by the early 1970s [9–11]. These studies helped scientists construct the concept of BER in the 1970s of a systematic DNA-repair pathway for small base damage [12]. This also had the important ramification for the understanding that cells are continuously attacked by not only exogenous DNA-damaging agents, but also by endogenously generated damage [13]. Since it was understood that the concept of “decaying DNA” was associated with mutation and genetic evolution, BER was then recognized as an essential cellular function. The identification, cloning, and characterization of many BER genes and recombinant proteins of *E. coli* occurred during the 1970s to 1980s. This was followed by similar advances in understanding BER in yeast and mammalian cells. Cloning the BER genes led to detailed genetic and biochemical characterization and the elucidation of X-ray crystal structures of many BER proteins [14,15]. By the end of the 1990s, a clear picture of the BER pathway was drawn. However, questions remained unsolved regarding the efficiencies of the recombinant BER proteins, particularly those of DNA glycosylases that carry out the first base removal steps. Purified DNA glycosylases exhibit very low catalytic activities when studied in vitro which questioned how BER proteins in cells succeeded in maintaining genomic integrity [16]. Based on studies carried out mostly in the 2000s, it became apparent that the BER reactions are coordinated to bring about efficient repair. A DNA–protein complex formed by a BER enzyme (eg, APE1) and the resulting cleaved DNA (eg, DNA cleaved by APE1) is in a conformation favored for interacting with a BER enzyme carrying out the next reaction (eg, DNA polymerase beta, Polβ) [17–19]. The BER coordination achieved by this “hand-off” mechanism is ensured by XRCC1, a BER scaffolding protein critical for facilitating the BER efficiency in vivo [20–24]. Understanding the coordination of BER that involves the scaffolding protein XRCC1 and the damage sensory protein poly(ADP-ribose) polymerases (PARPs) has greatly increased the possibility of modulation of BER in the intervention of diseases including cancer and neurodegeneration [25–27].

2.2 Types of DNA Damage Repaired by BER

We can define the BER pathway as a series of reactions by proteins that are capable of repairing abnormal bases, AP sites, and DNA single-strand breaks (SSBs).

2.2.1 Base Damage and DNA Single-Strand Breaks

DNA bases are vulnerable to alkylation, deamination, and oxidation.

2.2.1.1 Alkylation

A number of DNA-alkylating agents are known, including methyl methanesulfonate (MMS), 1-methyl-3-nitro-1-nitrosoguanidine (methylnitronitrosoguanidine; MNNG), and *N*-nitroso-*N*-methylurea (NMU) [1]. Temozolomide is an alkylating agent that is an FDA-approved chemotherapeutic drug used for glioblastoma treatment [28,29]. Alkylation of purines may also occur endogenously with S-methyladenosine [30]. N7- and N3-alkyl purines are the major adducts in DNA caused by alkylating reagents; more than 80% of adducts produced by MMS are N7-alkylguanine, and about 10% are N3-alkyladenine [31]. Alkylated purines become highly unstable, and readily undergo depurination (loss of purine bases) in physiological conditions [32]. It is noted that O⁶-methylguanine produced by alkylating agents is a highly mutagenic base adduct, but in mammals the lesion is repaired by a single enzyme MGMT (O⁶-methylguanine-DNA methyltransferase) through a direct reversal mechanism [33].

2.2.1.2 Deamination

Exocyclic amino groups in the bases are subject to deamination. Deamination at N4 of cytosine results in the conversion of cytosine to uracil. Similarly, deamination of adenine at N6 changes the purine base to hypoxanthine. These reactions are mutagenic as uracil pairs with adenine in DNA, and hypoxanthine with cytosine. Another important deamination reaction occurs at N4 of 5-methylcytosine (5mC). 5mC is the result of methylation in CpG di-nucleotide in mammalian cells. Deamination of 5mC converts cytosine to thymine, and thus generates a G:T mispair which is mutagenic. These incorrect uracil and thymine bases are removed by uracil DNA glycosylase and thymine DNA glycosylase in the BER pathway (see Section 2.3.1 and Table 17.1).

2.2.1.3 Oxidation

Reactive oxygen species (ROS) are continuously generated in cells [3]. The mitochondrial respiratory chain is the major source of ROS, as the electron transport system in the inner mitochondrial membrane builds a necessary redox gradient, and electron leaks inevitably occur and are trapped by oxygens to produce superoxide (O₂^{•−}) [34]. O₂^{•−} are effectively scavenged by mitochondria-specific superoxide dismutase Mn-SOD (SOD2). SOD2 is an extremely efficient enzyme that can easily prevent O₂^{•−} from accumulating inside cells. However, this reaction creates hydrogen peroxide, and in the presence of redox metals such as iron and copper, hydrogen peroxide may be further processed to hydroxyl radical (OH•) via the Haber–Weiss reaction [3]. OH• is highly reactive and readily attacks DNA to produce a plethora of different types of oxidative DNA base damage (reviewed in Hegde et al. [2] and Evans et al. [35]). Moreover, ROS also directly attacks the DNA backbone to produce SSBs [2]. SSBs produced by ROS often possess unusual 3′-end structures including 3′-phosphate and 3′-phosphoglycolate as the major products, and these have to be processed to 3′-OH termini in order for the repair process to be completed.

2.2.2 SSBs With Tyrosyl–DNA Covalent Linkage

Mammals possess three topoisomerases I, II, and III (TOP1, TOP2, and TOP3), that resolve higher-order supercoils and knot structures in DNA by introducing single-strand nicks in the DNA (TOP1 and TOP3), or DNA double-strand breaks (DSBs; TOP2) [36,37]. Topoisomerases form tyrosyl–DNA–phosphodiester covalent bonds as intermediate products during the reactions. The tyrosyl–DNA complex formation is transient and resolved in normal topoisomerase reactions. However, when steps of the reactions are inhibited or aborted, the covalent bonds become trapped. This can occur when the enzymes encounter sites of DNA damage such as AP sites and 8-oxoG, or when they are trapped by inhibitors of topoisomerases [38]. Tyrosine residues are trapped at either 3′- or 5′-termini depending on the type of topoisomerases. TOP1 generates a DNA 3′-tyrosyl-phosphodiester bond and a 5′-OH, while TOP2 generates a DNA 3′-OH and a 5′-tyrosyl-phosphodiester bond. In both cases the moieties that are formed block normal DNA synthesis and ligation, and thus they can be regarded as termini-blocking SSBs which require BER proteins repair them.

2.3 Mechanism of Mammalian BER

A model for the basic mechanism of mammalian BER was established by the mid-1990s. The entire BER pathway, the “single nucleotide gap-filling reaction” (SN-BER), could be reconstituted by five distinct reactions *in vitro* (the middle scheme in Fig. 17.1). (1) Base damage is recognized and removed by DNA glycosylases which leave AP sites. (2) AP sites are recognized by AP endonucleases and are incised, resulting in nicks in the DNA strand with a 3′-OH terminus and 5′-deoxyribose phosphate (dRP) structure [15]. (3) 5′-dRP is removed by DNA Polβ [39]. (4) Polβ fills a nucleotide in the

TABLE 17.1 Enzymes and Reactions in the BER Pathway

BER Sub-pathway	#	Reaction Description	Enzyme	Substrate	Product
1 nt-filling BER	A	Base removal	DNA glycosylases ^a	Abnormal bases	AP sites
	B	Incision upstream of AP sites	APE1	AP sites	SSB with 3'-OH/5'dRP
	C/C'	Incision downstream of dRP	Polβ(as a dRPase), DNA glycosylases ^b	dRP	SSB with 3'-OH/5'-P gap
	D	One nucleotide filling	Polβ	SSB with a 1 nt-gap	DNA with 3'-OH/5'-P nick
	E	DNA ligation	LigIIIα	DNA with 3'-OH/5'-P nick	Repaired DNA
Long-patch BER	F	Excision of flipped nucleotides	FEN1	Flipped strand breaks with 5'-dRP	SSB with multiple nucleotide gap
	G	Long-patch filling	Polβ, Polδ/Polε, PCNA	Multinucleotide gap	DNA with 3'-OH/5'-P nick
	H	DNA ligation	LigI	DNA with 3'-OH/5'-P nick	Repaired DNA
APE1-independent BER	I	δ-Elimination	NEIL1, NEIL2	3'-α,β-unsaturated aldehyde	SSB with 3'-P/5'-P gap
	J	3'-P removal	PNKP	SSB with 3'-P/5'-P gap	SSB with 3'-OH/5'-P gap
TDP ^c	K	3'-Tyrosyl-DNA-phosphodiesterase	TDP1	3'-Phosphotyrosyl linkage	3'-P
	L	Phosphate removal and addition	PNKP	3'-P and 5'-OH	3'-OH and 5'-P
	M	DNA ligation	LigIIIα (TDP1), LigIV (TDP2)	DNA with 3'-OH/5'-P nick	Repaired DNA
	N	5'-Tyrosyl-DNA-phosphodiesterase	TDP2	5'-Phosphotyrosyl linkage	3'-OH and 5'-P
Non-enzymatic reactions and enzymatic "mis"-reactions	1	Incision downstream of AP sites	Spontaneous β-elimination	AP sites	SSB with 3'-OH/5'-dRP gap
	2	Oxidation of AP site	Spontaneous oxidation	AP sites	Oxidized AP sites
	3	Stalled Topo I	Topoisomerase I	Normal DNA	3'-Phosphotyrosyl linkage
	4	Stalled Topo II	Topoisomerase II	Normal DNA	5'-Phosphotyrosyl linkage

Reactions are linked to the schemes (A–N) in [Figs. 17.1 and 17.2](#).

^aDNA glycosylases without AP lyase activity: Methylpurine DNA glycosylase (MPG), uracil DNA glycosylases, MutY-homology (MYH), Thymine DNA glycosylase (TDG).

^bDNA glycosylases with AP lyase activity: 8-oxoG DNA glycosylase (OGG1), EndoIII homology (NTH), EndoVIII-like 1 and 2 (NEIL1 and NEIL2). NEIL1 and NEIL2 also carry out βδ-elimination.

^cThe reactions do not involve DNA-repair synthesis.

