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Comprehensive Invited Review

DNA Damage and Repair: From Molecular Mechanisms to Health Implications

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ABSTRACT

DNA is subjected to several modifications, resulting from endogenous and exogenous sources. The cell has developed a network of complementary DNA-repair mechanisms, and in the human genome, >130 genes have been found to be involved. Knowledge about the basic mechanisms for DNA repair has revealed an unexpected complexity, with overlapping specificity within the same pathway, as well as extensive functional interactions between proteins involved in repair pathways. Unrepaired or improperly repaired DNA lesions have serious potential consequences for the cell, leading to genomic instability and deregulation of cellular functions. A number of disorders or syndromes, including several cancer predispositions and accelerated aging, are linked to an inherited defect in one of the DNA-repair pathways. Genomic instability, a characteristic of most human malignancies, can also arise from acquired defects in DNA repair, and the specific pathway affected is predictive of types of mutations, tumor drug sensitivity, and treatment outcome. Although DNA repair has received little attention as a determinant of drug sensitivity, emerging knowledge of mutations and polymorphisms in key human DNA-repair genes may provide a rational basis for improved strategies for therapeutic interventions on a number of tumors and degenerative disorders. *Antioxid. Redox Signal.* 10, 891–937.

I. INTRODUCTION

Cellular DNA is subjected to numerous alterations of a chemical and physical nature. The cell cannot tolerate damage to the DNA, and this results in compromising the integrity and the accessibility of fundamental information in the genome. At the cellular level, DNA lesions may hamper processes such as transcription and replication, resulting in cell-cycle arrest, cell death, and mutations. At the organism level, DNA lesions have been implicated in several inherited diseases, in carcinogenesis, in genetic disorders, and in aging (14, 72, 166).

II. SOURCES OF DNA DAMAGE

Although DNA is fairly stable, many sources of DNA alterations have an effect on its structure and integrity. Damage can be induced by several chemical reactive species and physical agents or may occur spontaneously through intrinsic instability of chemical bonds in DNA (14, 72, 99). Even under normal physiologic conditions, DNA is being damaged continuously (139). The base-sugar bonds in DNA are relatively labile, and several thousands of bases are lost each day in a mammalian cell, generating abasic (AP) sites. Purines are lost more easily than pyrimidines, and the brain is the most affected organ, followed by the colon and heart. In addition, deamination of DNA bases may frequently occur under physiologic conditions; the base most frequently undergoing deamination is 5-methylcytosine, followed by cytosine. The resulting modified bases will pair with adenine during the following round of DNA replication, giving rise to C→T transitions, a type of mutation frequently detected in inherited human diseases.

Examples of exogenous sources of DNA damage are sunlight, ionizing radiation, chemical compounds, and genotoxic drugs. In addition, cell-intrinsic sources also are known, such as replication errors, programmed double-strand breaks (in lymphocyte development), and DNA-damaging agents that are normally present in cells. The latter category, which mainly cause strand breaks and chemical modifications of nucleotides, includes reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, hydrogen peroxide, nitrogen-reactive species, such as nitric oxide, and others. These species are produced either by normal metabolism or by environmental stresses such as smoking, oxidizing chemicals, or ionizing radiation.

A. Damage to DNA by reactive species

Oxidation of DNA components is one of the major sources of induced DNA damage and can be generated by a variety of factors, including endogenous cell metabolism, chemicals, drugs, ionizing radiation, and solar light. Oxidation processes may involve hydroxyl radical, singlet oxygen, hydrogen peroxide, peroxynitrite, and one-electron oxidation, leading to several types of DNA modifications. These include chain breaks, DNA–protein crosslinks, abasic sites, purine-reactive aldehyde adducts, and oxidized DNA bases (for recent reviews. see 30, 71).

1. Damage to DNA by reactive oxygen species. Several modifications occurring in DNA are the result of chemical oxidation due to by-products of oxygen metabolism (30, 60, 99) released during normal respiration and also as part of the inflammatory response. Major sources of cellular ROS production are mitochondria, peroxisomes, cytochrome P450 enzymes, and the antimicrobial oxidative burst of phagocytic cells. The situation in which ROS exceed cellular antioxidant defenses is termed *oxidative stress*. Oxidative stress accelerates DNA damage and may contribute to aging (14, 72, 156, 166). ROS, including superoxide radical, hydrogen peroxide, and hydroxyl radical, can cause several types of DNA lesions, such as chemical modifications to the bases or to 2-deoxyribose moieties, chain breaks, adducts, and crosslinks.

Hydroxyl radicals can add to guanine and adenine at positions 4, 5, or 8 in the purine ring, generating a multitude of products. One of the most abundant lesions is 8-oxo-7,8-dihydroguanine (8-oxoG), also called 8-hydroxyguanine, which frequently mispairs with adenine. The addition of hydroxyl radical to C-8 of guanine produces a C-8 OH-adduct radical, which can be either oxidized to 8-oxoG or reduced to 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FAPγG), whose formation requires opening of the imidazole ring (Fig. 1). 8-oxoG is the most commonly used biomarker of DNA oxidation. It has been estimated that several thousand 8-oxoG lesions may form daily in a mammalian cell, representing >5% of all oxidative lesions. Until recently, measurement of 8-oxoG and other oxidized purine and pyrimidine nucleobases has been hampered by the occurrence of several drawbacks associated with their analysis. Optimized assays are now available through the efforts, in particular, of the European Standards Committee on Oxidative DNA Damage (ESCODD) network (88). The background level of DNA oxidation in normal human cells is likely to be around 0.3–4.2 8-oxoG per 10^6 guanine, which is orders of magnitude lower than has often been claimed in the past (43).

OH radical–mediated oxidation of pyrimidines, which occurs at positions 5 or 6 in the ring, producing several radical species, is another frequent lesion, whose major products are 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol) and 5,6-dihydroxy-5,6-dihydrocytosine (cytosine glycol). Conversion of hydroperoxides derived from OH radical addition across the 5,6-ethylene bond of thymine and cytosine leads to the formation of 5-hydroxy-5-methylhydantoin and 5-hydroxyhydantoin, respectively, through the rearrangement of the pyrimidine ring. The allylic radical, which results from abstraction of a hydro-

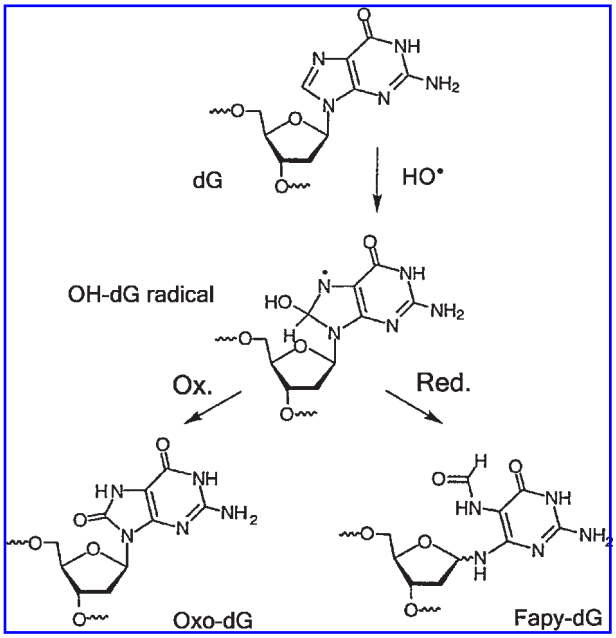

FIG. 1. Guanine modification by hydroxyl radicals. Addition of hydroxyl radical to C-8 of guanine produces an adduct radical, which can be oxidized to 8-oxo-7,8-dihydroguanine (8-oxoG) or reduced to give the ring-opened product 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FAPγG).

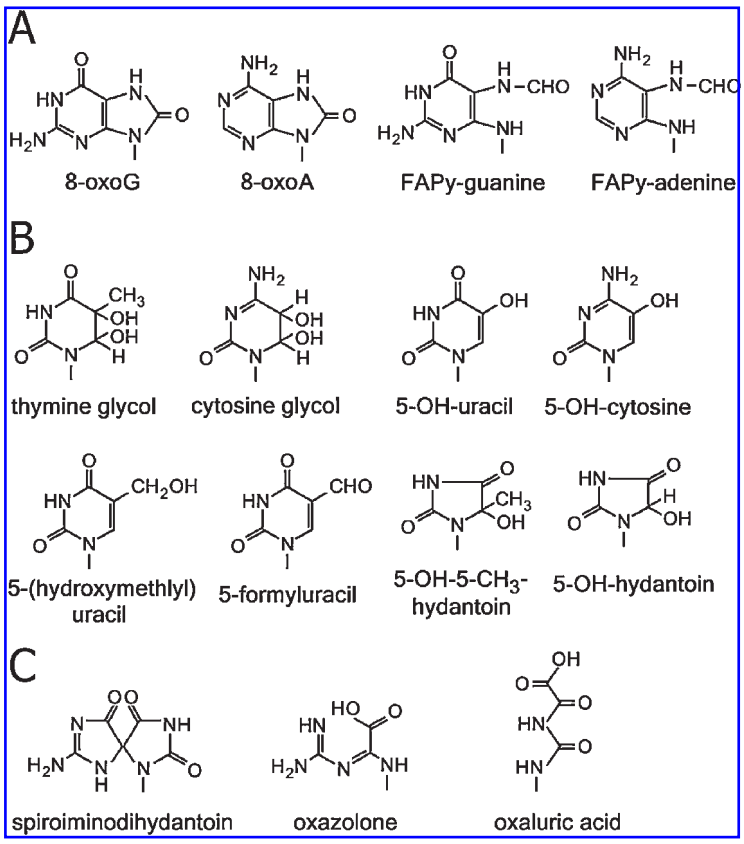

FIG. 2. Modified bases resulting from the attack of reactive oxygen species. Structure of 8-oxo-7,8-dihydroguanine (8-oxoG), 8-oxo-7,8-dihydroadenine (8-oxoA), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FAPγG), and 4,6-diamino-5-formamidopyrimidine (FAPγA), formed by hydroxyl radical addition to position 8 of the purine ring (**A**). Structure of main products formed by hydroxyl radical addition to positions 5 or 6 of the pyrimidine ring (**B**). Structure of other guanine oxidation products (**C**).

gen atom from the methyl group of thymine by the OH radical, gives rise to 5-(hydroxymethyl)uracil and 5-formyluracil (Fig. 2). The presence of O_2 or transition metal ions or both affects the mechanisms of radical reactions and hence the product obtained (72, 99).

Oxidatively generated lesions can lead to decomposition in base fragments that are essentially noncoding. Hydrogen abstraction from 2-deoxyribose and ribose by hydroxyl radicals leads to the formation of carbon-centered radicals. In the presence of O_2, these species convert to peroxyl radicals, which undergo different reactions giving rise, in most cases, to DNA strand breaks. It should be noted that oxidized residues, which may be released as reactive carbonyl derivatives or are still attached to the DNA strand, are also formed. C1′ and C4′ oxidation leads to either strand breaks or the formation of 2-deoxyribonolactone and other oxidized sugar residues, respectively (oxidized abasic sites), whereas C5′ radicals of 2-deoxyribose can react with C8 on the purine ring of the same nucleoside, resulting in cyclized products such as 8,5′-cyclo-2′-deoxyguanosine and 8,5′-cyclo-2′-deoxyadenosine.

The exposure of cells to H_2O_2 generates base-modification products and increases DNA strand breakage. The pattern of DNA damage observed in the presence of H_2O_2 indicates that it results from the formation and activity of hydroxyl radicals generated from H_2O_2 by a Fenton reaction. Singlet oxygen is inefficient at producing strand breakage and attacks only guanine to give unstable endoperoxides, which generate various products including 8-oxoG. DNA base-oxidation products are themselves subjected to further oxidation. 8-oxoG, which is more oxidizable than guanine, can be oxidated by singlet O_2, peroxynitrite, HNO_2 and HOCl to several products including spiroiminodihydantoin, oxazolone, and oxaluric and cyanuric acids (see Fig. 2).

2. Damage to DNA by other chemical species. Exposure of DNA to reactive nitrogen species such as N_2O_3 or HNO_2 can promote deamination of DNA bases and conversion of guanine to xanthine and oxanine. Peroxynitrite converts guanine into 8-nitroguanine, which is rapidly lost from DNA by spontaneous depurination (184) (Fig. 3).

Bases can be methylated endogenously by small molecules such as *S*-adenosylmethionine, and the O6 position of guanine can be alkylated by the action of nitrosamines. The most important lesion is 7-methylguanine (7-meG), with >4,000 residues generated per day. However, this base modification is relatively harmless, as it does not show marked miscoding or cytotoxic properties. In contrast, 3-methyladenine (3-meA) and O^6-methylguanine (O^6-meG) are miscoding lesions that may contribute to mutagenesis and must be efficiently repaired (Fig. 3).

Hypochlorous acid, endogenously generated by neutrophil myeloperoxidase activity, is shown to react with DNA, inducing DNA–protein crosslinks, chlorination of DNA bases, as well as pyrimidine oxidation products (133). The major end products include 5-chlorocytosine and 5-chlorouracil (Fig. 3). These modified bases have been detected at sites of inflammation and are indicative of HOCl-mediated DNA damage *in vivo* (133).

Other chemical sources of DNA damage are free radicals generated by cigarette smoke, environmental pollutants, and

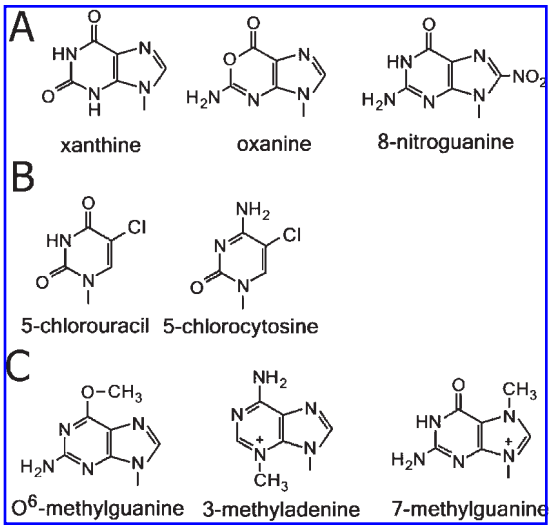

FIG. 3. Modified bases resulting from the attack of chemical-reactive species. Reactive nitrogen species such as N_2O_3 or HNO_2 can promote deamination of DNA bases (guanine to xanthine and adenine to hypoxanthine) and conversion of guanine to oxanine, whereas 8-nitroguanine is formed by the reaction of DNA with $ONOO^-$ (**A**). Examples of modified bases formed by the reaction of hypochlorous acid with DNA (**B**). Some of the modified bases resulting from alkylation (**C**).

chemotherapeutic drugs. *N*-Methyl-*N*-nitrosourea (MNU) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) belong to a class of alkylating agents that carry out their biologic effects principally by methylating the O^6 position of guanine to form O^6-methylguanine (O^6-meG). Platinum compounds (*i.e.*, cisplatin and carboplatin), by virtue of their high reactivity are capable of covalent binding to DNA bases and can form platinum–DNA adducts, including intrastrand and interstrand dipurinyl crosslinks, DNA–protein crosslink, and monoadducts with purines. The most abundant lesions produced are intrastrand crosslinks between the N7 atoms of adjacent purines.