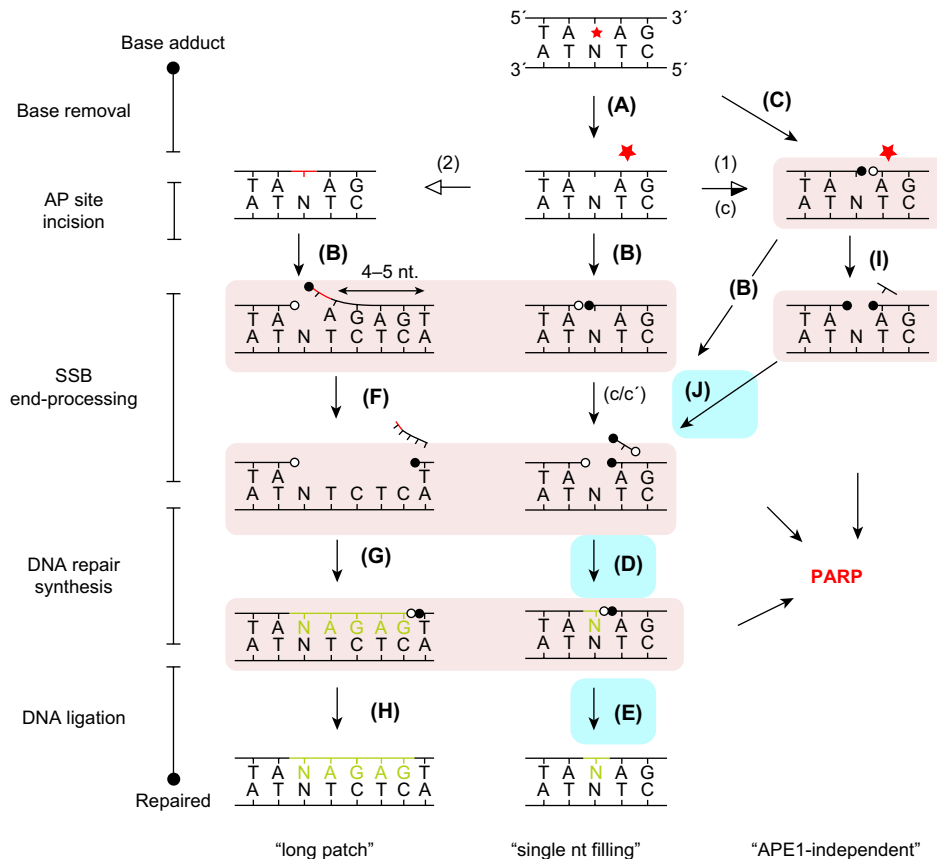


FIGURE 17.1 DNA base excision repair. The star in red represents abnormal bases including 8-oxoG and other oxidized and alkylated bases. An oxidized AP site after the reaction (2) is shown in red. Newly synthesized nucleotides are shown in green. Schemes (A–J) depict enzymatic reactions and (1) and (2) are spontaneously occurring reactions. The open circles at the 3'-end of SSBs denote 3'-OH termini, and the filled circles indicate 3'- or 5'-phosphate termini. Also see Table 17.1. XRCC1 and PARPs are not directly involved in the DNA processing but are pivotal for efficient BER in vivo. Reactions stimulated by XRCC1 (which is recruited to the DNA-damage sites by PARP1) are colored by light blue. PARP-activating DNA structures (ie, SSBs) are encircled in red.

gap and leaves a nick (3-OH and 5'-P without a gap). (5) The nick is sealed by DNA ligase III α [20,40]. Each step takes care of one type of DNA damage and leaves an intermediate lesion until the final nick-sealing reaction performed by DNA ligases occurs. Repair reactions may start at any of the intermediate lesions. For example, topoisomerase–DNA cross-links have been more recently characterized as forms of DNA damage, and repair of these trapped lesions does not follow the base removal step in the conventional BER pathway. Instead, resolution of the tyrosyl–DNA complex is followed by DNA end-processing reactions and by DNA synthesis and ligation, skipping the reactions described earlier as steps 1 and 2. This flexibility confers a versatility to BER and it can act on a plethora of different types of DNA damage that are generated endogenously or by exposure to exogenous DNA-damaging agents.

2.3.1 DNA Glycosylases

There are a total of 10 DNA glycosylases identified in mammals (Table 17.1). All DNA glycosylases cleave N-glycosylic bonds that link bases to the DNA-ribose backbone (Fig. 17.1, reaction A). This reaction creates AP sites that are processed further by an AP endonuclease (APE1). However, many DNA glycosylases further process the resulting AP sites using their intrinsic AP lyase activities (Fig. 17.1C). An AP lyase activity carries out a DNA strand–cleavage reaction through β - or $\beta\delta$ -elimination (Fig. 17.1B and I). The resulting 3'/5'-end structures are 3'-phospho- α,β -unsaturated aldehydes (3'-PUA)/5'-P by β -elimination and 3'-P/5'-P by $\beta\delta$ -elimination [15]. Importantly, these 3'-end structures cannot serve as primers for DNA-repair synthesis carried out by DNA polymerases, and require APE1 or polynucleotide kinase/phosphatase (PNKP) (Fig. 17.1B and J) to generate 3'-OH termini. Fig. 17.1 and Table 17.1 list the mammalian DNA glycosylases and summarize their reactions.

2.3.2 AP Endonuclease 1

Mammals appear to possess only a single active AP endonuclease; that is, APE1. APE1 not only incises AP sites to create 3'-OH/5'-dRP termini [15], but also it hydrolyzes 3'-phosphodiester bonds in 3'-PUA to generate 3'-OH (Fig. 17.1B) [40]. In both processes, APE1 generates 3'-OH ends which are absolutely required for DNA-repair synthesis carried out by DNA polymerases. Early studies of mice with homozygous knockouts of the Ape1 gene (Apex1) found that the gene disruptions resulted in early embryonic lethality [41,42], and APE1 was thought to be essential for cell viability [43,44]. However, in 2013, Masani et al. successfully created B cells defective in the APE1 gene [45]. Surprisingly, deleting the APE1 gene in the B cells did not affect the cell growth, although the cells exhibited a significant decrease in immunoglobulin class switch recombination [45,46], and were hypersensitive to treatment with MMS, an alkylating agent that produces AP sites. It needs to be determined whether cells other than B cells can survive without APE1, and this should be testable, given the advancement of the CRISPR gene-knockout technology. In a 2015 study, a particular mouse embryonic fibroblast cell line expressing APE1 at a level only 0.2% of normal cells was established [47]. While the cells with low APE1 grew normally, their mitochondrial respiratory activities and intracellular oxidative stress levels were greatly reduced. Thus, cells may be able to adapt to conditions with extremely low APE1 activity, which may explain why in previous studies that created an acute reduction in APE, the cells underwent apoptosis [43,44].

A second AP endonuclease, APE2, was identified based on amino acid sequence homology to APE1. However, its biological significance is not clear [45].

2.3.3 Enzymes That Process DNA Termini in BER

2.3.3.1 3'-End Cleaning (APE1 and PNKP)

As described earlier, for DNA-repair synthesis to be initiated, the 3'-terminal end used as a substrate for extension by DNA polymerases must possess a 3'-OH. However, when the AP lyase activity of a DNA glycosylase processes a damaged base instead of using the reaction of APE1 (Fig. 17.1B), a 3'-PUA is generated (Fig. 17.1, reactions 1 and C) and this differs from the requisite 3'-OH. Therefore, it is necessary for BER to process the "3'-blocking" damage. As depicted (Fig. 17.1B in the right scheme), APE1's phosphodiesterase activity has the capacity to remove these 3'-end structures including 3'-PUA and 3'-phosphoglycolate [40,48]. In contrast, APE1 has very weak activity on substrates containing 3'-phosphates (Fig. 17.1, product of I) [40]. Instead of APE1, PNKP has been shown to efficiently remove 3'-phosphate (Fig. 17.1J) [40,49,50].

2.3.3.2 5'-End Cleaning Enzymes

A 5'-phosphate is the end structure required for a DNA ligase reaction to occur with a 3'-OH (Fig. 17.1, prior to H and E reactions). Incision of AP sites by APE1 not only generates 3'-OH but also 5'-dRP which needs to be removed for the subsequent BER reaction (Fig. 17.1C). DNA glycosylases with intrinsic AP lyase activities (Table 17.1) remove the 5'-dRP moieties via β -elimination (Fig. 17.1C).

In addition to DNA glycosylases, DNA Pol β , the main DNA-repair DNA polymerase, has an intrinsic activity to remove 5'-dRP [39]. This "dRPase" reaction (Fig. 17.1C') is catalyzed via hydrolysis and usually requires Mg²⁺ as a cofactor. The reaction leaves 5'-phosphate at the 5'-termini of the DNA strand breaks. While 5'-phosphate termini can also be generated by DNA glycosylases with intrinsic AP lyase activity (Fig. 17.1C), dRPase and AP lyase are different enzymatic reactions. Although the role of dRPases and AP lyases in cleaning up the 5'-termini is identical (Fig. 17.1C and C'), AP sites can be incised by AP lyases (Fig. 17.1C) but not by dRPases [51].

The dRP or AP sites may be oxidized or reduced in cells (Fig. 17.1, reactions 1 and 2). This modification makes it impossible for the AP lyase/dRPase to remove the sugar moiety [52]. When AP sites are modified by oxidation/reduction, FEN1 (flap structure-specific endonuclease 1) can recognize the 5'-flap end structure (Fig. 17.1F in the left scheme), and incise the nucleotide a few bases downstream of the 5'-dRP.

2.3.3.3 TDP1 and TDP2: Resolving Tyrosyl-DNA Cross-links

Tyrosyl-DNA phosphodiesterases (TDPs) are enzymes that can resolve tyrosyl-DNA cross-links formed during aberrant activities by topoisomerases (Fig. 17.2).

TDP1 can resolve this unique structure to resolve the tyrosyl-3'-phosphodiester cross-link (Fig. 17.2, reaction 3), and leave 3'-phosphate termini in the DNA via hydrolysis (Fig. 17.2K) [38]. Similar to APE1, TDP1 can also remove 3'-phosphoglycolate [53]. The 3'-phosphate groups remaining after TDP1 reactions are then processed by PNKP to generate 3'-OH similarly to the 3'-end cleaning process in Fig. 17.1J. Of note, TDP1 also reacts on 3'-phosphoglycolate to generate 3'-phosphate [53], which may be further processed by PNKP to 3'-OH.

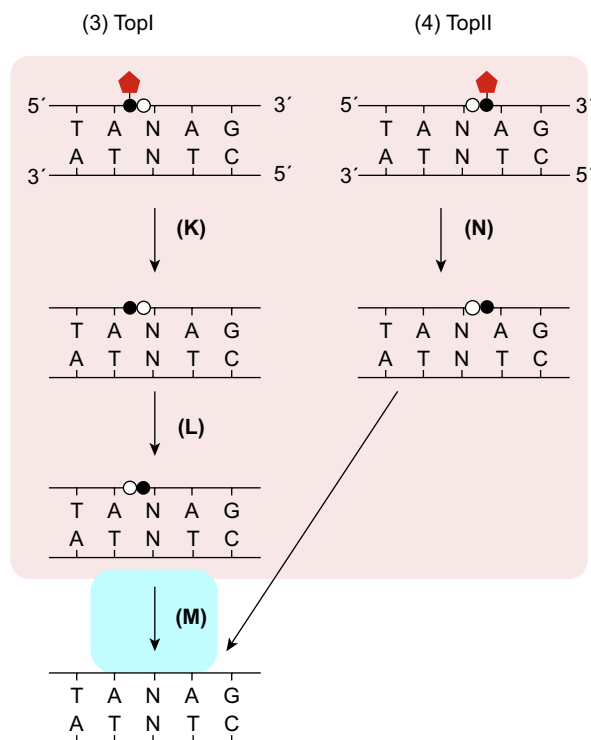


FIGURE 17.2 Resolution of DNA–protein covalent linkage. Stalled reactions on DNA by DNA topoisomerases I and II result in trapped 3′-phosphotyrosyl linkages which cause obstruction to transcription and replication, and result in cell death. Tyrosyl–DNA phosphodiesterase 1 and 2 (TDP1 and TDP2) resolve the stalled linkages followed by direct DNA ligation without DNA synthesis. In the case of TDP1, PNKP is required to generate proper 3′- and 5′-termini for DNA ligation. The same labels and colored areas as in Fig. 17.1 are used. Also see Table 17.1 for details.

Similar to TOP1, the catalytic Tyr–DNA intermediates of TOP2 may be trapped by TOP2 inhibitors such as etoposide [38]. Unlike TOP1, however, TOP2 incises the DNA to generate 3′-OH and 5′-P termini [37], and the Tyr residues form covalent cross-links to the 5′-phosphate termini (Fig. 17.2, reaction 4). TOP2 incises both strands and so it temporarily produces DSBs. When the TOP2 activity is inhibited and trapped, Tyr–5′-P intermediates are formed, DSB accumulate in DNA and become highly toxic. The trapped structure can be resolved by TDP2. The TDP2 reaction resolves the trapped linkage and releases the 5′-P termini in DNA (Fig. 17.2N).

2.3.4 Completion of an Entire BER Reaction: DNA Polymerases and DNA Ligases in Coordinated Reactions

The excision steps described earlier (Figs. 17.1A–C,F,I,J and 17.2K,L,N) are damage-specific BER reactions, and can only be processed by the enzymes that remove the particular lesions. In contrast, the DNA gap-filling (Fig. 17.1D and G) and sealing steps (Figs. 17.1E,H and 17.2M) do not involve damaged DNA, and thus theoretically any combinations of DNA polymerases and ligases should complete the processes. Although this may be the case in vitro, in cells there appears a stringent coordination that determines what DNA polymerases and ligases should follow each damage-specific BER process. It is believed that the coordination from the excision steps to the gap-filling/sealing reactions is to minimize the toxic effects of the intermediate DNA lesions. For example, gap-containing regions of DNA formed by BER might become even more toxic if they had to persist until they were randomly recognized by DNA polymerases. Instead, interactions of DNA polymerases and ligases with other BER proteins are known to improve the efficiency of the entire BER reaction. This is known as BER coordination [18,19,54,55], and it is controlled by interactions among the BER proteins and damaged DNA involved in each reaction step. The coordination of BER is further ensured by the presence of XRCC1 (see Section 2.3.5).

Thus, the DNA synthesis and ligation steps during BER should be viewed as sequential reactions that follow the damage-excision reactions. DNA polymerases beta, delta/epsilon, and lambda (Polβ, Polδ/ε, and Polλ) have been shown to function in DNA synthesis in BER, and DNA ligase I (LIGI) and III (LigIIIα) are the major DNA ligases in BER.

2.3.4.1 Single-Nucleotide Filling-BER

The involvement of Polβ has been studied since the 1980s. BER can be completed by Polβ with DNA LigIIIα in the simplest sub-pathway named single-nucleotide filling (SN)-BER which is shown in the middle column in Fig. 17.1A–E. As an example, uracil forms in DNA as the product of cytosine deamination (resulting in U:G mispair), and can be repaired by

SN-BER [14]. Uracil is removed by uracil DNA glycosylase (UNG) which produces AP site (Fig. 17.1A) [56]. APE1 then cleaves the DNA upstream of the AP site, and generates 3'-OH/5'-dRP termini (Fig. 17.1B). Pol β removes the dRP (Fig. 17.1C), fills the single nucleotide gap (Fig. 17.1D). This reaction leaves a nick with 3'-OH/5'-P termini, which is sealed by LigIII α tightly interacting with XRCC1 (Fig. 17.1E). Subsequent studies have shown that Pol λ , whose amino acid sequence shows high homology to Pol β , can substitute for Pol β in this BER sub-pathway [57]. Interestingly, Pol λ appears to be more critical than Pol β for cellular protection against oxidative DNA damage [58].

2.3.4.2 Long-Patch BER

An alternative repair pathway for oxidized (and reduced) AP sites was postulated that involves DNA Pol δ and Pole (Fig. 17.1F–H) [52]. Oxidized AP sites (Fig. 17.1, reaction 2) are incised by APE1 in the same way as intact AP sites (Fig. 17.1B), but the resulting oxidized 5'-dRP cannot be removed by Pol β or by AP lyase-associated DNA glycosylases. Instead, flap structure-specific endonucleases (FEN1) remove the dRP-containing 5'-termini (Fig. 17.1F), leaving gaps spanning several nucleotides. DNA synthesis from these gapped DNA structures was shown to be specifically carried out by Pol δ with PCNA as an essential elongation cofactor (Fig. 17.1G). Finally, DNA Lig I seals the nicked DNA to complete this BER sub-pathway (Fig. 17.1H).

2.3.4.3 APE1-Independent BER

As described earlier, two BER sub-pathways rely on APE1 to generate 3'-OH termini at damaged site in DNA, the essential primer for DNA polymerases. However, SSBs with 3'-phosphate termini are poor substrates for APE1, and thus the 3'-end cleaning step may become rate limiting.

The NEIL family of DNA glycosylases, NEIL1 and NEIL2, carry out β -elimination to generate 3'-PUA after the base damage is removed, and they further process PUA by δ -elimination to generate 3'-phosphate at the site ($\beta\delta$ -elimination) [59,60]. When PNKP was characterized for its pivotal role in SSB repair (SSBR) as a 3'-phosphatase/5'-kinase, Mitra and his colleagues examined the possibility of alternative BER sub-pathway that do not require APE1. Wiederhold et al. thus showed that AP sites can be processed to 3'-phosphate and 5'-phosphate by NEIL1 or NEIL2 (Fig. 17.1I), and then further processed by PNKP to generate 3'-OH (Fig. 17.1J) [40]. The concept that BER does not require an AP endonuclease has an important ramification in that APE1 can be dispensable in BER, and it also underscores the role of PNKP in BER.

2.3.5 Scaffolding Proteins in BER: Proteins That Do Not Directly Participate in DNA Processing

SSBs may be generated directly by DNA-damaging agents such as ROS or by enzymatic processing during BER. PARPs and XRCC1 play pivotal roles in SSBR. While PARPs and XRCC1 are not directly involved in DNA processing, they establish interactions with other BER enzymes for coordinated and efficient reactions. PARP1, the major PARP, binds to SSBs with a high affinity and protects the toxic DNA damage. PARP1 possesses an enzymatic activity that polymerizes ADP-ribosyl groups onto many cellular factors including itself. The PARylation activity of PARP1 is triggered by SSBs and by DSBs to some extent. PARP1 recruits XRCC1 which possesses a PAR-binding motif in its central domain [21] and thus interacts with PAR-modified PARP1 [61]. Auto-modification of PARP1 results in its decreased affinity for SSBs, and PARP1 is then dissociated from SSBs. XRCC1 then coordinates the BER-repair reactions by interacting with PNKP [23], Pol β [24], and LigIII α [20]. XRCC1–LigIII α interaction is essential for efficient SSBR. There are other BER proteins that reportedly XRCC1 interacts with to facilitate the whole BER pathway. These include PCNA, APE1, UNG, NEIL1, OGG1, MPG, NTL1, and NEIL2 [62–67]. However, XRCC1 is recruited on SSBs after PARP activation [21]. Although XRCC1 was shown to possess intrinsic affinity for DNA, SSBs are required for efficient interaction of XRCC1 with DNA [68,69]. Therefore, further studies should clarify how XRCC1 is recruited to DNA damage prior to the generation of SSBs to enhance the BER efficiency.

PARP1's role in BER has been studied for more than two decades, but new roles of PARP1 in enhancing BER are still being discovered [70,71]. This is partly because the PARylation reaction complicates cellular recovery from DNA damage. PARylation consumes cellular NAD⁺, whose synthesis requires energy. Thus, the overactivation of PARP has long been known to deplete intracellular NAD⁺ and ATP pools and cause cell death [72]. In addition, it was thought that a function of PARylation was to enhance the DNA-ligase reaction, given that PAR provides positive charges to the damaged sites, and this enhances activities of DNA ligases [73], particularly that of LigIII α [74]. Intriguingly, in 2015, Weinfeld et al. reported that DNA LigIII α , and not PARP1, is the SSB sensor and acts by recruiting XRCC1 and PNKP to affect the efficiency of SSB reactions in cells [75]. As PARP1 is involved in mitochondrial energy metabolism and apoptosis signaling [76,77], a definitive answer for PARP's role in BER needs additional investigation.