B. Physical sources of DNA damage

The major exogenous sources of DNA damage are ultraviolet (UV) and ionizing radiation. Ionizing radiation can generate highly reactive radical species and causes a broad spectrum of lesions in cellular DNA, similar to those produced during oxidative metabolism, including damage to the purine and pyrimidine rings, sites of base loss (AP sites), and strand breaks. UV-B radiation (290–320 nm) primarily induces photoproducts through direct excitation of the nucleobases. Further reactions proceed in an oxygen-independent manner that leads mainly to the formation of dimeric photoproducts. The two major photoproducts in DNA that are caused by UV radiation are cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6-4)-pyrimidone photoproducts (6-4PPs) (Fig. 4). The adjacent pyrimidines can be either thymine or cytosine, with some preferences at the 5′ and 3′ positions; CPDs involve mainly T<>T, whereas T<>C are more abundant in 6-4PPs (201). CPDs and 6-4PPs are among the most common environmentally induced bulky lesions in DNA and thus are major hazards to human health, with CPDs more cytotoxic and mutagenic than 6–4PPs in mammalian cells (193). Other photoreactions, which occur to a lesser extent, include photohydration of cytosine, with the formation of 6-hydroxy-5,6-dihydrocytosine, and oxidation of guanine into 8-oxoG (31). UV-C radiation (200–290 nm) can convert H_2O_2 to hydroxyl radical but primarily induces photoproducts, involving mainly pyrimidines. Most of the damaging effects of UV-A radiation (320–400 nm) on DNA involve photosensitization reactions, with the generation of cyclobutane dimers at TT sites. UV-A can also produce ROS, which are predominantly singlet oxygen. 8-oxoG has been shown to be generated in DNA of UV-A–irradiated cells, but the contribution of UV radiation in the oxidative damage to DNA is low (31).

III. SPECIFIC FEATURES OF THE VARIOUS DNA-REPAIR PATHWAYS

As mentioned earlier, DNA is subjected to several alterations. Spontaneous depurination and deamination, damage caused by radical species, adducts formed, as well as mispaired bases eventually incorporated during DNA replication, must be removed. To prevent the harmful consequences of DNA damage and recover the lost information, a variety of strategies and a complex network of complementary DNA-repair mechanisms have evolved, which depend on the type of damage inflicted to the double-helix structure of DNA (Fig. 5) (39, 61, 100, 104, 110, 122, 204, 261). One advantage of the DNA double helix is the redundancy of information. If one strand is damaged, the other one can be used to recover the information. Most of the repairing systems adopt an excision-repair mechanism by which the damaged strand is removed and resynthesized by using the other strand as a template. Thus, double-strand breaks are more

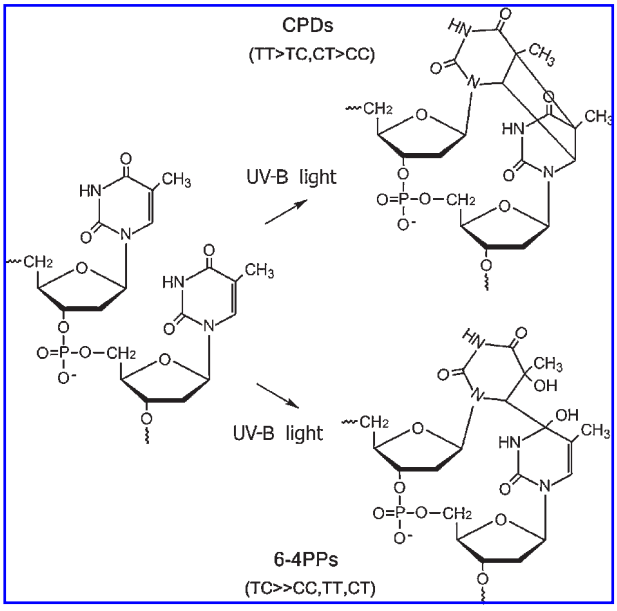

FIG. 4. Major classes of lesions induced in DNA by UV-B light: cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts (6-4PPs).

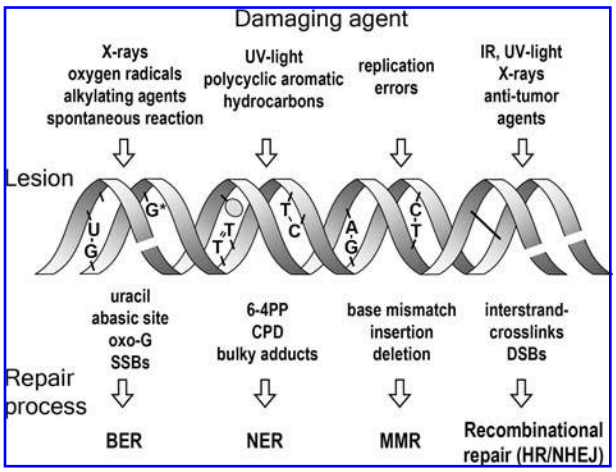

FIG. 5. DNA-damaging agents, DNA lesions, and repair mechanisms. DNA-damaging agents, examples of DNA lesions they introduce, and the relevant repair mechanisms involved. Each damaging agent can induce several types of lesions, in the same way as different damaging agents can induce similar DNA lesions, and DNA lesions repaired by each pathway may overlap. Adapted from De Boer and Hoeijmakers (52).

damaging because this backup system is not available. Defects in DNA that is being replicated or transcribed appear to be repaired more rapidly than those in DNA in condensed chromatin. This occurs, in part, because the DNA is more available to repair enzymes, but transcription-coupled repair also is important (169).

In the human genome, >130 genes have been found to be involved in repair mechanisms (260). As soon as the damage has been located, specific repairing molecules are recruited and bound to or near the damaged site, inducing other molecules to bind and make a specific complex that is able to repair the damage. Most small base modifications and nearly all oxidatively induced DNA lesions (except for double-strand breaks and purine cyclonucleosides), as well as single-strand breaks, are repaired *via* the DNA base excision repair (BER) pathway. Nucleotide excision repair (NER) is the most important repair process and removes the broadest spectrum of genomic damage, including UV-induced photoproducts, bulky and helix-distorting adducts, crosslinks, and oxidative damage. The general DNA mismatch repair (MMR) pathway is a critical mechanism responsible for maintaining genetic integrity by correcting base-substitution mismatches and insertion/deletion loop mismatches (IDLs) generated during DNA replication and recombination, as well as DNA lesions resulting from a variety of internal and external stresses. Finally, two distinct but interconnected pathways repair double-strand breaks: homologous recombination (HR)-dependent repair and non-homologous end-joining reaction (NHEJ). However, considerable overlap exists in the substrate specificity of repair pathways, and certain proteins are used in more than one pathway.

Response to DNA damage may also result in tolerance. Damaged sites not repaired before replication may cause an arrest of replicative machinery that can be relieved by passing the lesion. Cells have developed sophisticated mechanisms for switching off the replicative polymerase and switching on alternative polymerases that are able to replicate certain DNA lesions such as damaged or modified bases or bulky adducts or both. This process, whose mechanisms are largely unknown, is referred to as translesional DNA synthesis (TLS) and involves several specialized low-fidelity DNA polymerases and results in damage tolerance (85).

Unrepaired or improperly repaired DNA lesions, such as double-strand breaks, have serious potential consequences for the cell, leading to genomic instability and deregulation of cellular functions. Thus, DNA-damage response includes protein factors that can activate cell-cycle checkpoint machinery. A network of complex signaling pathways is then deciphered that detects DNA damage, arrests the cell cycle, and may ultimately lead to apoptosis (Fig. 6) (15, 179, 227).

A. Base excision repair

Base excision repair (BER) is one of the most active DNA repair processes that allows the specific recognition and excision of a damaged DNA base. The majority of damage processed by the BER pathway is generated by the attack of ROS, which are products of normal cellular metabolism (30, 60, 72). ROS are continuously generated as respiration by-products (1–5% of consumed O_2) in mitochondria and are the most abundant, endogenous toxic agents in aerobic organisms. Elevated cellular levels of ROS are formed when cells are exposed to redox agents and ionizing radiation. The result of these stresses is the development of DNA damage, which includes not only a multitude of base modifications, as reported earlier, but also base loss (*i.e.*, formation of AP sites) and single- or double-strand breaks containing 3′ sugar fragments or phosphates. All of these lesions are invariably cytotoxic or mutagenic or both. Nearly all oxidatively induced DNA lesions (except for double-strand breaks), as well as single-strand breaks that cannot be repaired directly by a DNA ligase without further processing, are repaired *via* the DNA BER pathway in organisms ranging from *Escherichia coli* to mammals (101, 108, 230, 258). The BER pathway is also active against many of the damages formed in DNA as a result of spontaneous base hydrolysis or modification by alkylating agents. Compared with other repair pathways, BER appears to be the simplest and most thoroughly defined of all repair processes and consists of five basic steps catalyzed by different enzymes. In this pathway,

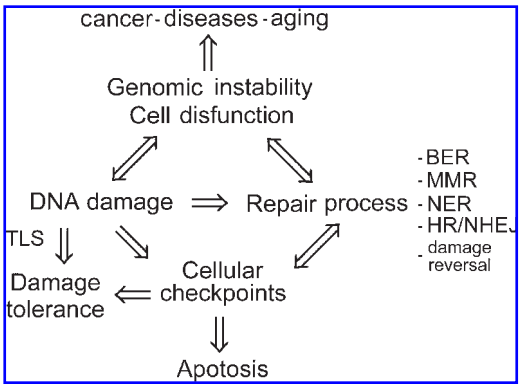

FIG. 6. Schematic representation of DNA-damage response.

Table 1. Proteins Involved in Base Excision Repair (BER) and Single-strand Break Repair (SSBR)

Protein	*Function*
Monofunctional DNA-glycosylases	Recognition and removal of altered base leaving an AP site
Bifunctional DNA-glycosylases	Recognition and removal of altered base, lyase activity generating a strand break containing 3′-blocking ends
APE1	AP endonuclease; low-efficiency 3′-phosphodiesterase and 3′-exonuclease activities
PNK	Polynucleotide kinase; 3′-phosphatase activity
POLβ	Gap-filling DNA polymerase; involved in both SP- and LP-BER; dRP lyase activity
POLδ/POLε	Replicative/repair DNA polymerases involved in LP-BER
FEN1	Flap (structure-specific) endonuclease; functions in LP-BER
PCNA	Proliferating cellular nuclear antigen; POLδ/POLε processivity factor, stimulates FEN1 activity and other LP-BER activities
RFC	Replication factor C; PCNA loading factor
RPA	Replication protein A; single-stranded DNA binding
LIG1	DNA ligase; functions in LP- and SP-BER
LIG3	DNA ligase; functions mainly in SP-BER; complex with XRCC1
PARP1	Poly (ADP-ribose) polymerase 1; sensor for DNA single-strand breaks
XRCC1	BER/SSBR scaffold protein; partner of LIG3, PARP1, and POLβ

Other additional proteins are not included (see text for details).

damaged bases are removed by a class of enzymes called DNA *N*-glycosylases. The resulting apurinic/apyrimidinic (AP) sites are processed by an AP-specific endonuclease activity or an AP-lyase activity associated with some *N*-glycosylases, leaving single-strand interruptions. These breaks, which may require an editing reaction to generate unblocked 5′ and 3′ ends, are then filled in by a DNA polymerase, either with a single-nucleotide, short patch or with a longer repair patch, followed by a ligation step. The relative involvement of different BER subpathways appears to depend, in part, on the type of initiating lesion (81, 117, 230). Proteins involved in the BER pathway are listed in Table 1.

1. DNA N-glycosylases. The critical step in the BER pathway is the recognition and the excision of damaged or mismatched substrate bases from the DNA by DNA *N*-glycosylases (56, 61, 108, 136). DNA *N*-glycosylases are conserved in all organisms, and a high degree of homology exists between eukaryotic and prokaryotic glycosylases, but eukaryotic *N*-glycosylases frequently have N- or C-terminal additions that are presumably involved in specifying intracellular location and in interaction with other proteins. Three-dimensional structure determinations allow BER *N*-glycosylases to be classified into several major structural families by architectural folds: helix-hairpin-helix (HhH), helix-two-turn-helix (H2TH), and uracil

Table 2. Examples of Human DNA *N*-Glycosylases, Their Activity and Substrate Specificity

	Full name	*Lyase activity*	*Substrates*
UNG	Uracil DNA *N*-glycosylase	No	Uracil (U:G > U:A), 5-formyluracil
TDG	G/T mismatch-specific thymine DNA glycosylase	No	G:T >> C:T > T:T
UDG	Uracil DNA glycosylase	No	Uracil (U:A), 5-OH-uracil
SMUG1	Single-strand-selective monofunctional uracil-DNA *N*-glycosylase 1	No	Uracil (ssU > U:G > U:A), 5-OH-uracil, 5-formyluracil
MBD4	Methyl-CpG-binding domain protein 4	No	T or U in T/UpG:5-meCpG
AAG (MPG)	*N*-Methyl purine DNA *N*-glycosylase	No	3-meA, 7-meG 7-meA, 3-meG
MYH	MutY homologue	No	8-oxoG, (A:8-oxoG), 2-OH-adenine, A:G mismatch
OGG1	8-Oxo-guanine glycosylase 1	β-lyase	8-oxoG, FAPyG
NTH1	Endonuclease three homologue 1	β-lyase	Tg, Cg, 5-OH-uracil, 5-OH-cytosine, DHU
NEIL1	NEI (endonuclease VIII)-like glycosylase 1	β,δ-lyase	FAPyA, FAPyG, Tg, 5-OH-uracil
NEIL2	NEI (endonuclease VIII)-like glycosylase 2	β,δ-lyase	Hydantoins (Sp, Gu) 5-OH-uracil, 5-OH-cytosine

Tg, thymine glycol; Cg, cytosine glycol; DHU, dihydrouracil; Sp, spiroiminodihydantoin; Gu, guanidinohydantoin.