2.4 BER Gene Knockout in Mice and Cells

Many BER genes have been studied using genetic knockout approaches in mice to understand the roles of BER in normal physiology and how alterations impact risks for disease. [Table 17.2](#) summarizes knockout studies of BER and NER genes. Homozygous deletions of many BER genes result in embryonic lethality in mice. Unsurprisingly, homozygous deletions of BER genes known to be required for essential activities in the cells (eg, DNA replication) result in embryonic lethality. Genes that belong to this category are Fen1, DNA ligases, and DNA polymerase genes required for DNA replication. However, many BER genes whose essential functions were not well defined can also result in embryonic lethality. These genes are Tdg, Apex1 (Ape1), Polb (Pol β), and Xrcc1. Notably, Parp1 homozygous knockout mice are viable, although cells lacking Parp1 are hypersensitive to many DNA-damaging agents, and double homozygous knockout of Parp1 and Parp2 result in embryonic lethality [78]. The deletion of individual DNA glycosylase genes does not produce serious phenotypic defects in mice except for the Tdg (thymine DNA glycosylase) gene. It is noted that TDG is required for demethylation of 5mC [79], and thus it is essential for the regulation of differentiation. Thus, losing this function is likely the cause of mouse embryonic lethality, rather than the deficiency in the repair of G:T mispairs in DNA [79]. These observations validate the belief that cells and the mammalian body cannot sustain the accumulation of endogenous DNA damage without BER, and they also underscore the role of BER in epigenetic DNA metabolism.

3. MAMMALIAN NER

3.1 History and Overview of NER

Excision-repair pathways involve the removal or “excision” of a stretch of DNA containing damaged DNA and the resulting gap is filled in by DNA replication using the undamaged DNA as a template. In the 1960s, several groups discovered key aspects of the NER pathway in bacteria and in mammalian cells. Paul Howard-Flanders, Richard Setlow, and their colleagues found that bacteria treated with UV light remove small fragments of DNA containing pyrimidine dimers [80,81]. At roughly the same time, Philip Hanawalt and David Pettijohn demonstrated that “DNA-repair synthesis” coincides with excision of fragments containing pyrimidine dimers in bacteria treated with UV light [82]. Robert Painter developed a novel technique to detect “DNA-repair synthesis” in mammalian cells treated with UV light [83]. This technique is still used today to measure “unscheduled DNA synthesis” or DNA synthesis that occurs outside of S phase as part of the NER pathway after cells are treated with a DNA-damaging agent. An additional seminal observation was made by James Cleaver, who working together with Robert Painter, found that cells from patients with the sun-sensitive and cancer-prone syndrome, xeroderma pigmentosum (XP), are deficient in NER [84]. This observation was groundbreaking for many reasons. It provided evidence that deficiencies in the NER pathway can predispose humans to the development of cancer. It also led to a cell complementation analysis of the clinically heterogeneous disease, XP and this paved the way to identifying many different genes involved in the NER pathway [85]. Seven genetic complementation groups have been identified in XP, designated XPA through XPG, that represent different genes required for the NER pathway [86–88].

Studies performed by many groups around the world during the 1980s and 1990s resulted in the cloning and biochemical characterization of many genes required for mammalian NER [89–93]. As suggested by the seminal observations made in the 1960s, the overall general strategy of NER in mammalian cells is similar to that found in bacteria. An initial step in the pathway involves DNA-damage recognition. This is followed by the introduction of two incisions in the damaged strand, one on each side of the damage. An oligonucleotide containing the DNA damage is removed, and this is followed by synthesis of new DNA to replace the excised, damaged DNA. Finally, there is ligation of the newly synthesized DNA to the parental DNA. While the overall strategy of NER has been conserved in mammals and bacteria, it has been estimated that NER in mammalian cells, *in vivo*, requires 30–50 different gene products, and hence it is much more complicated than that found in bacteria.

A perhaps unique characteristic of the NER pathway is that it can be coupled to the process of transcription (reviewed in Refs. [94,95]). This surprising aspect of NER was first documented by the investigation of DNA repair in specific regions of the genome. Using this approach, it was discovered that DNA damage can be preferentially removed from genes active in the transcription process [96,97], and this preferential repair is actually targeted to only the transcribed strand of an active gene while the nontranscribed stand is unaffected [98]. Subsequent investigations have provided evidence that many of the same genes are involved in NER and transcription-coupled NER (TC-NER), but the processes differ at the steps involving recognition of the DNA damage.

TABLE 17.2 Summary of Phenotypes of Homozygous Knockout Mice of BER and NER Genes

Pathway	Gene Symbol	MGI ID ^a	Reaction ^b	Homozygous knockout mouse phenotype	References
BER (Fig. 17.1)	Ung	109352	A	Viable; no significant phenotype	[195]
	Smug1	1918976	A	Viable; no significant phenotype	[196]
	Mpg (Aag)	97073	A	Viable; no significant phenotype	[197]
	Nthl1	1313275	C	Viable; no significant phenotype; increased tumors in Nthl1 Neil1 double knockout mice	[198,199]
	Mutyh	1917853	A»C	Viable; increased intestinal tumors, particularly with exposure to KBrO ₃	[200]
	Ogg1	1097693	A>C	Viable; KBrO ₃ induces renal cancer	[201,202]
	Tdg	108247	A	Nonviable; critical to controlling epigenetic status	[79]
	Neil1	1920024	C, then I	Viable; reduced germinal B cell	[203]
	Neil2	2686058	C, then I	Viable; accumulation of oxidative DNA damage in transcriptionally active genes in aged mice	[204]
	Neil3	2384588	C?	Viable; reduced proliferation and sensitive to genotoxic stress	[205]
	Apex1 (Ape1)	88042	B	Nonviable; apoptotic	[41,42,206]
	Polb	97740	C', then D	Neonatal lethality; immune deficiency	[207]
	Pold1	97741	G	Null likely nonviable; proofreading deficiency to elevated mutation and tumors; shortened longevity	[208]
	Pole	1196391	G	Null likely nonviable; proofreading deficiency to elevated mutation and tumors; shortened longevity	[209]
	Poll	1889000	D	Viable	[210]
	Fen1	102779	F	Nonviable; heterozygous knockout mice predisposed to adenocarcinoma; E359K mutation oncogenic	[211,212]
	Lig1	101789	H	Nonviable	[213]
	Lig3	109152	E	Nonviable	[214]
	Xrcc1	99137	–	Nonviable; increased spontaneous SSBs	[215]
	Parp1	1340806	–	Viable; cells sensitive to DNA damage; improved ischemic injury recovery; resistance to diabetes; Parp1 Parp2 double knockout embryonic lethal	[72,216–218]

NER (Fig. 17.3)	Ddb1 (Xpe)	1202384	A	Nonviable	[219]
	Ddb2 (Xpe)	1355314	A	Susceptible to UV-induced DNA damage and skin tumor	[220]
	Xpc (Rad4)	103557	A	High incidence of UV-induced skin tumors; high mutation frequency	[221]
	Rad23b	105128	A	Neonatal mortality; growth retardation and other abnormality; shortened life	[222]
	Ercc8 (Csa)	1919241	D	Increased skin tumor by UV irradiation	[223]
	Ercc6 (Csb)	1100494	C	UV sensitivity; increased skin and eye tumors; circling behavior; low body weight	[224]
	Ercc3 (Xpb)	95414	E, F	Null nonviable	[225]
	Ercc2 (Xpd)	95413	E, F	Null nonviable; a missense knock-in with brittle and graying hair, cachexia	[226,227]
	Xpa	99135	G, H	Predisposition to skin tumors induced by UV and other bulky DNA damage	[228]
	Ercc5 (Xpg)	103582	G, J	Postnatal mortality; hypersensitive to UV	[229]
	Ercc1 (Rad10)	95412	I	Growth and liver failure; postnatal death; early aging and sensitive to oxidative stress	[230]
	Ercc4 (Xpf)	1354163	I	Impaired growth; short life (~several weeks); cells hypersensitive to UV	[231]

^aMGI, Mouse Genome Informatics (<http://www.informatics.jax.org>).
^bReactions depicted in Fig. 17.1 (BER) and in Fig. 17.3 (NER).

3.2 Types of DNA Damage Repaired by NER

The NER pathway is unusual, in that it recognizes and removes a wide spectrum of different types of DNA damage and the damage is usually formed by some covalent alteration or modification to one of the DNA bases [99–102]. It is generally held that the NER pathway actually recognizes a distortion in the localized structure of the DNA helix produced by the presence of a damaged base and it does not directly recognize the modified base in “a hand in glove” manner (described in Section 2.3.1 in more detail) [103,104]. Hence, NER can recognize and remove structurally unrelated base modifications including those formed by exposure to UV light, benzo(a)pyrenes and other aromatic hydrocarbons, aflatoxins, 2-acetylaminofluorenes and chemotherapeutic agents, such as platinum.

UV light has been used extensively to investigate the NER pathway. UV light results in the covalent linkage of adjacent pyrimidines and produces two predominant types of DNA damage; the cyclobutane pyrimidine dimer (CPD) and 6-4 photoproduct (6-4PP) [105,106]. Several organisms have developed additional strategies for removing UV photoproducts by the process of photoreactivation. However, humans and other placental mammals appear to lack photoreactivation pathways, and hence, NER is their sole means of removing CPDs and 6-4PPs. Left unrepaired, CPDs and 6-4PPs can produce mutations and contribute to the development of skin cancer. One of the hallmarks of the disease XP is an extremely elevated incidence of skin cancer. Many XP patients develop a form of skin cancer within the first decade of their life and develop many tumors in sun-exposed regions of their body. It is likely that UV photoproducts are formed in the skin of XP patients beginning early in life; however, since XP patients have a deficiency in NER, the photoproducts persist and lead to the formation of mutations, a driving force in cancer etiology and progression [107]. Efforts to protect XP patients from the harmful effects of sunlight and UV radiation are prolonging their lives but their deficiencies in NER appear to contribute to the development of other forms of cancer.

Thousands of compounds have been identified in the vapor and particulate phases of cigarette smoke and they include carcinogens, co-carcinogens, mutagens, and tumor promoters. About 70 of these compounds have been classified as carcinogens [108,109]. Different classes of carcinogens are present in tobacco smoke and include the polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene (BP), dibenz(a,h)anthracene, and dibenzo(a,i)pyrene. Metabolic activation of these and other chemical compounds found in tobacco smoke can create intermediates that react with DNA bases and produce DNA adducts that are substrates of NER. Hence, DNA adducts are likely continually formed in the lung tissues of people who smoke, and if they are not removed by DNA-repair processes, their persistence could lead to the formation of mutations and ultimately to lung cancer.

3.3 Mechanisms of Mammalian NER

Advances in the 1980s and 1990s led to the development of mammalian cell-free systems to investigate detailed mechanistic steps in NER [89,91,93,110]. During the mid-1990s, NER was reconstituted *in vitro* using the purified repair proteins: XPC-RAD23B, TFIIH (containing XPB and XPD), XPA, XPG, and ERCC1-XPF and the purified replication proteins: RPA, PCNA, and DNA Pol δ [89,111]. Subsequent studies indicate that these and additional proteins function in the cell through an ordered and sequential assembly onto damaged DNA (reviewed in Refs. [99,101,107]).

3.3.1 DNA-Damage Recognition and Unwinding of the Damaged DNA Duplex

The properties that govern the ability of NER to recognize structurally diverse types of DNA damage were originally described in a model described as “bipartite recognition” [103,104]. In this model, the more favorable substrates for NER are those in which the DNA damage destabilizes the DNA helix and is bulky. Damage substrates that destabilize the helix can promote disruption of hydrogen bonding and bending of the DNA helix. The bipartite recognition model has been supported by studies that have compared the efficiency of NER on damaged substrates that differ in the degree to which they destabilize helix. Hence, it was discovered that 6-4PPs have a strong destabilizing effect on the DNA helix and are efficient substrates for NER, while CPDs do not and can be poorly repaired [112].

DNA-damage recognition in NER is achieved by the XPC protein (Fig. 17.3A) [113]. XPC binding to damaged DNA is promoted by destabilization of the DNA helix and XPC can even bind destabilized DNA in the absence of DNA damage as seen using substrates containing small loops or bubbles [114]. XPC resides in a complex with RAD23B and centrin-2, a member of the calmodulin family of calcium-binding proteins [113,115]. RAD23B stabilizes XPC and may help deliver it to the site of damage (Fig. 17.3A) [116]. The role of centrin-2 is less clear. Since the presence of CPDs in DNA do not promote disruption of hydrogen bonding or destabilizing of the helix, they are not efficiently recognized by XPC. An additional protein, UV DNA damage-binding protein 2 (UV-DDB2), is required for the removal of CPDs in cells and may directly and indirectly promote binding of XPC to CPDs (Fig. 17.3A) [115].

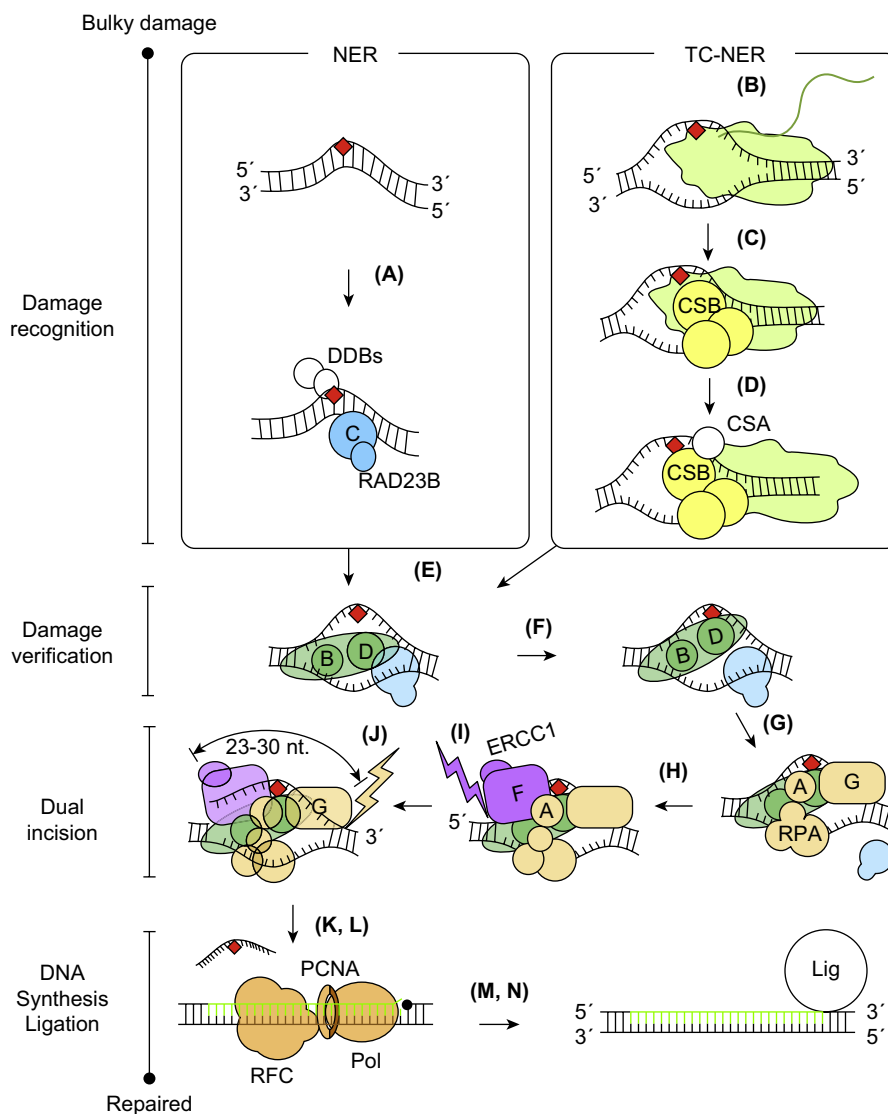


FIGURE 17.3 DNA nucleotide excision repair. (DNA-damage recognition) In the regular NER (A, global genome NER), the XPC/RAD23B complex is critical for the damage recognition. The XPC/RAD23B complex (blue) senses distortion in DNA structure containing damage. In the case of cyclobutane pyrimidine dimer (CPD), XPC/RAD23B requires UV-DDB1/2 (XPE) complex for DNA binding. (B–D) In TC-NER, RNA Pol II (light green) stalled by DNA damage (B) initiates NER. (C) CSB–UVSSA–USP7 complex (yellow) is recruited to the damage site, and (D) CSA–CSB complex formation facilitates RNA PolII backtracking to set up a NER platform. **(Damage verification)** (E) Transcription elongation factor TFIIF opens DNA double-strand at the damage site, and XPD plays a key role in damage verification step (F). **(DNA strand incision)** (G) XPA, RPA, and XPG bind to the open complex. (H) The ERCC1/XPF complex is recruited to the repair complex through interaction with XPA, (I) and XPF incises the damage-containing DNA strand at the 5'-upstream of the lesion. (J) The incision by XPF is immediately followed by downstream incision carried out by XPG. **(DNA-repair synthesis and ligation)** (K and L) Polδ/Polε or Polε with PCNA and RFC fills the gap created by XPF and XPG, (M and N) and DNA LigIIIα/XRCC1 or DNA LigI seals the 3'-OH/5'-P DNA termini to complete the NER reaction. Also see [Table 17.2](#).

TFIIF is a large complex that functions in both NER and transcription [117]. It is loaded onto sites of damaged DNA through interactions with XPC–RAD23B (Fig. 17.3E) [118–120]. It is comprised of 10 subunits that can be divided into the core complex which contains XPB and the cyclin-activated kinase (CAK) sub-complex which is not required for NER. XPD appears to serve as bridge between the core and CAK complexes. XPB and XPD are both helicases and ATPases and the roles of these activities in NER have been extensively studied. XPB helicase activity functions in 3'–5'-translocation and XPD helicase activity functions in 5'–3'-translocation (Fig. 17.3F, Table 17.3). In contrast to XPD, the ATPase activity of XPB is required for NER but not its helicase activity [121]. A major function of XPB in NER appears to be in disrupting the DNA helix, which assists in the loading of TFIIF onto damaged DNA. Once TFIIF is loaded onto DNA, XPD helicase activity results in its translocation along the DNA, unwinding the damaged duplex in the 5'–3'-direction until it encounters

TABLE 17.3 Enzymes and Reactions in the NER Pathway

NER Pathways	#	Reaction Description		Protein	Interaction With Preexisting Factor	DNA
NER	A	Damage recognition	Bulky damage recognition	XPC/Rad23B	DNA kink	Distortion in DNA due to bulky damage; CPD recognition requires UV-DDB
			CPD recognition ^a	DDB1/2	CPD	
				XPC/Rad23B	DDB 1/2	
TC-NER	B	Damage recognition	Transcription stalling	RNA Pol II	DNA damage	
	C		CSB recruitment	CSB/UVSSA/USP7	RNA Pol II	
	D		Backtracking RNA Pol II	CSA-CSB	CSB	
Downstream reactions common to NER/TC-NER	E	Strand opening		TFIIH		DNA strand opening
	F	Damage verification		XPB⇒XPD	Part of TFIIH	
	G	3'-Incision complex (XPG) formation		XPA-RPA-XPG		
	H	5'-Incision complex (ERCC1/XPF) formation		ERCC1/XPF	XPA	
	I	5'-Incision		ERCC1/XPF		Nick upstream of damage
	J	3'-Incision		XPG		Nick downstream of damage
	K	DNA synthesis		Polδ/Polε/PCNA		22–30 nt incorporation
	L			Polε/PCNA		
	M	DNA ligation		LigIIIα/XRCC1		Repaired
	N			LigI		

Reactions (#) are linked to the schemes (A–N) in Fig. 17.3.