DNA *N*-glycosylases (UDGs) (108, 136). Furthermore, *N*-glycosylase structures can possess a variety of additional functional domains, such as a [4Fe–4S] iron sulfur cluster or β-sheets. Each DNA *N*-glycosylase is responsible for repairing a subset of target bases, and most are highly specific for a certain type of altered base. However, some of them demonstrate broad substrate specificity, but with a preference for either pyrimidine or purine derivatives. This results in significant redundancy at the recognition step of the BER repair mechanism, whose true significance remains unclear. Table 2 provides examples of *N*-glycosylases found in human cells; similar *N*-glycosylases also are found in other organisms.

The BER pathway involves two kinds of DNA *N*-glycosylase, monofunctional and bifunctional. Monofunctional DNA *N*-glycosylases possess only base excision (*N*-glycosylase) activity that hydrolyzes the *N*-glycosidic bond between the base and the 2-deoxyribose, generating an abasic site on DNA (Fig. 7). This is the case for alkylpurine-DNA *N*-glycosylase (AAG, also known as MPG), which mainly removes methylpurine residues or hypoxanthine-DNA, and uracil-DNA *N*-glycosylases (UNGs), which remove hypoxanthine and uracil residues derived from deamination of adenine and cytosine, respectively. The structural biochemistry of these enzymes shows similar folds and common motifs, suggesting a universal mode of action (reviewed in 113). DNA *N*-glycosylases scan DNA searching for kinks or bends caused by the presence of damaged or mismatched bases. This recognition step involves bending of DNA at the damage site followed by a universal nucleotide flipping-out mechanism, as was first discovered for the uracil-DNA *N*-glycosylase/DNA complex, in which the glycosylase exchanges the DNA base/base pairing into a DNA base/protein pairing. This mechanism situates the lesion in the active site, designed differently in each DNA *N*-glycosylase for substrate specificity, and if the binding site offers a good fit, the base is excised. Most monofunctional *N*-glycosylases share an HhH DNA-binding motif and invariant Asp residue common to the active sites. The AP site created by monofunctional DNA *N*-glycosylase is identical to that created by spontaneous DNA depurination or depyrimidination.

Bifunctional DNA *N*-glycosylases form a class of proteins that both excise the damaged base and cleave the DNA backbone on the 3′ side of the AP site *via* an intrinsic AP lyase activity. These enzymes use a common reaction mechanism that involves several steps. Similar to monofunctional *N*-glycosylases, the initial recognition of the damaged site is followed by bending of DNA and subsequent flipping of the lesion into the active site (113, 224). The damaged base is then displaced by a nucleophilic attack at C1′, which is responsible for glycosidic bond cleavage and 2-deoxyribose ring opening by C1′–O4′ bond cleavage (245). This reaction leads to the formation of an abasic Schiff base intermediate, which can proceed with a single lytic reaction at the 3′ phosphodiester bond through a β-elimination, or with a double lytic reaction occurring at both the 3′ and 5′ phosphodiester bonds (consecutive β- and δ-elimination). The β-lyase reaction generates a strand break with one base gap containing 5′-phosphate and 3′-phospho-α,β-unsaturated aldehyde (4-hydroxypentenal phosphate) ends, whereas the β,δ-lyase reaction gives phosphates at both the 3′ and 5′ ends (245) (Fig. 8).

Almost all the DNA *N*-glycosylases involved in the repair of oxidative DNA damage possess intrinsic AP lyase activity. The best-known oxidized base-specific DNA *N*-glycosylases identified in mammals are NTH1 (thymine glycol–DNA glycosylase) and OGG1 (8-oxoguanine-DNA *N*-glycosylase). Both are orthologues of *E. coli* Nth endonuclease and belong to the endonuclease III superfamily. This class of enzymes catalyzes a β-lyase reaction promoted by specific lysine and aspartate residues and is characterized by a conserved HhH motif, which acts as the DNA-binding domain (40). A [4Fe–4S] cluster loop can be present, and this has been proposed to operate as an architectural element positioning the DNA-binding loop. NTH1 recognizes a wide range of oxidized pyrimidine derivatives, such as thymine glycol, 5-hydroxycytosine, and 5,6-dihydrouracil. OGG1 is primarily responsible for the repair of oxidation products of guanine, such as 8-oxoG and ring-opened FAPyG (136).

Two other DNA *N*-glycosylases, named NEIL1 and NEIL2, which are structural orthologues of *E. coli* endonuclease

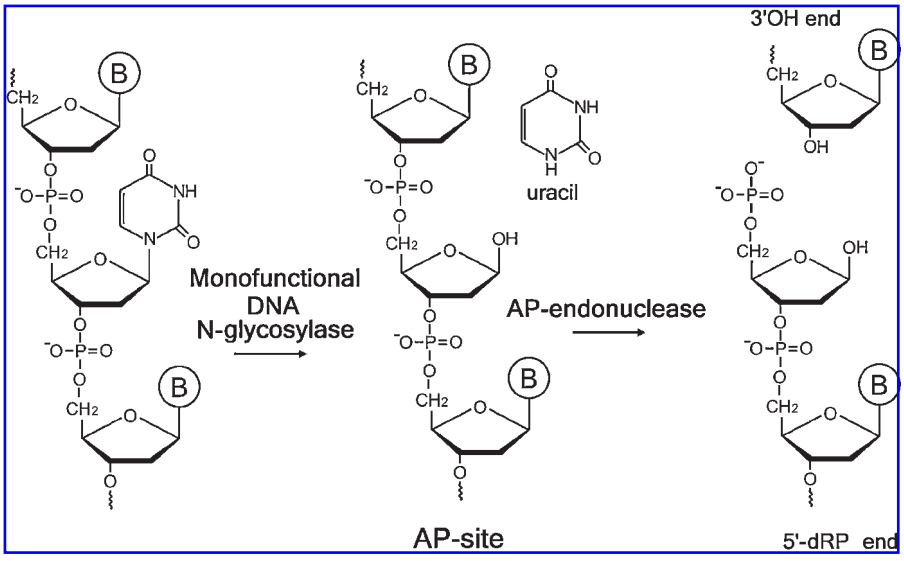

FIG. 7. Schematic reaction of monofunctional DNA *N*-glycosylases. The damaged base is removed by hydrolysis of the *N*-glycosylic bond between the base and the 2-deoxyribose, generating an abasic site. The resulting product is further processed by an AP-specific endonuclease, generating a 3′-OH terminus and a 5′-terminal 2-deoxyribose-5-phosphate (dRP) moiety.

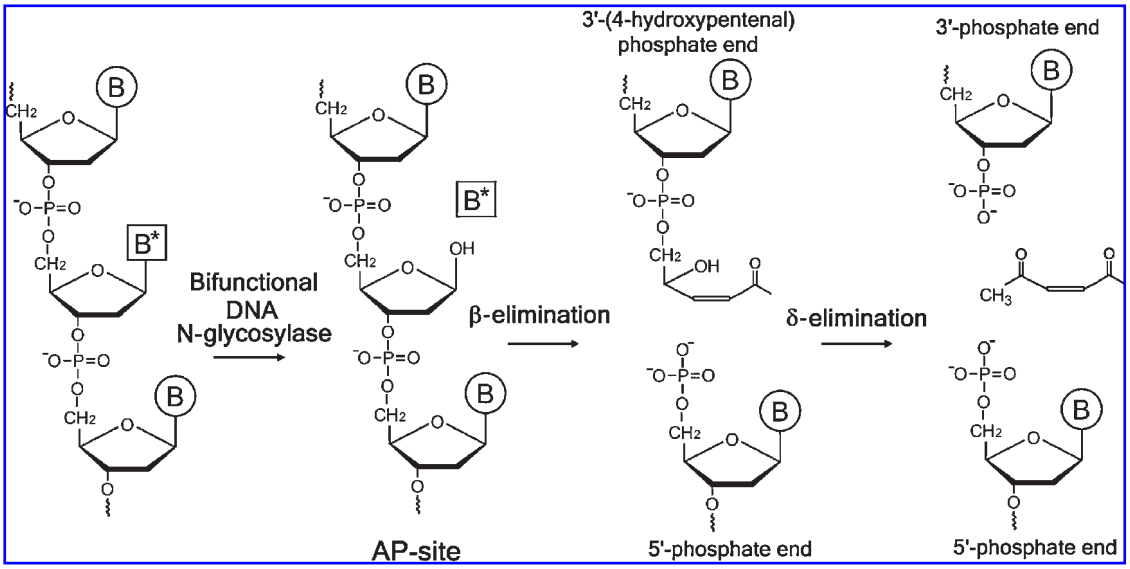

FIG. 8. Schematic reaction of bifunctional DNA *N*-glycosylases. These proteins both excise the damaged base and cleave the DNA backbone on the 3′ side of the AP site *via* an intrinsic AP lyase activity. The β-lyase reaction generates a strand break with one base gap containing 5′-phosphate and 3′-phospho-α,β-unsaturated aldehyde (4-hydroxypentenal phosphate) ends, whereas the β,δ-lyase reaction gives phosphates at both 3′ and 5′ ends and releases 4-oxo-2-pentenal.

VIII–like enzymes and prefer ROS-derived lesions of pyrimidines, were cloned, expressed, and characterized in mammals (101). NEIL1 and NEIL2 *N*-glycosylases are characterized by a H2TH DNA-binding motif and catalyze a β,δ-lyase reaction. The N-terminal proline and a glutamate residue have been identified as being responsible for catalyzing base excision and β-elimination, whereas a conserved internal lysine residue acting as proton donor is proposed to promote an additional activity resulting in a final β,δ-elimination product (40, 101, 245). NEIL2 contains a zinc-finger motif essential for maintaining structural integrity and positioning a conserved arginine residue, which interacts with the minor groove of DNA and may be critical for glycosylase activity in both zinc-containing (NEIL2) and zinc-less fingers (NEIL1). NEIL1 shows high affinity for ring-opened purines, FAPyA and FAPyG, excises 8-oxoG, and also is active with thymine glycol generated by oxidation of thymine. NEIL2 appears to prefer cytosine-derived lesions, particularly 5-hydroxyuracil and 5-hydroxycytosine. As an additional function, NEILs *N*-glycosylases, unlike OGG1 and NTH1, are functional in the recognition and removal of subsequent oxidation products of 8-oxoG (*e.g.*, spiroiminodihydantoin and guanidinohydantoin) (101).

Several DNA *N*-glycosylases physically interact with other nuclear proteins and are subjected to posttranslational modifications (73, 108, 117). Among them, the interaction with two proteins of the NER pathway, XPG and XPC, promotes binding and excision activity of NTH1 and AAG DNA *N*-glycosylases, respectively. Conversely, the interactions of DNA *N*-glycosylases and replication-related factors, such as PCNA and RPA, as well as proteins of MMR, MSH6, and MLH1, suggest a BER response, together with MMR, in postreplicative repair. The rate-limiting step in the action of many DNA *N*-glycosylases is the dissociation process. The enzyme exhibits a better affinity for the AP site product than for the initial damaged substrate, and in this way could hide the harmful AP site until it is processed in the subsequent step of the BER pathway. It has been reported that the release of human TDG can be facilitated by covalent modification with the ubiquitin-like proteins SUMO-1 and SUMO-2/3 (223).

2. APE1-dependent pathway. Once the damaged or mismatched base has been removed by DNA *N*-glycosylase activity, the BER pathway requires a strand-processing step before DNA-repair synthesis can be performed. This generates unblocked 3′ and 5′ ends, which are substrates for the action of DNA polymerase and ligase. The key enzyme in this step is an apurinic/apyrimidimic endonuclease (APE1) (55, 259). This enzyme is a multifunctional protein, also known as redox factor 1 (Ref1), that is involved in the redox activation of several transcription factors (237). The C-terminal domain of APE1 has an endonuclease activity highly specific for AP sites and a 3′-phosphodiesterase activity, which are responsible for the two major enzymatic functions of APE1 in BER: strand incision at the AP site and excision of 3′ abasic fragment generated by monofunctional or bifunctional DNA *N*-glycosylase, respectively. Recently, the strand incision activity of APE1 has been involved in the repair of oxidized abasic sites, such as 2-deoxyribonolactone (55). These sites represent a harmful lesion, which can either undergo chemical rearrangement, yielding a strand break, or form covalent DNA–protein crosslinks with repair enzymes such as DNA polymerase. APE1 can incise DNA duplex at 5′ of several oxidatively damaged bases, such as 5-OH-2′-deoxycytidine, in a DNA *N*-glycosylase–independent manner. This alternative nucleotide incision-repair pathway may represent a backup system for the BER pathway, but its physiologic relevance is unclear (114). APE1 also displays an additional editing function with a $3' > 5'$ exonuclease activity preferential for certain 3′-mismatched nucleotides (259). More-

over, the redox state of APE1 can alter its AP endonuclease activity (129). It has been observed that APE1 can enhance the base-excision activity of several DNA *N*-glycosylases. APE1 may increase *N*-glycosylases' turnover, facilitating their dissociation from the AP site by competing for the same site, although a direct stimulation of base-excision activity has been reported (73, 258).

In the APE1-dependent BER pathway, strand processing proceeds differently for monofunctional and bifunctional *N*-glycosylases (Fig. 9). In the case of monofunctional *N*-glycosylases, APE1 cleaves the DNA backbone immediately adjacent to the abasic site by its AP endonuclease activity, generating a 3′-OH terminus and a 5′-terminal 2-deoxyribose-5-phosphate (dRP) moiety. Reaction proceeds with the repair synthesis to fill the gap by DNA polymerase β (POLβ). POLβ has intrinsic dRP lyase activity and carries out both 5′-end cleaning, with the removal of the 5′-abasic terminal fragment, and DNA-repair synthesis, with incorporation of the appropriate nucleotide at the site of the base damage. In the case of bifunctional glycosylases, the repair procedure requires the additional 3′-excision activity of APE1, which can remove the 4-hydroxypentenal phosphate blocking ends resulting from AP β-lyase activity. The gap is then filled by POLβ, likewise with monofunctional DNA glycosylases.