^aCPD, cyclobutane pyrimidine dimer; CSA(B), cockayne syndrome protein A (B); RNA Pol II, RNA polymerase II holoenzyme; DDB1/2, UV-damage DNA-binding protein 1 and 2; UVSSA, UV-stimulated scaffold protein A.

a bulky covalent DNA base modification that results in blockage of additional translocation [114,121]. This blockage of XPD-mediated translocation of TFIIH at sites of damage is viewed as one step in DNA-damage verification which serves to prevent or reduce gratuitous NER at undamaged locations in the DNA (Fig. 17.3F, Table 17.3).

For many years it was held that XPA rather than XPC was involved in DNA-damage recognition. Instead, XPA appears to hold a central role in coordinating the loading of additional NER proteins at the site of damage and perhaps serves as an additional step in DNA-damage verification (Fig. 17.3G) [122–127]. When the translocation of XPD becomes stalled at a damaged site, XPC–HHR23B dissociates and XPA, RPA, and XPG bind the damaged site. A stable pre-incision complex is formed and comprised of TFIIH, XPA, RPA, and XPG. XPA serves an important role in assembling the pre-incision complex in its interaction with other NER proteins and single-stranded DNA. XPG binds through interactions with TFIIH.

3.3.2 Incision, Repair Synthesis and Ligation

Once the pre-incision complex is formed, XPA recruits ERCC1–XPF complex (Fig. 17.3H) [125,127]. ERCC1–XPF and XPG are junction-specific endonucleases that cleave DNA at junctions between double-stranded and single-stranded DNA. The unwinding of DNA by TFIIH and the assembly of XPA, RPA, XPG, and TFIIH produce a bubbled structure at the site

of DNA damage. Evidence supports an ordered sequence of incisions; ERCC1–XPF makes the first incision on the 5′ side of the DNA damage (Fig. 17.3I) and XPG makes the second incision on the 3′ side of the damage (Fig. 17.3J) [128]. The oligonucleotide containing the DNA damage and TFIIH are released (Fig. 17.3K) [129]. Once the first incision is made by ERCC1–XPF, a free 3′-OH is formed that can be used by DNA polymerases in repair synthesis, and this could even occur before the second incision is made by XPG (Fig. 17.3L). The repair synthesis step in NER, about 25–30 nucleotides in length, was once assumed to be relatively straightforward. However, the discovery that the error-prone DNA polymerase kappa (Polκ), participates in repair synthesis during NER, in addition to Polδ and Polε suggests that this step in NER is complex [130–132]. Similarly, the ligation step which seals the final phosphodiester bond between the newly synthesized DNA and the parental DNA (Fig. 17.3N) appears more complicated than originally thought. It appears to be regulated by the proliferative state of the cell with DNA ligase I used in proliferative cells and DNA ligase IIIα used in quiescent and replicating cells (reviewed in Ref. [130]).

3.4 Transcription-Coupled NER

The process of TC-NER has been studied for several decades (reviewed in Refs. [94,95]). The existence of mechanisms that couple DNA repair to transcription was indicated many years ago by studies that followed the recovery of RNA synthesis and DNA-repair levels after cells were exposed to UV light [133]. It was found that RNA synthesis, which is initially inhibited by UV light, recovered before significant amounts of DNA damage were found to be removed from total cellular DNA. Subsequently, it was found that UV-induced CPDs were selectively removed from transcriptionally active genes in mammalian cells and the selective or preferential repair of DNA damage from active genes was due to selective repair of only the transcribed strands of the genes [96–98]. The selective repair of DNA damage from the transcribed strands of active genes was first documented in mammalian cells and then subsequently documented in *E. coli* and in yeast. These observations led to models of transcription-coupled repair in which recognition of DNA damage present in the transcribed strand of an active gene was a direct consequence of the stalling or arrest of RNA polymerase when it encountered the damage. This was supported by subsequent studies that found that certain types of bulky damage arrest elongation of RNA polymerase when they are located in the transcribed strand of an active gene but they do not block it when they are present in the nontranscribed strand (reviewed in Ref. [134]).

Investigating TC-NER in cell-free systems has been challenging and this is likely due to the combined complexities involved in the transcription elongation process, in NER and in chromatin structure. Biochemical and genetic studies indicate that damage recognition in TC-NER occurs through blockage or stalling of the RNA polymerase complex when it encounters damage in the transcribed strand (Fig. 17.3B). Many of the subsequent events, loading of TFIIH, XPA, RPA, ERCC1–XPF, and XPG, are likely similar to those found in global NER (Fig. 17.3E–N). However, a notable and major difference between NER and TC-NER involves processing of the RNA polymerase when it becomes stalled or arrested at DNA damage. Due to the large size of the RNA polymerase complex, some processing events are required to remove or displace it in order for the subsequent loading of essential NER proteins to occur. Different models for these processing events have been proposed and include the backward translocation of the RNA polymerase complex away from the damage (backtracking) and/or ubiquitin-mediated modification of damage-stalled RNA polymerase and subsequent degradation of the complex. It remains unclear how these processes occur in mammalian cells. However, genetic and biochemical studies support roles for Cockayne syndrome A (CSA), Cockayne syndrome B (CSB), UV-sensitive syndrome A (UVSSA), and XPA-binding protein 2 (XAB2) in TC-NER (Fig. 17.3C and D). Cell lines with defects in each of these genes exhibit deficiencies in TC-NER or recovery of RNA synthesis following treatment with DNA-damaging agents [118,135–138]. Studies since mid-2000s suggest that degradation of damage-stalled/arrested RNA polymerase complexes may not be a common event; instead, actual degradation of the RNA polymerase complex may serve as a less frequent method of simply clearing the polymerase from the damaged site which might then allow global NER to act at the damage. Similarly, in some rare or unusual instances, the RNA polymerase complex may actually bypass the damage [139]. However, for TC-NER to occur, the polymerase more likely backtracks or is transiently displaced or altered, and this movement of the polymerase serves as a mechanism for loading TFIIH and subsequent NER factors which ultimately results in DNA-damage removal, DNA-repair synthesis, and ligation [140–142].

Mutations in a gene required for NER or TC-NER generally renders cells more sensitive to treatment with agents that introduce bulky types of DNA damage. Their sensitivity to DNA damage can be severe to moderate depending on the gene that is mutated. Clearly, mutations in NER genes can predispose humans to the development of skin and other forms of cancer as illustrated by the disease XP and discussed in more detail later. However, mutations in genes specifically required for TC-NER such as CSA, CSB, and UVSS2 do not generally predispose humans or mice to cancer (reviewed in Ref. [107]). Instead, CS patients display complex phenotypes that include developmental and neurological abnormalities, growth arrest,

mental retardation, and premature death. Both CS and UVSS2 patients show cutaneous sensitivity to UV irradiation. These observations together with biochemical and genetic studies may indicate that deficiencies in proteins required for the coupling of NER to transcription may lead to the persistence of RNA polymerase complexes arrested at sites of damage which in turn may trigger apoptotic events leading to cell death.

3.5 NER and Chromatin Structure

The recognition of DNA damage and the functions of many proteins involved in NER and TC-NER described earlier must take into consideration the packaging of DNA into chromatin when repair takes place *in vivo*. The presence of nucleosomes and the assembly of nucleosomes into higher-order chromatin structures likely impede DNA-damage recognition and NER. Hence, “an access, repair, restore” model proposes that chromatin and nucleosomes must be altered or displaced during DNA-damage recognition and repair, and this is followed by restoration of the nucleosome and chromatin structure following repair [143]. It is likely that this involves alterations in the posttranslational modifications of the histone tails such as by acetylation, alterations in the distribution of histone variants, and the recruitment of chromatin-remodeling complexes. Early studies indicated that nucleosomes become rearranged during NER and that the acetylation of histones stimulated NER. More studies conducted between 2012 and 2014 have provided more detailed mechanistic insights into how alterations in chromatin impact DNA-damage recognition and processing by NER and TC-NER (reviewed in Refs. [95,107,144–146]).

The access step which allows NER proteins to recognize and bind DNA damage appears to be influenced by many proteins. UV-DDB promotes ubiquitylation of core histones and associates with PARP1 to mediate PARylation of chromatin to open it up [116,147,148]. Histone acetylation by the histone acetyl transferases, p300 and GCN5, can also contribute to relaxing chromatin and the ATP-dependent chromatin-remodeling complexes, SWI/SNF and INO80, can promote repair by displacement of nucleosomes and by influencing the recruitment of XPC–RAD23B–centrin complex to the damage [149,150]. After repair is completed, the restore step to assemble the newly synthesized DNA into nucleosomes involves histone chaperones CCRF-associated factor (CAF1) and alternative splicing factor, ASF1 [151–153].

TC-NER occurs during the elongation stage of transcription since it serves and is signaled by RNA polymerase complex blocked at DNA damage. Hence, this state of chromatin is likely different from chromatin that is not transcriptionally active. For TC-NER, the chromatin has already been “opened” to allow transcription initiation and elongation. CSB is required for TC-NER and studies have found that it can remodel chromatin *in vitro* [154]. Whether it has chromatin remodeling functions during TC-NER is unclear. CSA and UVSSA can play different roles in targeting CSB for ubiquitylation and degradation. CSA promotes ubiquitylation of CSB, while UVSSA inhibits ubiquitylation of CSB [155,156]. CSA and CSB appear to promote the association of histone acetyl transferases and proteins that promote chromatin remodeling and chaperones to incorporate histones into newly reassembled nucleosomes [142].

3.6 Alterations in NER and Cancer Predisposition

It is clear that heritable mutations in NER genes can predispose individuals to the development of skin cancer and other forms of cancer. Many XP patients develop nonmelanoma skin cancer within the first decade of life. This is in sharp contrast to the development of nonmelanoma skin cancer in the general, non-XP population that occurs, on average, when people are well into their 60s. XP patients can also develop tumors in internal, non-UV-exposed organs including tumors of the brain and central nervous system and the lung [87]. Genetically modified mice with deficiencies in certain NER genes are also predisposed to UV-induced skin cancer and carcinogen-induced and spontaneous forms of lung cancer [157].