In the final step, ligation to seal the remaining nick is performed by a DNA ligase. This step can be performed by two ligase activities. DNA ligase 3 (LIG3) is active primarily in short-patch repair. LIG3 is physically associated with the x-ray repair cross-complementing protein1 (XRCC1) and this interaction plays a critical role in stabilizing LIG3 activity. XRCC1 is a protein with no known enzymatic activity that interacts with many proteins of BER, thus functioning as a molecular scaffold and recruiting BER repair factors at the damaged site (73). XRCC1 can interact with POLβ, as well as with APE1, increasing both its AP endonuclease and 3′-phosphodiesterase activities. The other ligase activity is provided by DNA ligase 1 (LIG1), which is active in long-patch repair as well as DNA replication. Reconstitution experiments have demonstrated that APE1-dependent BER can be completed by using just four proteins. As an example, the repair of uracil in DNA was reconstituted *in vitro* with UNG *N*-glycosylase, APE1, POLβ, and LIG3 (137). BER also has been reconstituted *in vitro* with a bifunctional DNA glycosylase, by using OGG1, APE1, POLβ, and LIG1 (189).

3. Short-patch and long-patch BER. In mammalian cells, the repair event generally involves the replacement of a single nucleotide mediated by POLβ, which incorporates the correct nucleotide and eventually removes the 5′-dRP terminus through its dRPase activity. This process has been termed short-patch (or single nucleotide) BER (SP-BER). When the short-patch pathway is used, the final ligation step is carried out by the LIG3/XRCC1 complex. Sometimes BER synthesis extends beyond a single nucleotide, with incorporation of two to 10 nucleotides. This alternative pathway is known as long-patch BER (LP-BER) and is usually used in yeast cells. The longer-patch pathway depends on enzymes normally involved in DNA replication: DNA polymerase δ (POLδ) or ε (POLε), replication factor C (RFC), and proliferating cell nuclear antigen (PCNA), which act as cofactors for both POLδ and POLε (117, 230). The replaced strand does not incur degradation during polymerization but rather is displaced and cut away by DNAase IV or flap endonuclease 1 (FEN1), whereas the ligation step is performed by LIG1 (Fig. 9). *In vitro* reconstitution experiments have shown that LP-BER can be completed with one of the PCNA-dependent polymerases, POLδ or POLε, although it has been reported that POLβ can perform the strand-displacement synthesis. The mechanisms that influence polymerase selection are still unclear, but a crucial role appears to be played by protein–protein interactions (81, 101, 230). For example, the interaction with APE1 and FEN1 can play a role in patch-type selection by POLβ. LP-BER can be promoted through the stimulation of the strand-displacement activity of POLβ by FEN1 and the flap-cleavage activity of FEN1 by APE1. Conversely, SP-BER can be promoted by XRCC1, which inhibits the strand-displacement activity of POLβ.

4. APE1-independent pathway. Recently, an APE1-independent pathway was identified in mammals (101). This process is initiated by bifunctional DNA glycosylases with a β,δ-lyase activity, such as NEIL1 and NEIL2 (see Fig. 9). These enzymes produce a single-strand break with a 5′-phosphate residue and a 3′-phosphate blocking end, which has to be removed to provide the 3′-OH terminus required for subsequent repair synthesis by a DNA polymerase. APE1-homolog enzymes in *E. coli* (Xth and Nfo) can process both the 3′-blocking end products of β- and β,δ-elimination. Conversely, mammalian APE1 demonstrates a weak DNA 3′-phosphatase activity and primarily removes the 3′-phospho-α,β-unsaturated aldehyde generated by the β-lyase activity of OGG1 and NTH1, whereas 3′-phosphate ends generated by the β,δ-lyase activity of NEIL glycosylases are poor substrates. However, 3′-phosphate ends appear to be favored substrates of polynucleotide kinase/phosphatase (PNK), a protein highly expressed in mammalian cells that possesses both 3′-phosphatase and 5′-kinase activities. Mammalian PNK also has been shown to be involved in repair of 3′-phosphate termini at DNA single-strand breaks induced by ionizing radiation and ROS (126). These damages generate a 3′-blocking end, which is not a good substrate for APE1 but can be processed by PNK. Thus, in mammalian cells, a combination of NEIL glycosylases and PNK could generate the 3′-OH termini essential to complete BER repair by DNA polymerase without involving APE1. A PNK-mediated repair in the absence of APE1 was observed *in vitro* for both NEIL1 and NEIL2 by using a reconstituted system containing DNA with oxidative damages, PNK, POLβ, and the ligase complex LIG3/XRCC1 (49). AP sites and 3′-dRP generated by other DNA glycosylases also can be processed through a NEIL-PNK–dependent pathway. Moreover, NEIL1, similar to APE1, can enhance OGG1 turnover by competing for the AP site. Thus, the NEIL-PNK–dependent repair may represent an alternative repair pathway, providing redundancy in mammalian BER and an important protection against oxidative and spontaneous DNA damage (101).

5. Single-strand break repair. Single-strand break (SSB) lesions are both mutagenic and cytotoxic because they interfere with DNA replication, and therefore they need to be repaired. SSBs are directly produced by certain DNA-damaging agents (ionizing radiation and other oxidizing compounds)

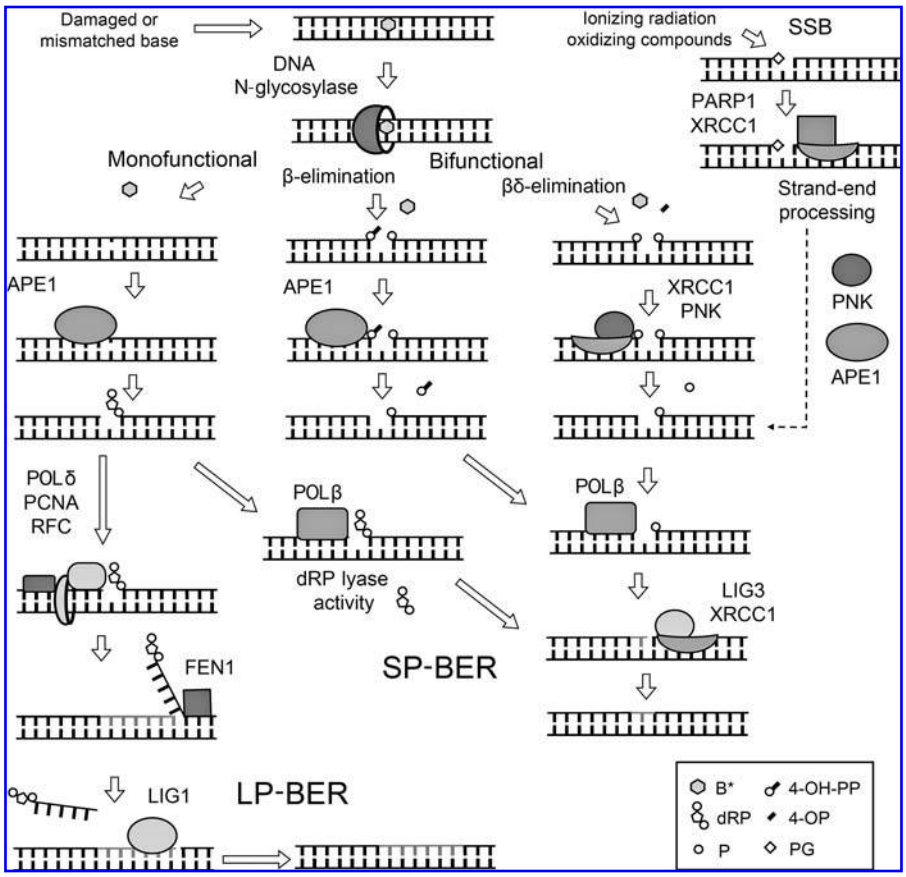

FIG. 9. Schematic representation of the BER pathway. The key enzymes in the BER pathway are DNA *N*-glycosylases, which recognize and remove damaged or mismatched bases (**B***). The following steps depend on the activity associated with each DNA *N*-glycosylase and can be grouped in APE1-dependent and APE1-independent repair. In the APE1-dependent pathway, the abasic site left by monofunctional *N*-glycosylases is processed by the endonuclease activity of APE1, generating a 3′-OH terminus and a 5′-terminal 2-deoxyribose-5-phosphate (dRP) moiety (*left*). The strand processing takes place in a different way for bifunctional glycosylases with β-lyase activity. In this case, the exonuclease activity of APE1 removes the 4-OH-pentenal phosphate (4-OH-PP) 3′-blocking end (*middle*). In the APE1-independent pathway, the process is initiated by bifunctional DNA *N*-glycosylases with a β,δ-lyase activity. Their activity releases 4-oxo-2-pentenal (4OP), generating 3′-phosphate ends, which appear to be favored substrates of PNK (*right*). Once the 3′-unblocked ends are generated, DNA polymerase can perform repair synthesis. This event generally involves the replacement of a single nucleotide mediated by POLβ, which incorporates the correct nucleotide and eventually removes the 5′-dRP termini through its dRPase activity. The final ligation step is carried out by the LIG3/XRCC1 complex (SP-BER). Sometimes DNA synthesis extends beyond a single nucleotide, with incorporation of two to 10 nucleotides. This pathway depends on enzymes normally involved in DNA replication, such as POLδ or POLϵ, RFC, PCNA, and the ligation step is carried out by LIG1 (LP-BER). Single-strand breaks (SSBs) are both intermediates of the BER process or induced by ionizing radiation and oxidizing compounds. Their repair can be performed through the BER pathway but requires additional activities. The PARP1/XRCC1 complex initially detects the SSBs and the 3′-blocking ends, such as 3′-phosphate, 3′-phosphoglycolate (PG), or 3′-4-OH-pentenal phosphate, require a strand processing catalyzed by PNK and/or APE1.

or created during DNA metabolism. Because SSBs are also intermediates of the BER process, their repair can be performed through this pathway, although it may require additional activities (see Fig. 9). PARP1, an enzyme that catalyzes the poly(ADP-ribosyl)ation of several protein substrates, including itself, is implicated in many cellular processes, including DNA repair, and has been proposed to function in the SSBs detection (36, 216). PARP1 has two zinc-finger motifs that mediate binding to strand breaks to enable its enzymatic activity. A physical interaction occurs between PARP1 and XRCC1, whose biologic function is to recruit XRCC1 and other associated proteins to the strand break (185). This interaction involves the central domain of XRCC1 and the N-terminal zinc-finger and the automodification region of PARP1, and is dependent on poly(ADP-ribosyl)ation activity of PARP1. Moreover, XRCC1 interacts with PNK, leading to an increase in both its kinase and phosphatase activities. PNK activity is important in SSB repair because it can process the 3′-blocking ends of SSBs, including 3′-phosphoglycolate and 3′-phosphate ends, which are generated mainly by ionizing radiation and OH radical, as well as by DNA-repairing enzymes. A complex containing XRCC1, PNK, and TDP1 also has been reported. TPD1 has a tyrosyl-DNA phosphodiesterase activity that removes topoisomerase I from 3′-trapped protein–DNA intermediates. These DNA/protein complexes are transiently formed during the activity of topoisomerase I, whose function is to relax supercoiled DNA, and their accumulation can lead to the formation of double-strand breaks (197).

6. Protein interactions and post-translational modifications in BER. Although it is able to be reconstituted *in vitro* with a few proteins, BER involves the participation of several proteins and auxiliary factors. Physical or functional protein–protein interactions or both may play a role in determining BER efficiency, subpathway selection, and patch size (73, 81, 117). Furthermore, several BER protein activities are regulated by posttranslational modification, and some of the physical protein–protein interactions link BER to other DNA

transaction pathways. WRN, a protein with both helicase and 3′-exonuclease activities, can interact with APE1 and POLβ. APE1 modulates the helicase activity of WRN, thus preventing unwinding of strand-break intermediates and facilitating gap filling in SP-BER, whereas WRN stimulates strand displacement synthesis by POLβ, unwinds strand-break intermediates, and facilitates LP-BER. p53, a protein involved in DNA damage signaling, is associated with APE1 and POLβ, and these interactions account for the observed enhancement of BER activity by p53. A detailed description of protein–protein interactions and posttranslational modifications involving BER proteins is well reviewed in (73).

7. Reversing the chemical damage. As a complement to the BER machinery, specialized proteins contribute to base-damage recognition and removal. Mammalian O^6-alkylguanine-DNA methyltransferase (AGT) directly removes alkyl groups at the O^6 position of guanine (as O^6-meG) in a stoichiometric irreversible reaction that reverses the DNA base damage (191, 217). The protein transfers the O^6-alkyl group to a cysteine residue at its active site, simultaneously inactivating itself, and is then degraded by the proteasome. ABH2 and ABH3, human homologues of *E. coli* AlkB, belong to the superfamily of 2-oxoglutarate- and iron-dependent oxygenases (2, 65) and restore normal base from alkylation damage in both DNA and RNA. These enzymes require Fe^{2+} and O_2 as the oxygen donor to catalyze a hydroxylation reaction in which the *N*-alkyl group modifying a base is oxidized into an alcohol then leads to the regeneration of the normal base with the release of formaldehyde. These oxygenases couple the substrate oxidation to conversion of 2-oxoglutarate into succinate and CO_2. Substrate specificity of human enzymes is restricted to 1-methyladenine and 3-methylcytosine, followed by 1,N^6-ethenoadenine.

Agents that give rise to a base damage in DNA can also modify the nucleotide precursors. Thus, modified nucleoside triphosphates generated in the nucleotide pools can be dephosphorylated by a sanitization enzyme. MTH1, the homolog of *E. coli* MutT, recognizes several base-modified 2-deoxyribonucleoside triphosphates, 8-oxo-GTP, 8-Cl-GTP, 8-OH-ATP, and 2-OH-ATP, and prevents their incorporation into DNA by hydrolyzing them to nucleoside monophosphate (86, 115).