It is unclear how alterations in NER impact cancer etiology in the general, non-XP population. Deficiencies in NER could render an individual with a greater predisposition to the development of cancer and conversely, enhancement of NER capacity in an individual could render them less susceptible to the development of cancer. Alterations in individual repair capacity could also impact how an individual responds to treatment with chemotherapeutic agents that damage DNA. An individual’s capacity to carry out NER could be influenced by the inheritance of polymorphic alleles of NER genes, by exposure to agents in the environment that impact NER efficiency, or by some combination of the two. These interactions are likely highly complex. There have been numerous studies that have investigated correlations between polymorphisms in NER genes and many different forms of cancer including those that occur in the lung, stomach, breast, skin, or blood. However, while linkages have been reported in some studies, many of these are either not supported or are found to be weak associations when studies are combined and subjected to meta-analyses [158–163]. In addition, while XP is a rare disease, the frequency of single mutant alleles is much greater, but it remains unclear if individuals containing only one mutant allele of an NER gene are more highly predisposed to the development of cancer.

4. BIOLOGICAL IMPLICATIONS BEYOND DNA DAMAGE AND REPAIR

Some functions of excision repair are indispensable for the organisms. The central theme in this section is the versatility of BER and that some proteins involved in BER can be utilized in fundamental cellular activities unrelated to DNA repair. As there are excellent reviews on these subjects [163,164], this section briefly describes recent studies investigating additional roles of proteins involved in BER.

4.1 Diversity of Immune Cells by Activation-Induced Deaminase

Somatic hypermutation (SHM) and class switch recombination (CSR) are necessary for antibody diversification in antigen-specific memory B cells, and both mechanisms require activation-induced deaminase (AID) [164–166]. Because AID deaminates cytosine to generate uracil in DNA, a well-known BER substrate, involvement of BER in this pathway is being established [45,166]. The canonical BER reactions depicted in Fig. 17.1 do not likely occur during SHM and CSR. Instead, when uracil is generated in DNA by AID, it serves as a flag to recruit error-prone bypassing DNA polymerases for SHM and components of DNA DSB repair cooperate with some of the BER enzymes to lead to CSR [46,164]. Continued understanding of the mechanisms of SHM and CSR involving BER, other DNA-repair and -signaling pathways should illuminate the sophisticated crosstalk among the DNA-repair pathways.

4.2 DNA Demethylation

Methylation of cytosine at CpG dinucleotides generates 5mC. 5mC is a major epigenetic DNA modification that controls gene expression. While abnormalities in the distribution of 5mC in the genome are a hallmark of cell transformation in cancer genomics, DNA methylation is pivotal in controlling normal cell differentiation during development.

Processes are required to regulate demethylation of DNA and this is necessary to remove 5mC and introduce cytosine. Studies in the past several years have established an essential role of the demethylation process not only in cell differentiation, particularly for the stem cell research, but also in cancer development [79,167,168].

An initial event in the demethylation process, described in detail in a previous review by Wu and Zhang [169], is the conversion of 5mC to 5-hydroxymethyl cytosine (5hmC) by Tet methylcytosine dioxygenases (Tet1, Tet2, and Tet3; ten-eleven translocation 1, 2, 3 gene protein). The Tet proteins further process 5hmC to 5-formylcytosine (5fC) and then to 5-carboxylcytosine (5caC) [170]. Both 5fC and 5caC are processed by BER, as TDG recognizes and removes these unusual cytosine derivatives as its substrates, and leaves AP sites at these locations (Fig. 17.1A). The reactions that follow the generation of AP sites are not entirely clear. However, a 2010 study showed that the BER proteins including TDG, APE1, PARP1, and XRCC1 are upregulated during developmental stages in embryonic mice when whole-genome demethylation takes place [171]. In zygotic cells, PARP1 and XRCC1 were found to be physically associated with the paternal genome where demethylation takes place [171]. Several studies have found that the BER proteins, XRCC1, PARP1, and APE1, are utilized in the demethylation process in *Arabidopsis* and mammalian cells [167,172–175]. Because of the impact of demethylation on many study fields of study including stem cells, cell differentiation, cancer, and cancer stem cells, the advanced technology demonstrating the involvement of BER in the distribution of C, 5mC, 5hmC, 5fC, and 5caC [168,176,177] has broadened the role of BER beyond DNA repair and toward epigenetic maintenance.

5. INTERPLAY BETWEEN NER AND BER: THE KEY ROLE OF THE DNA-DAMAGE RESPONSE FOR PREVENTION OF CELLULAR DEGENERATION

5.1 Overlapping Substrate Specificity Between BER and NER

BER and NER enzymes may recognize the same types of DNA damage, and hence, this class of substrates could be repaired by either pathway. A role of NER in the repair of endogenously generated DNA damage has been suggested since it could explain the neurodegenerative phenotypes associated with some NER deficiencies. However, UV damage does not occur in neurons and hence the substrates for NER that may produce the neurodegenerative phenotype are an unsolved question.

Overlapping substrates for BER and NER were reported in *E. coli* and yeast [178–183]. Memisoglu et al. found that a deficiency in rad13, an NER protein in yeast, produced increased sensitivity to alkylating reagents, and they proposed that alkylated bases may be repaired directly by the NER pathway [180]. In 2010, the repair of AP sites was reported to be associated with TC-NER in yeast [184], and the investigators proposed a detailed mechanism for this observation.

Some studies have also reported overlapping roles of BER and NER in the removal of oxidative DNA damage in human cells. A biochemical study showed that human NER proteins could recognize and remove 8-oxoG in DNA [185]. Although it has been difficult to clearly show a role of NER in the removal of 8-oxoG in vivo, primary cells from XPC patients have been found to be hypersensitive to treatment with oxidizing reagents [186].

5.2 A Nuclear–Mitochondria Signaling Network as a Main Platform of BER/NER Interplay

An emerging field of study, how mitophagy is regulated by a DNA-damage response, is being formed that may finally delineate the crosstalk between BER and NER, and perhaps it also involves other DNA-repair pathways.

Mitophagy is a cellular process that degrades damaged mitochondria and facilitates generation of new mitochondria. Mitophagy and autophagy require common factors and reactions involving ubiquitin-dependent proteasome systems and LC3 conjugation [187,188]. However, compared to autophagy, mitophagy is a mechanism that provides a quality check for maintaining the integrity of mitochondria, and its biological role resembles that of apoptosis.

Mitophagy requires a ubiquitin ligase, Parkin [188]. Deficiencies in the gene for Parkin, PARK2, are a major cause of both early and late onset of Parkinson's disease. Parkin is a RING domain containing E3 ligase and it requires an essential cofactor, PINK1, which is also a Parkinson's disease-causative gene [189]. The astonishing finding that ubiquitin Ser65 phosphorylation regulates mitophagy was reported by several studies [190–193]. Impaired mitochondria lose the inner membrane potential, which induces phosphorylation at Ser65 of ubiquitin by PINK1. The Ser65-phosphoubiquitin facilitates Parkin's translocation from the cytosol to the surface of mitochondria, and enhances its ubiquitination reaction. Therefore, cells proactively maintain the quality of mitochondria with Parkin, for which PINK1 plays a central regulatory role.

PINK1 is highly sensitive to proteolysis; the truncated PINK1 loses the kinase activity, and thus becomes incapable of activating Parkin and mitophagy. Although the sensitivity of PINK1 to proteolysis may provide an auto-regulation of the Parkin–PINK1 protein degradation system, in 2014 it became apparent that PINK1 is susceptible to oxidative stress in causing its proteolysis [194]. An unexpected finding was that lack of XPA was associated with increased PINK1 cleavage, resulting in the impairment of the cellular function to check mitochondrial integrity [194]. This study proposed the following degenerative cellular events (Fig. 17.4): (1) Elevation of PARP1 activities in XPA-deficient cells causes insufficient NAD⁺ concentration. (2) Low NAD⁺ causes down-modulation of SIRT1 activity which in turn lowers PGC-1 α activity. PGC-1 α is the master regulator of mitochondrial regeneration and energy-generating activity in the cells. (3) Low PGC-1 α activity causes lower UCP2 levels. Because UCP2 is an uncoupler that maintains the proper mitochondrial membrane potential, when UCP2 is increased, it is an inducer of mitophagy. In contrast, lower levels of UCP2 result in degradation of PINK1 and suppression of mitophagy. This effect is additive and results in the accumulation of damaged mitochondria. The unusually high mitochondrial and oxidative stresses are unsustainable and thus cause apoptosis. This phenotype and the novel link to mitochondria was not only found to be associated with deficiencies in XPA; it was also associated with Cockayne syndrome B (CSB) and ataxia telangiectasia-mutated (ATM) deficiencies. Surprisingly, it was not associated with deficiencies in XPC. Therefore, although both XPA and XPC are essential for the NER process, XPA appears to have an independent function in the maintenance of mitochondrial integrity.

These are remarkable discoveries in the field of BER and NER, and further studies may provide critical information as to why deficiencies in NER cause neurodegenerative diseases. However, an important question regarding the mechanism of NER has not been answered: Does XPA's direct involvement in repairing endogenous DNA damage help cells maintain intact mitophagy, or is XPA a signal transducer in this particular DNA-damage response? In other words, what initiates mitochondrial degeneration which is exacerbated by a deficiency in XPA? The unusually high oxidative stress caused by mitochondrial degeneration and UCP2 down-regulation may be a consequence rather than the cause of cellular degeneration. Similarly, PINK1 degradation may be induced by the elevated oxidative stress. The fundamental cause of these molecular events could be endogenous DNA damage (Fig. 17.4). Endogenous DNA damage is continuously generated under the normal physiology, and keeps PARPs at its equilibrium balance between the activated and dormant forms. It is hypothesized that, in a yet unidentified reaction scheme, the presence of XPA suppresses the activation of PARP, either by facilitating repair or by inhibiting PARP. Hence, a deficiency in XPA could result in the accumulation of active PARP, which results in a gradual, yet irreversible, degeneration of mitochondrial and an increase in oxidative stress that ultimately kills the cell. Crucial experiments remain to be carried out to identify interactions of XPA with molecules involved in the DNA-damage response, including endogenous DNA damage and DNA-repair intermediates (eg, AP sites, DNA-strand breaks, protein–DNA cross-links, and 5meC), and NER/BER proteins such as PARPs.