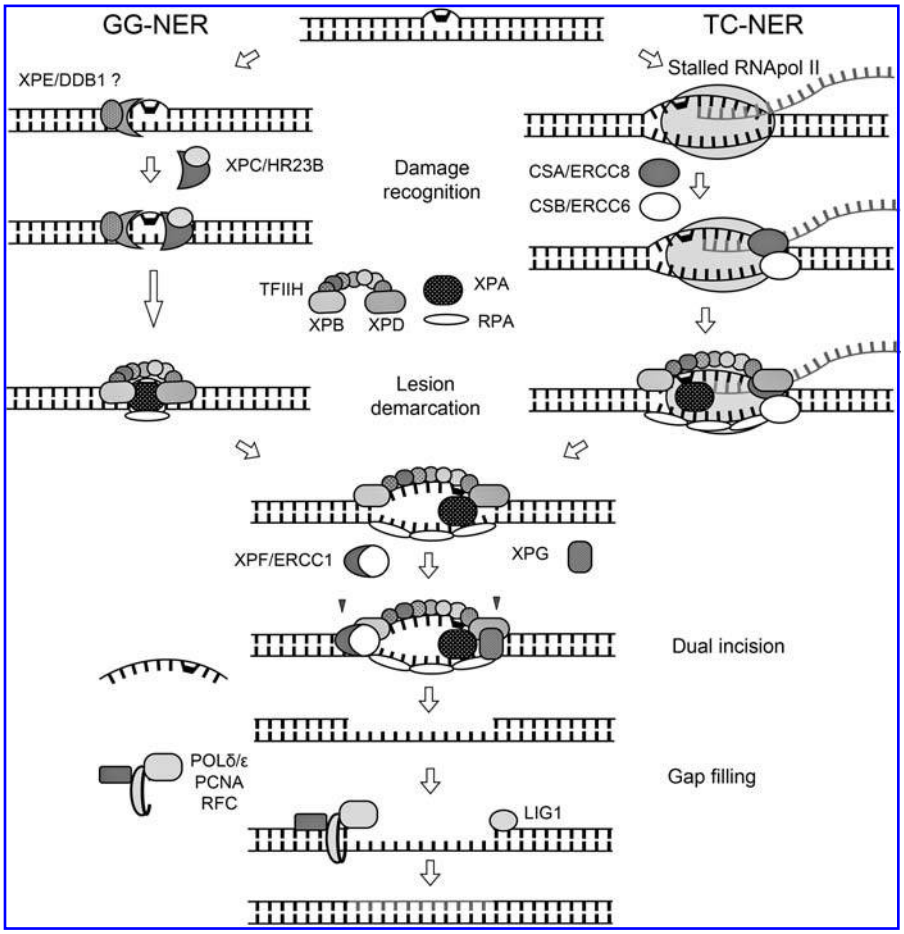

FIG. 10. Schematic representation of the NER pathway. The NER pathway is responsible for the removal of bulky adducts induced by UV radiation or some chemicals. Global genome repair (GG-NER) eliminates lesions from the entire genome, whereas transcription-coupled repair (TC-NER) specifically repairs damages on DNA strands of actively transcribed genes. The two NER pathways share the same repair mechanism with a difference in the DNA damage-recognition step where specific sets of proteins are involved. In GC-NER, the XPC/HR23B complex in cooperation with XPE and DDB1 recognizes lesions. The following step is lesion demarcation, through the formation of an open complex that includes XPA, RPA, and the transcription factor IIH (TFIIH) complex. XPB and XPD, both subunits of TFIIH complex, are ATP-dependent DNA helicases containing, respectively, 3′–5′ and 5′–3′ DNA unwinding activity, that generates an open stretch of 20 nucleotides around the lesion. XPA and RPA stabilize the open complex and are required for recruitment of additional components. In addition, RPA binds and stabilizes the undamaged strand. In the final step, the damaged strand is excised by the activity of two structure-specific endonucleases, XPG and XPF/ERCC1. The 3′-incision is made by XPG, close to the lesion site, whereas XPF/ERCC1 is responsible for the 5′-incision, localized 15–25 nucleotides away. The resulting gap is filled by the PCNA-dependent DNA polymerases δ and ϵ, and sealed by DNA ligase. In TC-NER, the stalled RNA polymerase II on the damaged site, assisted by CSA (ERCC8) and CSB (ERCC6) proteins, attracts the core NER machinery.

TABLE 3. PROTEINS INVOLVED IN NUCLEOTIDE EXCISION REPAIR (NER)

Protein	*Function*
Damage recognition in global genomic NER (GG-NER)	
DDB1	Binds damaged DNA with XPE; component of E3 ubiquitin ligase
XPE (DDB2)	Binds damaged DNA with DDB1; recruits other NER proteins
XPC	Binds damaged DNA with HR23B; recruits other NER proteins
HR23B	Binds damaged DNA with XPC; recruits other NER proteins
TFIIH complex	
XPB (ERCC3)	$3' > 5'$ helicase activity; early and late DNA unwinding
p62 (GTF2H1)	?
p44 (GTF2H2)	Regulation of XPD
p34 (GTF2H3)	?
p52 (GTF2H4)	Regulation of XPB
XPD (ERCC2)	$5' > 3'$ helicase activity: late DNA unwinding
Cdk7	Cdk (C-terminal domain kinase); transcription only; participates in the formation of the cdk-activating kinase (CAK) complex
Cyclin H	Cyclin; transcription only; participates in the formation of the CAK complex
MAT1	Cdk assembly factor; transcription only; participates in the formation of the CAK complex
Excision complex	
XPA	Binds and stabilizes open complex; confirms damage and recruits RPA
RPA	Single-stranded DNA binding; binds undamaged strand in open complex
XPG (ERCC5)	Structure specific endonuclease (3′ incision); stabilizes full open complex
XPF (ERCC4)	Structure specific endonuclease (5′ incision); complex with ERCC1
ERCCI	5′ incision with XPF
Damage recognition in transcription-coupled NER (TC-NER)	
CSA (ERCC8)	Complex with DDB1; recruited by CSB
CSB (ERCC6)	Recognizes stalled RNA polymerase II

Gap filling and ligation require POLδ/POLε, PCNA, RFC, and LIG1 activities.

B. Nucleotide excision repair

Although base-excision repair is an important pathway, it is insufficient to deal with all types of damage. Any damage to be corrected by base-excision repair would require a specific DNA glycosylase capable of recognizing it. The presence of a wide variety of DNA-reactive chemicals combined with a huge diversity of oxidative pathways that can be induced by ionizing radiation and OH radical is susceptible to generating many types of DNA alterations. Therefore, it would be difficult, if not impossible, for the evolutionary development of damage-specific DNA *N*-glycosylases to deal with all of them. Thus, a different and more flexible damage-repair mechanism has evolved in living organisms, known as nucleotide excision repair (NER), which recognizes damaged regions by their abnormal structure and then proceeds with their excision and replacement. NER has the ability to eliminate a wide diversity of structurally unrelated DNA lesions, the most relevant of which are CPDs and 6-4PPs, formed by adjacent pyrimidines and constituting the two major classes of lesions induced by UV radiation. NER can also remove bulky chemical adducts induced by large polycyclic aromatic hydrocarbons, such as those present as benzo[α]pyrene in cigarette smoke, and the distorting intrastrand crosslinks induced by chemotherapeutic agents, such as cisplatin. Finally, NER has been shown to be involved in the removal of minor base damages induced by alkylating and oxidizing agents, which are generally not helix distorting, and thus functions as a backup system (16). In mammalian cells, NER is the major repair pathway for the removal of bulky adducts induced by UV radiation or other environmental carcinogens, but it may help remove oxidatively generated DNA lesions such as 8-oxoG and 8,5′-cyclo-2′-deoxyadenosine (48). The latter lesion is not repaired by DNA *N*-glycosylases, which, in general, deal with tiny modifications. This confirms the importance of the NER mechanism in oxidative damage response because of its capacity to remove bulky lesions.

The NER process involves the action of 20 to 30 proteins in successive steps: DNA-damage recognition and assembly of a multiprotein complex at the damaged site; double incision through endonuclease activity of the damaged strand several nucleotides away from the lesion, on both the 5′ and 3′ sides; and removal of the damage-containing oligonucleotide between the two nicks. The resulting gap is filled by a DNA polymerase, and the newly synthesized strand is sealed by a DNA ligase. The names of many of the genes involved in NER start with the letters "XP," because they were first identified in genetic complementation studies of the human DNA-repair disease, xeroderma pigmentosum (XP). Nucleotide-excision repair can be categorized into two classes: global genome repair (GG-NER) and transcription-coupled repair (TC-NER). GG-NER eliminates lesions from the entire genome, whereas TC-NER specifically repairs damage on DNA strands of actively transcribed genes. The two subpathways of NER share the same repair mechanism, with a difference in the DNA-damage recognition step, in which a specific set of proteins is involved (52, 100, 187) (Fig. 10, Table 3).

1. Global genome repair. GG-NER acts on DNA lesions throughout the genome, but the kinetics of repair can be

influenced by a number of parameters related to DNA lesion structure and chromatin configuration. In this process, the damage-recognition step is the rate-limiting one, whereas the binding strength of proteins involved in damage recognition depends on the chemical structure of DNA lesions and the way in which these interfere with the DNA helical structure. Large bulky lesions, such as 6-4PP and CPD, are located in the minor groove of the DNA helix and are recognized by NER proteins as being abnormal structures in the DNA, which interfere with its dynamic properties such as bending, twisting, unwinding, and rewinding. Lesions that are good substrates for NER often cause local unwinding of a few DNA bases around the damaged site, thus promoting DNA bending and facilitating further unwinding by NER enzymes. The efficiency of GG-NER may be influenced by accessibility of DNA lesions to repair proteins, which, in turn, is dependent on chromatin structure.

2. DNA damage recognition in GG-NER. The XPC/HR23B heterodimeric complex is the first NER factor to detect a lesion and recruit the rest of the repair machinery to the damaged site in GG-NER (141). XPC is a 125-kDa protein that interacts directly with DNA and other protein factors involved in the initial step of recognition, such as HR23B and transcription factor IIH (29). HR23B is a 58-kDa human protein, homologue to the yeast protein RAD23, important for the proper function of the recognition complex through its modular structure. It contains, as well as the XPC-binding domain, an N-terminal ubiquitin-like and two ubiquitin-association domains (132).

The XPC/HR23B complex recognizes specifically damaged DNA on the basis of the extent of distortion induced by the damage on DNA helical structure. It has been observed that 6-4PPs, which are more helix distorting, are recognized much more readily than CPDs, indicating that recognition of the latter lesions requires additional factors. Recently, centrin 2, which interacts with the XPC/HR23B complex, was shown to stimulate NER activity by enhancing damage recognition of XPC (180). Another factor that cooperates in the recognition of the damage is the protein complex known as UV-DDB (UV-damaged–DNA-binding protein), consisting of two proteins, DDB1 and DDB2 (also known as XPE). UV-DDB binds selectively to a variety of UV-induced DNA lesions and may represent an initial damage sensor, in particular for CPD lesions. Genetic experiments indicate that recognition of CPDs is heavily dependent on the XPE protein (229). DNA bending, induced by UV-DDB binding to damaged DNA sites, may enhance the binding affinity of the XPC/HR23B complex. The UV-DDB heterodimer is also a component of an E3 ubiquitin ligase, and it appears to convey the other components of the ubiquitination system to UV-damaged DNA sites (229). A reversible ubiquitination of XPC upon UV irradiation, which is dependent on the presence of UV-DDB, has been recently reported (229). Therefore, the UV-DDB–associated E3 ubiquitin ligase may help to recruit XPC to the damaged site. Ubiquitination of XPC by E3 ligase does not cause XPC degradation, probably because of the protective interaction with the ubiquitin-association domains of HR23B. Instead, this modification appears to enhance the affinity of XPC for damaged DNA. XPC is also subjected to SUMO conjugation induced by UV irradiation. This prevents XPC degradation and is dependent on the activity of XPA, a factor responsible for the next step of NER (253). Thus, UV-induced posttranscriptional modifications of XPC appear to be crucial for the ordered and specific assembly of proteins at the DNA-damaged site.

3. Lesion demarcation. The increased distortion induced by XPC/HR23B permits the entry and the binding of additional factors, which lead to the formation of an open complex, with a local unwinding of the DNA helix, and to the demarcation of the lesion. In this step, the factors initially required are XPA, RPA, and transcription factor IIH complex (TFIIH). Several protein–protein interactions take place to allow the formation of a multicomponent complex that specifically recruits two specialized endonuclease activities, XPG and XPF. These activities are required to excise the damaged strand by incision on both the 3′ and 5′ sides of the open complex and complete the repair machinery (209). XPF is physically associated with the excision cross-complementing protein 1 (ERCC1) to form a functional complex.

TFIIH is a protein complex of nine subunits (XPB, XPD, p62, p52, p44, p34, cdk7, cyclin H, and MAT1) that was originally identified as an essential factor in basal transcription initiation. XPB and XPD are ATP-dependent DNA helicases that contain, respectively, 3′–5′ and 5′–3′ DNA-unwinding activity that is required for the transcription initiation of RNA polymerase II at the promoter site. In the NER pathway, TFIIH binds to the damaged strand, and through the helicase activity of XPP and XPD uses the energy of ATP to unwind a stretch of 20 to 30 nucleotides of DNA helix around the damaged site, forming a preincision structure (59). The binding of TFIIH is mediated by its p62 subunit that specifically interacts with the recognition complex XPC/HR23B, thus allowing the recruitment of TFIIH to the damaged site. Among the other subunits of the TFIIH complex, Cdk7, cyclin H, and MAT1 constitute the cdk-activating kinase (CAK) complex associated with TFIIH. The CAK complex is able to phosphorylate cyclin-dependent kinases (CDKs) involved in cell-cycle regulation and is also required for phosphorylation of the C-terminal domain of RNA polymerase II. The CAK complex is loosely associated with TFIIH and is not required for NER activity reconstituted *in vitro*. However, TFIIH complexes that have been released from the NER repair complex can support mRNA synthesis by RNA polymerase II in a reconstituted transcription assay, suggesting that TFIIH can be shuttled between repair and transcription.

Besides the unwinding function, the TFIIH complex also is responsible for the recruitment of XPA and XPG (the endonuclease required for 3′ incision). The N-terminal region of the p62 subunit contains a pleckstrin homology/phosphotyrosine-binding domain that associates with the endonuclease XPG (92). The same domain can also interact with several transcriptional activator proteins, such as p53 and VP16, and this reinforces the multiple role of TFIIH in repair and transcription.

The initial unwinding by XPB helicase opens up a small stretch and permits access of XPA to the damaged region. XPA is a 36-kDa zinc metalloprotein that, in the central part, contains a DNA-binding site, consisting of a loop-rich subdomain binding preferentially to structurally distorted DNA molecules, especially in a single-stranded context. Thus, XPA binding pro-

vides a second (after XPE and XPC) level of selection for damaged DNA, ensuring that normal DNA will not be subjected to excision repair. XPA also interacts with many other NER components such as RPA, ERCC1 (binding partner of XPF, the endonuclease required for 5′ incision), and TFIIH. The binding of XPA to TFIIH allows the complete unwinding of DNA helix to generate an open stretch of 20 to 30 nucleotides, with the assistance of RPA.

RPA is the last protein involved in lesion demarcation, and its recruitment is facilitated by its physical interaction with XPA. RPA is the major eukaryotic single-stranded DNA-binding protein required for eukaryotic metabolism and is involved in several processes such as DNA repair, replication, and recombination. RPA consists of a heterotrimeric complex composed of 70-, 32-, and 14-kDa polypeptide subunits, and its ssDNA-binding activity resides mainly in the central region of the 70-kDa subunit, which contains two tandem oligonucleotide binding folds (20). The same region contains a zinc-finger domain that interacts with XPA; thus, RPA–XPA interactions might be modulated by ssDNA-RPA binding (50). RPA participates in multiple steps of the NER process; at first RPA facilitates DNA unwinding by TFIIH through its ssDNA binding activity and, after the interaction with XPA, it binds to the single, undamaged strand thus protecting it from nuclease attack (151). The optimal binding site of RPA has a size of 20 to 30 nucleotides, similar to the fully opened repair complex and to the damaged strand released after DNA excision. Concomitant with the binding of RPA and XPA, the initial recognition complex XPC/HR23B is released, allowing its recycling for other damaged sites where the repair process must start.

4. Dual incision step. In the following step, the damaged strand in the preincision-opened complex is excised by the activity of two endonucleases, XPG and XPF/ERCC1 (28). Both XPG and XPF/ERCC1 are structure-specific endonucleases effective on junctions between single- and double-stranded DNA. XPG is a structure-specific nuclease closely related to the FEN1 nuclease that participates in base-excision repair, and cuts at the 3′ side of such junction in the open complex. XPF/ERCC1 is a heterodimeric protein complex that cuts at the 5′ side. The 3′-incision made by XPG is close to the lesion site (few nucleotides), and in most studies appeared to be made before and independent of the 5′-incision by XPF/ERCC1, which is 15 to 24 nucleotides away. This is the result of several protein–protein interactions that specifically direct the positioning and activity of both endonucleases, avoiding incision of the undamaged strand. XPG is directed at the preincision complex through its interaction with TFIIH, and the following interaction with XPA and RPA plays a role in their mutual association with the DNA substrate (11). XPG contains two nuclease motifs separated by a large insertion, which is responsible for the interaction with TFIIH and contributes to the substrate specificity (64).

XPF/ERCC1 is the last protein complex to join the NER preincision complex. The association of XPF/ERCC1 is mediated by hydrophobic interactions between the C-terminal helix-hairpin-helix domains present in both proteins (38). The recruitment of XPF/ERCC1 is dependent on its binding with XPA and RPA, confirming the pivotal role of these two proteins in the final assembly of the preincision complex. The specific interaction between ERCC1 and XPA properly localizes the XPF nuclease domain, just as the interaction between XPF and RPA coordinates the nuclease activity toward the damaged strand. XPG is also required for the binding of XPF/ERCC1 to the damaged site, inducing a structural change in the preincision complex. Thus, various molecular interactions among NER components coordinate both endonuclease activities to ensure correct timing and the specific excision of the damaged strand (28, 248).

5. Gap filling and ligation. After removal of the lesion, DNA repair is completed by DNA polymerase, which binds to the 3′-OH group generated by the XPF/ERCC1 cut and synthesizes the new DNA strand, leading to the displacement of the damage-containing oligonucleotide and of NER components TFIIH, XPA, XPG, and XPF/ERCC1. After gap filling, the newly synthesized DNA is sealed by DNA ligase. Reconstitution experiments have demonstrated that repair synthesis is efficiently performed by DNA polymerase δ and ε with the aid of accessory proteins RFC and PCNA, which assist DNA polymerase in the reaction, whereas DNA ligase I is a likely candidate for the sealing reaction. As mentioned earlier, RPA protects the undamaged DNA strand from degradation.

6. Transcription-coupled repair. Transcription-coupled (TC)-NER is an alternative pathway that specifically removes DNA lesions in the genomic area where transcription is occurring simultaneously (169, 213). TC-NER was first described for cultured mammalian cells, but has subsequently been shown to operate in a variety of organisms including bacteria and yeast. A major obstacle that prevents its complete understanding is the lack of a cell-free system capable of performing TC-NER. At present, it is not completely clear how repair is coupled with active transcription, but it is generally assumed that a stalled transcript provides a strong signal to attract the repair machinery. In this case, the recognition factors mediate the dissociation of RNA polymerase II from the DNA strand to allow the repair process to proceed (213). Slow removal of DNA lesions from transcription templates would prevent efficient transcription, and this could lead to cell death if essential genes are involved.

7. DNA damage recognition in TC-NER. In humans, TC-NER requires all the proteins needed for GG-NER except XPE, XPC, and HR23B, suggesting that a different mechanism (not requiring XPE and XPC) is involved in recognizing the damage in transcribed strands. Numerous experiments suggest that this different mechanism involves the stalling of RNA polymerase at damaged sites. RNA polymerase stops when it runs into minimally distorting damage in the template strand. This probably explains why CPDs, which are less distorting than 6-4PPs, are repaired much more rapidly by TC-NER than by GG-NER. The stalled RNA polymerase II complex itself seems to be the damage-recognition signal in TC-NER and attracts the core of the NER machinery. Because a stalled RNA polymerase II sterically hinders accessibility of NER proteins, it has to withdraw or dissociate from the lesion for repair to take place. CSA (ERCC8) protein, which is thought to be involved in formation of multiprotein complexes, and CSB

(ERCC6), which is a member of the SWI/SNF family of DNA-dependent ATPase implicated in chromatin remodeling, are involved in processing of a stalled RNA polymerase complex. However, the precise role of CSA and CSB in the process of TC-NER remains unclear, and several *in vitro* systems have been constructed to gain more information (83, 145, 214). It has been shown that CSB can interact in a cooperative manner with stalled RNA polymerase and with XPG. Although CSB did not increase the recruitment of NER factors and was not implicated in the release of stalled RNA polymerase, it was absolutely essential for dual-incision activity. Moreover, in another reconstituted system, CSB bound to stalled RNA polymerase recruited the CSA/DDB1 complex, and this enhanced the interaction of stalled complex with chromatin-remodeling factors such as histone-acetyl-transferase p300 and the nucleosome-binding protein HMGN1.

The possibility for oxidized nucleosides, including 8-oxoG and thymidine glycol, to be repaired by TC-NER, which was well accepted a few years ago, has been questioned and subsequently ruled out (147, 149). Recently, it was reported that CSA and CSB are constituents of 8-hydroxyguanine repair and that CSA is also involved in the repair of 8,5′-cyclopurine-2′-deoxynucleosides (48).

The fate of stalled RNA polymerase is not clear, but it has been observed that a fraction of RNA polymerase II became ubiquitinated after treatment of cells with UV in a CSA- and CSB-dependent manner (152). Thus, it is possible that ubiquitination is a signal for the degradation of the protein so that the lesion becomes accessible to repair enzymes. The following steps of TC-NER are essentially the same as for GC-NER with the formation of the open complex and the lesion demarcation by XPA, RPA, and TFIIH, the excision of damaged strand, the filling by DNA polymerase, and the sealing by a ligase. Additional evidence suggests that the XPB and XPD helicase subunits of TFIIH, and also the XPG nuclease, play special roles in TC-NER, roles that go beyond their roles in GG-NER. It may be that these three proteins assist in the resumption of transcription.

C. Mismatch repair

The mismatch repair (MMR) is a system for the correction of errors introduced during DNA replication, when an incorrect base is incorporated into the daughter strand that evades the proofreading activity of DNA polymerase. These mismatches, if uncorrected, will give rise to permanent mutation in the genome during the subsequent round of DNA replication. As well as single-base mismatches, the postreplicative MMR system efficiently corrects loops of one to a few extrahelical nucleotides (insertion deletion loops, IDLs) that arise during the replication of repetitive DNA tracts. IDLs are the consequences of spontaneous slippage-dependent misalignment between primer and template DNA strands. The MMR system is essential to all organisms because it maintains the stability of the genome during repeated duplication.

1. Mismatch repair in prokaryotes. The process of mismatch repair is highly conserved throughout evolution, and this single fact has greatly facilitated its study in human cells (116, 122, 138, 165). Essential components of the MMR system were initially identified in *E. coli* by genetic analysis and were named "Mut" genes because their inactivation generated hypermutable strains. Subsequent studies led to the characterization of the MMR system in *E. coli* that involves at least 10 factors (116, 138). Three proteins are essential in detecting the mismatch and directing repair machinery: MutS, MutH, and MutL. MutS has two functional domains, a DNA-binding domain and an ATPase/dimerization domain, and detects mismatch in DNA duplex. MutS forms a homodimer that nonspecifically binds and bends DNA to search mismatches, discriminating between homoduplex and heteroduplex DNA. When MutS recognizes a specific mismatch, it undergoes a conformational change and initiates the MMR pathway through direct or indirect interactions with other proteins, including MutL, MutH, and UvrD. MutL has ATPase activity, and its function is to connect the recognition of a mismatch with the excision of the same mismatch from the newly synthesized strand. This is an ATP-dependent process by which MutL dimerizes and interacts with MutS, activating the latent endonuclease activity of MutH. MutH recognizes the temporarily unmethylated newly synthesized strand and cleaves it at hemimethylated GATC sites located within 1,000 bp of the mismatch. MutL also recruits UvrD at the damage site. UvrD, also known as MutU, is a DNA helicase II that unwinds the DNA duplex from the nick generated by MutH, allowing the excision of the newly synthesized strand between the nick and the mismatch sites, through the action of four redundant single-strand DNA-specific $3' > 5'$ (ExoI and ExoV) and $5' > 3'$ (RecJ and ExoVII) exonucleases. The final step consists of the repair synthesis of DNA carried out by the replicative DNA polymerase III, single-strand DNA-binding proteins, and DNA ligase.

2. Proteins involved in mammalian mismatch repair. All eukaryotic organisms, including yeast, mouse, and human, have MutS and MutL homologues, MSHs and MLHs, respectively. Different from bacterial MutS and MutL, which function as homodimers, mammalian MSHs and MLHs form heterodimers with multiple proteins. Five highly conserved MSHs (MSH2–MSH6) are present in both yeast and mammals, whereas MSH1, which is present in mitochondria, exists only in yeast. MSH2 is required for all mismatch correction in nuclear DNA, whereas MSH3 and MSH6 are required for the repair of some distinct and overlapping types of mismatched DNA during replication. MSH4 and MSH5 show reproductive tissue-specific expression and are probably involved in meiotic recombination. Mammalian cells possess two MutS activities that function as heterodimers and share MSH2 as a common subunit: MutSα (MSH2–MSH6 heterodimer) and MutSβ (MSH2–MSH3 heterodimer). Studies of DNA binding by MutSα and MutSβ and of MMR activity in mammalian cell–free systems indicate that MutSα, which represents 80–90% of the cellular MSH2, is primarily responsible for repairing single base–base and IDL mismatches, whereas MutSβ is primarily responsible for repairing IDL mismatches containing up to 16 extra nucleotides in one strand. The two complexes can share responsibility for repairing some IDL mismatches, especially those with one extra base (91, 96, 167).

The four homologs of MutL in both yeast and mammals were identified by their great similarity to prokaryotic MutL proteins, especially at their N-termini, or as genes whose mutation gives

rise to a failure to repair mismatches in meiotic recombination intermediates. Three eukaryotic MutL activities have been identified and, similar to eukaryotic MutS activities, function as heterodimeric complexes, with MLH1 serving as the common subunit. MutLα, a heterodimer of MLH1 and PMS2, is the primary MutL activity in human mitotic cells and supports repair initiated by either MutSα or MutSβ. MutLα accounts for ≈90% of the MLH1 in human cells (200), but two low-abundance complexes involving MLH1 also have been identified. MutLβ, a human MLH1-PMS1 heterodimer, has been isolated, but its involvement in mismatch repair has not been demonstrated (200), whereas MutLγ, a MLH1/MLH3 complex, has been reported to support modest levels of base–base and single-nucleotide ID mismatch repair *in vitro*, events that are presumably initiated by MutSα (34).

Eukaryotes have no known homologue of *E. coli* MutH, and no DNA helicase activity has been shown to participate in replication-error repair. Repair is initiated when a complex of MutS homologues, either MutSα or MutSα, binds to a mismatch. In contrast to *E. coli*, in which mismatch repair is directed by the transient absence of adenine methylation at GATC sites within newly synthesized DNA, the strand signals that direct replication-error correction in eukaryotes have not been identified (120, 172). However, it has been observed that strand discontinuities, as gaps between Okazaki fragments associated with replication, may direct the repair. At present, most of the information on the mechanism of eukaryotic MMR is derived mainly from the analysis of nick-directed repair of circular heteroduplexes in mammalian cell extracts. The strand break that directs repair may reside either 3′ or 5′ from the mismatch site, with the excision of a DNA tract extending up to ~200 nucleotides beyond the mismatch. Thus, the mammalian repair system displays a bidirectional capability, responding to both 3′ and 5′ heteroduplex orientations, and its functionality is retained at nick-mismatch separation distances as large as 1,000 bp.

Genetic and biochemical evidence indicates that the exonuclease activity is provided by Exo1, which, however, hydrolyzes duplex DNA with a $5' > 3'$ polarity (150). Surprisingly, this enzyme is required for excision and repair directed by strand breaks located either 5′ or 3′ to the mismatch, and this finding suggests the existence of alternative excision activities. An involvement has been proposed of $3' > 5'$ editing exonuclease activity of DNA polymerase during repair, whereas Vo *et al*. (247) suggested that Mre11, a $3' > 5'$ exonuclease, participates in 3′-directed mismatch repair. Several DNA-binding proteins have been found to be involved in eukaryotic MMR. The single-stranded DNA-binding protein RPA promotes repair DNA synthesis, stimulating the excision of the mismatched strand and stabilizing the gap against endonuclease attack. HMGB1 interacts with MutSα and may play a role in the initiation of DNA excision (266). DNA resynthesis is catalyzed by DNA polymerase δ, PCNA, and RFC, which load PCNA onto the helix. PCNA confers processivity to polymerase δ and plays multiple roles in mismatch repair. PCNA has been proposed to function in the mismatch-recognition stage of MMR by helping MutSα to search for mismatched DNA (148) or increasing the mismatch-binding specificity of MutSα (80). Moreover, PCNA is required for control mismatch-provoked excision (see later). Proteins involved in mammalian mismatch repair are listed in Table 4.