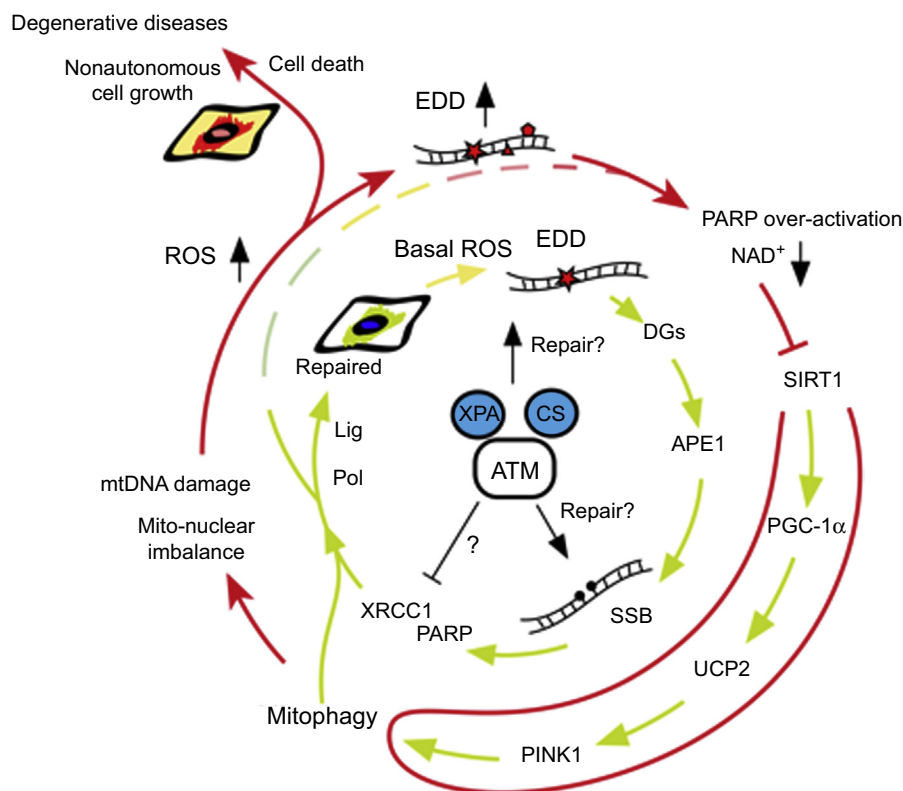


FIGURE 17.4 A model of cellular degeneration caused by endogenous DNA damage. (Green, inner ring) A normal cellular cycle in which EDD (endogenous DNA damages) are continuously generated but under the control of BER, and PARPs are activated to facilitate the repair process. (Red, outer ring) EDD generation at a high rate causes overactivation of PARP. Depletion of NAD⁺ suppresses SIRT1-dependent PGC-1 α activation, which abrogates mitochondrial quality check by Parkin/PINK1 [194]. Cells enter a vicious cycle involving ROS elevation and mitochondrial degeneration. XPA, CSB, and ATM, all appear to take part in keeping EDD at normal levels [194]. To determine what type of EDD exactly causes the PARP overactivation, and to understand the coordination of BER with XPA, CSB, and ATM will help improve precision medicine of degenerative diseases. DGs, DNA glycosylases.

6. CONCLUDING REMARKS

Here, we reviewed advances in understanding two mammalian DNA excision-repair mechanisms. We described the basic mechanisms of BER and NER, and reviewed recent studies regarding the interplay of BER and NER, revealing a novel role of NER which is independent of the repair of UV-induced DNA damage. The versatility of BER was also illustrated by describing how BER is also involved in processing modified DNA bases such as 5mC and this role in epigenetics is an indispensable function of BER. The stepwise reaction scheme of BER makes it a flexible pathway and thus an ideal DNA-modifying machinery that can adapt to different types of unusual bases.

Endogenous damage must be repaired by DNA-repair pathways to avoid pathophysiological conditions. NER has been studied mainly to understand its role in removing bulky DNA damage generated by UV radiation and by exposure to carcinogens. However, recent studies have led to the discovery of novel DNA-damage responses involving NER as well as BER that likely play roles in disease. One key to understanding the impact of these novel pathways on disease may be to identify the endogenous targets of NER.

GLOSSARY

3'-blocking damage Non-3'-OH termini at DNA strand breaks that cannot serve as DNA synthesis primers and therefore require 3'-end processing to generate 3'-OH termini. 3'-blocking damage includes 3'-phosphate, 3'- α,β -unsaturated aldehyde, 3'-phosphoglycolate.

AP lyase A lyase that catalyzes DNA-strand breakage at AP sites, and removes 5'-dRP from 5'-ends of DNA strand breaks. The AP lyase reaction occurs via β - or $\beta\delta$ -elimination through formation of Schiff base. Many DNA glycosylases possess an AP lyase activity. Also see dRPase.

AP sites (apurinic/apyrimidinic sites) A type of DNA damage where a base (either purine or pyrimidine) is removed. Also known as abasic sites.

Bipartate recognition of DNA damage during NER The efficient recognition and removal of DNA damage by NER generally requires that the damage to DNA possesses two important features. One feature is that the damage represents a covalent modification to the DNA. The second feature is that the presence of the covalent modification creates a significant alteration in the overall structure of the DNA helix.

Deamination Deamination may occur at exocyclic amino groups of cytosine, 5-methyl cytosine, adenine, and guanine, which are converted to uracil, thymidine, hypoxanthine, and xanthine, respectively. The bases resulted from deamination may form base pairs different from original pairs (U to A, T to A, HX to C) and thus potentially mutagenic.

Demethylation A process wherein 5-methyl cytosine is converted to cytosine. An active demethylation process in cells involves enzymes of BER. BER proteins that are shown to function in demethylation include TDG, PARP, XRCC1, and APE1.

DNA-alkylating agents Chemical compounds with electrophilic alkyl groups that attack nucleophilic groups in DNA. Commonly used alkylating agents in research include methyl methanesulfonate (MMS), methylnitronitrosoguanidine (MNNG), *N*-nitroso-*N*-methylurea (MNU), temozolomide.

dRPase An enzyme capable of removing 5'-dRP from 5'-ends of DNA strand breaks via hydrolysis. The term dRPase is often used to describe an AP lyase, due to the fact that their roles in the BER pathway in producing 5'-phosphate from 5'-dRP are identical. By definition, unlike AP lyases, dRPase does not incise AP sites. The difference between AP lyases and dRPases is described in detail by Piersen et al. [51].

Mitophagy An active process to digest damaged mitochondria involving protein degradation via ubiquitination catalyzed by Parkin and PINK1. It is a specialized autophagy for maintaining quality of mitochondria.

Oxidative DNA damage Bases and backbone of DNA can be oxidized spontaneously or induced by oxidizing reagents. These include 8-oxoguanine, thymine glycol, DNA strand breaks with 3'-blocking damage. Find details in Refs. [3,35].

RNA-polymerase backtracking Instead of moving along the template DNA strand in the 3' to 5' direction synthesizing new RNA, the RNA polymerase complex can translocate in the opposite direction and move backwards in the 5' to 3' direction.

TET Tet methylcytosine dioxygenase or ten-eleven translocation gene protein. TET enzymes catalyze base conversion reactions using 5-methylcytosine (5mC) as the starting substrate to generate 5-hydroxymethylcytosine (5hmC), then 5-formylcytosine (5fC), and finally 5-carboxycytosine (5caC). The converted cytosine derivatives are recognized and removed by TDG. Evidence indicates that AP sites, generated by TDG, are repaired by the traditional BER pathway involving APE1, PARP1, XRCC1, Pol β , and DNA ligase III. TET1, TET2, and TET3 belong to the TET enzyme family.

LIST OF ABBREVIATIONS

3'-PUA 3'-Phospho- α,β -unsaturated aldehydes

5caC 5-Carboxylcytosine

5fC 5-Formylcytosine

5hmC 5-Hydroxymethyl cytosine

5mC 5-Methylcytosine

6-4PP 6-4 Photoproduct

AID Activation-induced deaminase

APE1 AP Endonuclease 1

Apex1 Ape1 gene

AP-site Apurinic/aprimidinic sites

ASF1 Alternative splicing factor

ATM Ataxia telangiectasia mutated

BER Base excision repair

BP Benzo(a)pyrene

CAF1 CCRF-associated factor

CAK Cyclin-activated kinase

CPD Cyclobutane pyrimidine dimer

CSA Cockayne syndrome A

CSB Cockayne syndrome B

CSR Class switch recombination

DDR DNA-damage response

dRP 2-Deoxyribose 5-phosphate

EDD Endogenous DNA damages

FEN1 Flap structure-specific endonucleases

LIG1 DNA ligase

MGMT O⁶-methylguanine-DNA methyltransferase

MMS Methanesulfonate

MNNG Methylnitronitrosoguanidine

MPG Methylpurine DNA glycosylase

MYH MutY homology

NEIL1 and NEIL2 EndoVIII-like 1 and 2

NER Nucleotide excision repair

NMU *N*-nitroso-*N*-methylurea

NTH EndoIII homology

O_2^- Superoxide
OGG1 8-oxoG DNA glycosylase
OH• Hydroxyl radical
PARPs Poly(ADP-ribose)polymerases
PNKP Polynucleotide kinase/phosphatase
Polβ DNA polymerase beta
Polδ/ε DNA polymerase delta/epsilon
Polλ DNA polymerase lambda
ROS Reactive oxygen species
SHM Somatic hypermutation
(SN)-BER Single-nucleotide filling base excision repair
SOD2 Manganese superoxide dismutase 2
SSBs Single-strand breaks
Tet1, Tet2, and Tet3 Tet methylcytosine dioxygenases
TC-NER Transcription-coupled NER
Tdg Thymine DNA glycosylase
TDPs Tyrosyl-DNA phosphodiesterases
TOP1, TOP2, TOP3 Topoisomerases I, II, and III
UNG Uracil DNA glycosylase
UV-DDB2 UV DNA damage-binding protein 2
UVSSA UV-sensitive syndrome A
XAB2 XPA-binding protein 2
XP Xeroderma pigmentosum

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