3. Mismatch-provoked excision and repair. Biochemical studies and reconstitution experiments have indicated that a complete excision of the mismatched section of DNA requires at least the heterodimeric complexes MutSα and MutLα, Exo1, RPA, and ATP (90,268) (Fig. 11). In this simple excision system, MutSα activates and confers high processivity to Exo1 in a mismatch- and ATP-dependent manner (90). Exo1 initiates its $5' > 3'$ hydrolytic activity at the strand break on a 5′-heteroduplex, and excision terminates upon mismatch removal in a manner that depends on RPA and MutLα. RPA has both negative and positive regulatory effects on the reaction. RPA restricts the hydrolytic activity on excision products by reducing processivity of the MutSα/Exo1 complex and promotes a turnover of the system after mismatch removal by binding to gaps, controlling the access of Exo1 to 5′ termini in excision intermediates/products, and facilitating MutSα/Exo1 complex displacement from DNA (90). MutLα seems not to be

TABLE 4. PROTEINS INVOLVED IN MISMATCH REPAIR (MMR)

Protein	*Function*
MutS homologues	
MSH2	Forms heterodimers with MSH6 (MutSα, 80–90%) and MSH3 (MutSβ)
MSH3	MutSβ (MSH2/MSH3); repair of large insertion/deletion loops
MSH6	MutSα (MSH2/MSH6); repair of base mispairs and small insertion/deletion loops
MSH4 and MSH5	Heterodimer MSH4/MSH5 (MutSγ); processing recombination intermediates in meiosis
MutL homologues	
MLH1	Forms heterodimers with PMS2 (MutLα, >90%), PMS1 (MutLβ), and MLH3 (MutLγ); adaptors recruited by MutS heterodimers
PMS2	MutLα (MLH1/PMS2); repair of base mispairs and small insertion/deletion loops
PMS1	MutLβ (MLH1/PMS1); role unknown
MLH3	MutLγ (MLH1/MLH3); repair of insertion/deletion loops
Excision and repair synthesis	
Exo1	Exonuclease; mismatch excision ($5' > 3'$ exonuclease activity)
RPA	Single-stranded DNA binding; regulatory effect on Exo1
RFC, PCNA	Nick identification, POLδ processivity factors
POLδ, LIG1	Gap filling and sealing

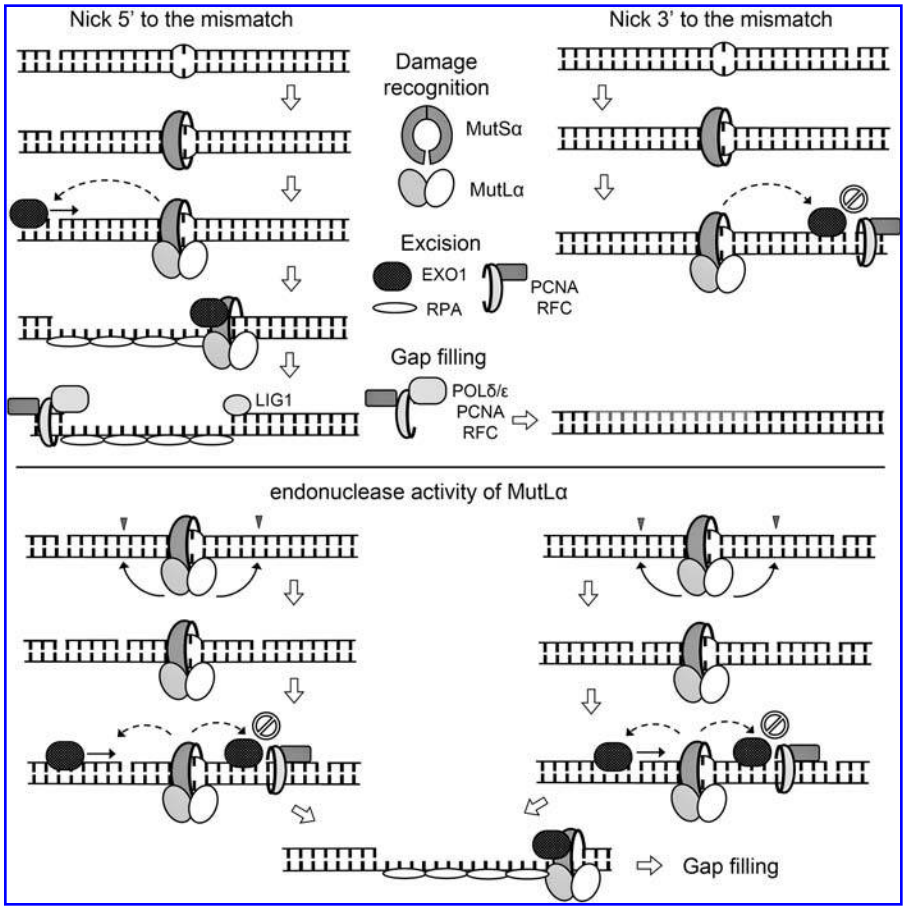

FIG. 11. Models for nick-directed mismatch excision. The MMR system corrects base-pairing errors that are introduced in the newly replicated DNA strand, evading the proofreading activity of DNA polymerase. *Top*: In eukaryotes, signals that differentiate the newly synthesized DNA strand are probably nicks and discontinuities, such as those between Okazaki fragments associated with replication. At present, most of the information on the mechanism of eukaryotic MMR is derived from the analysis of nick-directed repair of mismatched DNA, which can be classified in 5′- and 3′-heteroduplexes, depending on the position of the nick in respect to the mismatch. Base mismatches are primarily recognized by MutSα, which activates Exo1 in a mismatch- and ATP-dependent manner. Exo1 is the exonuclease responsible for mismatch excision, and its activity is further regulated by MutLα, associated with MutSα, and by RPA. RPA restricts the hydrolytic activity on excision products and protects the single-stranded DNA generated during the reaction. Because Exo1 has only 5′ > 3′ exonuclease activity (*straight arrow*), a different scenario can be depicted for 5′- and 3′-heteroduplexes. In 5′-heteroduplex, once the strand is excised beyond the mismatch, DNA is resynthesized by the PCNA-dependent DNA polymerase δ/ϵ. In contrast, hydrolysis by Exo1 in 3′-heteroduplex will proceed with the wrong polarity for mismatch removal, and, in this case, it has been proposed that Exo1 activity can be repressed by the presence of PCNA and RFC. *Dotted-curved arrows* indicate a signaling that must occur between mismatch and strand break. *Bottom*: Recently, a new model has been proposed, in which MutSα, RFC, and PCNA activate a latent endonuclease activity of MutLα (*curved arrows*). This activity occurs on both 5′- and 3′-heteroduplexes and is strongly restricted to the nicked strand, introducing additional incisions near the mismatch that provide a further entry site for the excision system. In the case of 3′-heteroduplex, the incision distal to the mismatch provides an initiation site for mismatch removal by the 5′ > 3′ exonuclease activity of Exo1 (*straight arrows*). DNA repair is completed by the PCNA-dependent DNA polymerases δ/ϵ. *Dotted-curved arrows* indicate a signaling that must occur between mismatch and strand break. Models are based on those reported by Modrich (172) and Kadyrov *et al*. (124).

required for the activation of Exo1, but enhances the mismatch dependence of the reaction, functioning as a molecular matchmaker. MutLα also participates in excision termination, and, to this end, two different mechanisms have been proposed. Genschel *et al*. (90) attributed the involvement of MutLα in termination to its role in suppressing Exo1 activity on mismatch-free DNA. In this mechanism, MutLα simply stabilizes excision products against nonspecific hydrolysis by Exo1. In contrast, Zhang *et al*. (268) concluded that MutLα, acting in concert with RPA, plays an active role in excision termination on mismatch removal.

MutSα also activates Exo1 on a 3′-heteroduplex, but in this case, hydrolysis would proceed with the wrong directionality for mismatch removal. Different from 5′-heteroduplex, excision on a 3′-heteroduplex is supported when PCNA and RFC are supplemented to the reconstitution system (66). It has been observed that RFC and PCNA act to suppress nonproductive Exo1 hydrolytic 5′ > 3′ activity when the strand break that leads the hydrolysis is located 3′ to the mismatch, and this effect requires a structural integrity of RFC (66). This has led to a model wherein the 3′ or 5′ placement of a strand discontinuity produces a differential hydrolytic response that is due to the orientation-dependent binding of PCNA onto primer templates and the ability of PCNA to interact with the MutSα/MutLα complex and Exo1 (66). Once the strand is excised beyond the mismatch, DNA can be resynthesized by the PCNA-dependent DNA polymerase δ (POLδ) in the presence of RPA, and the remaining nicks sealed by a ligase activity. However, this system may require additional activities that play significant roles.

Because Exo1 is required for both-direction excision-directed repair, a cryptic 3′ > 5′ exonuclease activity for Exo1 has been proposed, only present within the context of MMR, which is

responsible for 3′-directed excision. However, this paradigm has been recently resolved by the finding of a latent MutLα endonuclease activity that can be stimulated in an ATP- and mismatch-dependent manner by the interaction with MutSα, RFC, and PCNA (124). This activity occurs on both 5′ and 3′-heteroduplex and is strongly restricted to the nicked strand, introducing additional incisions near the mismatch that provide a further entry site for the excision system. In the case of 3′-heteroduplex, the incision distal to the mismatch provides an initiation site for mismatch removal by the $5' > 3'$ exonuclease activity of MutSα-activated Exo1 (124) (see Fig. 11).

Recently, several purified systems have been described to support mismatch correction. MutSα, MutLα, Exo1, RPA, HMGB1, and POLδ are sufficient to carry on repair of 5′-heteroduplexes containing a G-T mismatch or a three-nucleotide ID mispair, and covalently closed repair products are obtained on supplementation of these proteins with DNA ligase I (268). However, this system does not support 3′-directed excision even in the presence of RFC and PCNA, and MutLα is not required for repair. In contrast, a reconstituted repair system that supports bidirectional mismatch repair has been described (45). This system is dependent on MutSα, MutLα, Exo1, RPA, POLδ RFC, and PCNA. Whereas MutLα is dispensable for 5′-directed repair but is required for 3′-heteroduplex repair, RFC and PCNA are always required for the DNA synthesis step and also for the excision step on a 3′-heteroduplex.

4. Functions of the mismatch repair system. In addition to correct replication errors, the MMR system is involved in controlling the fidelity of recombination events, both mitotic and meiotic, and in DNA damage surveillance participating in the earliest step of checkpoint responses (122, 123, 138). MMR machinery can also recognize certain DNA lesions generated by normal intracellular metabolism (oxidative stress) and by physical and chemical insult from the external environment, including certain chemotherapeutic agents (211, 265). Oxidation is a significant and constant source of spontaneous DNA damage. The oxidized purine 8-oxoG is a particularly frequent DNA lesion that, during replication, can form base pairs with adenine to promote the formation of G→T transversions. Purine dNTPs are also subject to oxidative damage, and the oxidized products are substrates for incorporation into DNA during replication. To avoid this, human cells sanitize the dNTP pool by hydrolyzing oxidized purine dNTPs. Evidence suggests that MMR removes 8-oxodGMP erroneously incorporated during replication (211), suggesting that this new role for MMR represents a further level of protection against the dangers of oxidized DNA bases. In addition, MMR proteins bind to O^6-meG DNA adducts formed by some alkylating agents, specifically S_N1 methylating agents (*e.g., N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, MNNG), and loss of MMR confers strong resistance to these agents. Similarly, MMR proteins bind to DNA containing *cis*-diamminedichloro-platinum (cisplatin) adducts, and loss of MMR confers partial resistance to cisplatin (128).

MMR proteins are involved in DNA damage surveillance, contributing to cell-cycle arrest and apoptosis in response to a wide range of DNA damage, but the molecular mechanisms are still unclear. In a simplified model, MMR proteins bind to damaged DNA and recruit several checkpoint proteins such as the signal-transducing kinases ataxia–telangiectasia mutated (ATM) and ataxia–telangiectasia Rad3-related (ATR), and the effector kinases Chk1 and Chk2. These kinases, in turn, activate p53 and p53-related proteins, which are key components in DNA-damage responses, cell-cycle checkpoint activation, and programmed cell death (apoptosis) (120, 123). Moreover, MMR proteins are involved in other pathways such as antibodies' diversification through somatic hypermutation (35), and regulation of genetic recombination, by modulating meiotic crossover and preventing recombination of divergent sequences (231); however, their exact role in these processes is poorly understood. The recent interactome analysis of human MutL homologues provided a starting point for further studies on the importance of MMR proteins in DNA metabolism and other biologic pathways (33).

D. Double-strand break repair

DNA double-strand breaks (DSBs), such as those induced by exogenous DNA-damaging agents or endogenously produced reactive oxygen species, can promote genome rearrangements that initiate carcinogenesis or apoptosis (110). Interestingly, despite the potential danger of DSBs, mammals have evolved clever ways of exploiting the intentional generation of DSBs to control biologic processes, such as development of the immune system and generation of genetic diversity in meiosis (162, 257). To combat the risk of large-scale sequence rearrangements that could potentially result from both intentional and unintentional DSBs, mammals have evolved intricate DNA damage-response and -repair mechanisms. Break-repair possibilities also are influenced by the manner in which the break is created. Historically, attention to DSBs has been focused primarily on DSBs generating two-ended DNA molecules that can be formed when a DNA duplex is fractured into two parts (Fig. 12). Such two-ended breaks can be formed at any time during

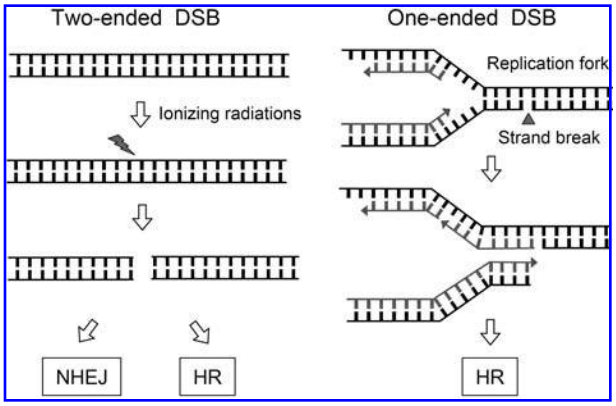

FIG. 12. DNA double-strand breaks (DSBs) formation. Two-ended DSBs can be formed when a DNA duplex is fractured into two parts at any time during the cell cycle and may be caused by ionizing radiation. Two-ended DBSs can be repaired by homologous recombination (HR) with the intact sister chromatid, or by nonhomologous DNA end joining (NHEJ) that may result in sequence rearrangement. One-ended DSBs are created when the replication fork collides into an unrepaired DNA single-strand break. In this case, homologous recombination between sister chromatids close by and linked at the replication fork provides a mechanism for accurate repair.

the cell cycle and may be caused, for instance, by ionizing radiation. Two-ended DSBs can be accurately repaired by the nonhomologous end-joining pathway in a process that directly rejoins the broken ends. If DNA ends are not directly ligatable, as in the case of ionizing radiation–induced DNA strand-breaks, an editing step is required. Although such two-ended DSBs are important DNA lesions, it is becoming increasingly clear that a significant proportion of DSBs do not arise from direct fracture of a DNA duplex, but rather occur indirectly as a result of damage or discontinuities in one strand encountered during DNA replication. For example, one-ended DSBs, which generate only one free DNA molecule end, can arise when the replication fork collides into an unrepaired DNA single-strand break (SSB) (see Fig. 12). Replication forks may also stall or break down when they run into certain base lesions leading to daughter strand gaps that must be corrected. Thus, DNA replication is associated with the risk of converting base damage and SSBs into highly toxic DSBs, and these one-ended breaks require complex signaling and processing to be accurately repaired. This eliminates nonhomologous DNA end joining as a means of repair. Homologous recombination between sister chromatids close to and linked at the replication fork provides a mechanism for accurate repair of such damage (12). Two-ended DSBs can also be repaired by homologous recombination with the intact sister chromatid, or the homologous chromosome, as repair template, thus limiting the possibility of joining ends from independent loci that would result in sequence rearrangement.

Proteins involved in the repair of DSBs are listed in Table 5.

1. Non-homologous DNA end joining. The simplest mechanism to repair a DSB is nonhomologous DNA end joining (NHEJ) (104, 261). This repair pathway rejoins juxtaposed ends in a manner that does not need to be error free, and it is usually precise for simple or two-ended breaks, characterized by blunt ends. It can, however, lead to sequence alterations at the break point when the ends are not compatible. Although the term "nonhomologous" is used to describe this repair pathway, a short 1- to 6-bp region of sequence homology (microhomology) near the DNA end often facilitates rejoining. In contrast to NHEJ, homology-directed repair (HR) is directed by longer stretches of homology, generally more than 100 bp, and thus a major difference between NHEJ and HR is the span of homologous sequences associated with repair processing.

The structure of the DSB end will direct the substrate into differential use of end-processing factors (Fig. 13). A "clean" two-ended DSB, with either blunt ends or small 5′ or 3′ complementary overhangs, is a substrate for an NHEJ reaction that requires just its "core" components: Ku70, Ku80, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), XRCC4, XLF, and DNA ligase IV (222). The Ku70/80 heterodimer is the DNA-binding component forming a ring that can specifically bind to DNA ends. The main function of DNA/Ku complex is to recruit and activate the catalytic subunit (DNA-PKcs), a serine/threonine protein kinase. The precise role of DNA-PKcs is not clear, but its association near DNA ends might be important for their juxtaposition (256). In addition, DNA-PKcs binding causes Ku70/80 to move about one helical turn inward from the end, thereby facilitating access of other proteins involved in the processing of the break. After juxtaposition of the two DNA ends, DNA-PKcs undergoes autophosphorylation (205), and the DNA ends become available for the ligation step. This is performed by a protein complex containing the DNA ligase IV (LIG4) associated with XRCC4 in a dimeric form that acts as cofactor and is probably required for proper targeting of the ligase to DNA ends (163). XRCC4 is required for the stability of LIG4 *in vivo* and can stimulate its adenylation and ligase activity. Recently, an XRCC4-like protein, XLF (also known as Cernunnos), has been identified as an interaction partner of the LIG4/XRCC4 complex (7, 25). Its function in NHEJ

Table 5. Proteins Involved in Double-strand Break Repair

Protein	*Function*
NHEJ core components	
Ku80 (XRCC5)	Form Ku70/80 heterodimer; bind DNA ends, recruit and activate DNA-PKcs
Ku70 (XRCC6)	
DNA-PKcs (XRCC7)	DNA-dependent protein kinase catalytic subunit; recruits LIG4/XRCC4 complex
Ligase IV (LIG4)	Joins DNA ends
XRCC4	Complex with LIG4; targets LIG4 to DNA ends and stabilizes its activity
XLF/Cernunnos	Partner of the LIG4/XRCC4 complex
NHEJ end-processing activities	
Artemis	Nuclease activity regulated by DNA-PKcs; processes DNA ends, making them suitable for ligation activity
PNK	Generates 3′-OH and 5′-phosphate ends suitable for ligation activity
WRN	Werner syndrome protein; $3' > 5'$ exonuclease and helicase activities
TdT, POL μ and λ	Fill in 5′ single-stranded extensions
MRN complex	
MRE11	Exonuclease, endonuclease, and helicase activities
RAD50	DNA ends tethering
NBS1	Nibrin; promotes recruitment of MRN complex to DSBs

Several other proteins, including RAD51, BRCA1/2, RAD52, RAD51 paralogues, helicases, and topoisomerases, are involved in homologous recombination for strand invasion, synthesis-dependent strand annealing, Holliday junction formation, migration, and resolution.

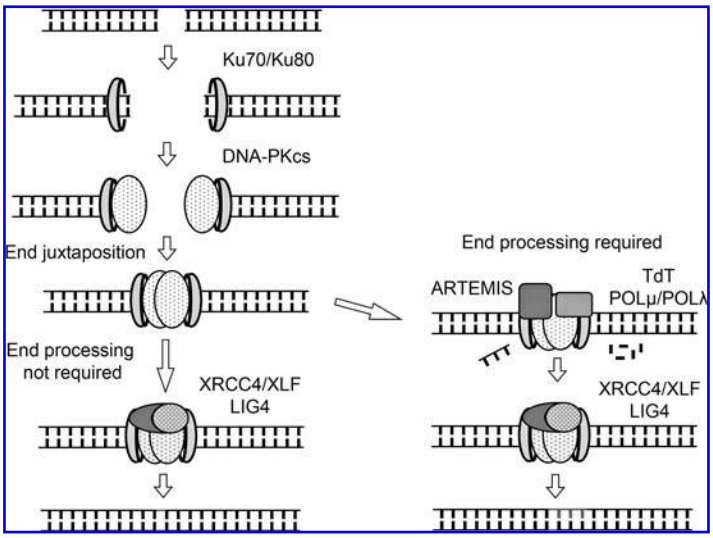

FIG. 13. Schematic representation of DNA double-strand break repair through nonhomologous DNA end joining (NHEJ). A clean two-ended DSB, with blunt ends, is a substrate for NHEJ reaction that requires just its core components: Ku70, Ku80, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), DNA ligase IV (LIG4), XRCC4, and XLF. The Ku70/80 heterodimer forms a ring that can specifically bind to DNA ends. The main function of the DNA–Ku complex is to recruit and activate DNA-PKcs, which in turn promotes DNA ends juxtaposition. If no further processing of ends is required, the complex attracts the additional core components, LIG4, XRCC4, and XLF, which together form the ligase complex and seal the DNA ends. DNA end joining in the presence of 3′- and 5′-overhangs, hairpins [intermediates in V(D)J recombination], flaps, and gaps, characterized by single-strand/double-strand transitions, requires an additional end processing before sealing. This can be done by the structure-specific Artemis nuclease and/or the DNA polymerases POLμ, POLλ, and TdT. The central player in this process is likely to be the Ku heterodimer, which can interact with Artemis (through DNA-PKcs), and the LIG4/XRCC4 complex, thus orchestrating the activities and the reversible interaction of the processing factors with the core components.

has not yet been defined, but cells from patients with mutations in the XLF gene are radiosensitive and DSB repair defective. The patients themselves are immunodeficient because of their inability to process properly the DSB intermediates required for the assembly of active immunoglobulin genes.

Another protein complex is associated with DNA ends resulting from a breakage. It contains two homodimers of MRE11 and RAD50 as core components and a third subunit, NBS1, whose stoichiometry is less well defined. This complex, also known as MRN complex, plays multifaceted roles, acting as a DNA damage sensor and as an enzymatic effector in repairing DNA (47, 226). During NHEJ, the MRN complex may facilitate tethering of the two DNA ends, and its function seems to be less critical in conditions in which ends can be directly ligated than in those when ends require processing (53).

2. End processing before NHEJ. DSBs differ not only with respect to the number of DNA ends but also in the chemical composition of the ends. For example, ionizing radiation–induced DNA strand breaks are not directly ligatable because they are not proper substrates for DNA ligases. In addition to DNA-strand break, reactive oxygen species resulting from radiation cause base and sugar damage. Such dirty DNA ends require editing and can be processed by polynucleotide kinase, PNK, which interacts with XRCC4 (134) and generates 3′-hydroxyl and 5′-phosphate ends suitable for ligation activity. Another subclass of incompatible DNA end structures are 3′- and 5′-overhangs, hairpins [which are intermediates in V(D)J recombination], flaps, and gaps. These DNA structures, characterized by single-strand/double-strand transitions, can be specifically processed by the structure-specific Artemis nuclease, a versatile endonuclease (159). Artemis is recruited to damaged DNA ends through the interaction with DNA-PKcs, which is required for its activity (see Fig. 13). Another exonuclease activity probably involved in the polishing of damaged DNA ends is WRN. Finally, several DNA polymerases have been implicated in end-processing for NHEJ, including the terminal deoxynucleotidyl-transferase (TdT) and the alternative DNA polymerases μ and λ (POLμ and POLλ) (157, 177). Whereas TdT can add untemplated nucleotides to DNA ends, POLμ and POLλ can fill in 5′-single-stranded extensions. In addition to these activities, several other factors are required for efficient joining of "difficult" breaks, and probably involve the MRN complex, the damage sensor ATM, and the phosphorylation of histone H2AX (208).

The core components of NHEJ must play a central role in orchestrating all these activities. The central player of this platform is likely to be the Ku heterodimer, which can interact with Artemis (through DNA-PKcs), POLμ, POLλ, and the LIG4/XRCC4 complex (157). The reversible interaction of the processing factors with the core components does not require a strict order of engagement of the processing factors nor their simultaneous action on both DNA ends or even on each of the two strands of one end. This provides great flexibility in the combination of different ends that can be rejoined (104, 157, 261).

3. PARP1 and Ku compete for DSB repair by distinct NHEJ pathways. Recent work has identified DNA ligase III (LIG3) as a candidate factor in alternative pathways of NHEJ and indicates PARP1 as an additional potential contributor (13, 252). A prerequisite for PARP1 activation, and therefore involvement in DSB repair, is binding to DNA ends. In a directly relevant biochemical study using a novel two-step *in vitro* DNA end-joining assay, not sensitive to interference by Ku proteins, Audebert *et al.* (13) reported that DNA end joining requires the synapsis activity of PARP1 and the ligation activity of the LIG3/XRCC1 complex. Thus, the repair module PARP1/LIG3/XRCC1 (PLX), which plays a central role in SSB repair (32), also is implicated in the repair of DSBs. This function may not rely on novel activities as DSBs form when SSBs occur in close proximity, in opposite DNA strands. The affin-

ity of PARP1 for DNA ends generates an activity analogous to Ku and offers an alternative mode of recognition of this type of lesion. The much higher affinity of Ku for DNA ends will limit the contribution of PARP1/LIG3-dependent end joining to instances in which the classic pathway is compromised, helping the cell to restore its genome stability. Biochemical studies revealed that Ku70/Ku80/DNA-PKcs complex is a substrate of PARP1 activity, and this modification impairs its binding to DNA. HR and NHEJ pathways compete for DSBs repair, with NHEJ acting primarily in the G_1 phase and HR in the late S and G_2 phases. It has been reported that PARP1 may protect the HR pathway from interference by Ku70, suggesting a new function for PARP in controlling DSB repair (109).

4. Homologous recombination. DNA recombination, the exchange of strands between homologous DNA molecules is an essential biologic process that ensures accurate genome duplication, DNA-damage repair, and chromosome segregation. DSB repair through homologous recombination (HR) is generally accurate because the undamaged sister chromatid is used as a repair template. Whereas NHEJ can function in DSB repair throughout the cell cycle, HR is largely restricted to late S/G_2 phases. Assembling a complete mechanistic picture of homologous recombination repair in eukaryotes is complicated by the abundance of proteins that can perform some of the required steps and by the absence of identified proteins needed for others (104, 261).

The HR pathway is initiated by a 5′- to 3′-strand resection at the DSB ends, through a nuclease activity, to generate 3′-single-stranded DNA tails that are coated with RPA protein (Fig. 14). The MRN complex, the first factor detected at DSB sites, facilitates this step (47, 226). MRE11 and RAD50 form the core complex, as a heterotetrameric assembly (M2R2), which then interacts with NSB1. MRE11 possesses several biochemical properties, such as DNA exonuclease activity, which can be stimulated by RAD50, single-strand DNA endonuclease activity, and DNA unwinding activity. However, nuclease activities of MRE11, described to date, would create a single-strand end with the incorrect polarity, so other nucleases or factors that modulate MRE11 activity are probably required. RAD50 contains motifs that are responsible for nucleotide binding and are required for its DNA end-tethering activity (53, 112, 173). Recruitment of MRN complex to DSBs is promoted by the binding of NBS1 to phosphorylated-histone-H2AX (112, 208), a modification catalyzed by the kinase activity of the signaling protein ATM in the presence of DSBs (235). After resection and protein binding, the resulting nucleoprotein filament invades the complementary sequence of a sister chromatid, forming heteroduplex DNA. This process requires the activity of the breast cancer susceptibility protein BRCA2 and RAD51. BRCA2 is involved in control of the recombinase activity of RAD51 and its loading onto single-stranded DNA (87, 220). RAD51 is the central player in almost all homology-directed repair events and directs the 3′-single-stranded DNA tail of the nucleoprotein filament to search out, invade, and pair with undamaged homologous sequences, thus facilitating strand recombination. RAD51 is assisted by a number of protein factors that include, besides BRCA2, RAD52, RAD54, RAD54B, and probably also the RAD51 paralogues RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 (104, 220, 241, 261). The process of strand invasion and formation of heteroduplex DNA leads to the displacement of a DNA strand in the sister chromatid forming a so-called D-loop. After D-loop formation, the annealed 3′-end is then extended by repair synthesis beyond the original break site to restore the missing sequence information at the break point. The sister chromatid provides an ideal template for such error-free repair synthesis and, indeed, it is the preferred template for homology-directed repair. On the other side of the D-loop, an "X" produced structure, called a Holliday junction, is formed at the border between hetero- and homoduplex. Several proteins can bind Holliday junctions and modulate the ability of these junctions to slide in either direction, but their exact role has not yet been fully elucidated. For example, although it is clear that RAD54, WRN, and BLM facilitate Holliday junction migration (27, 46, 127), it is not clear how the direction of migration is controlled, nor is it clear whether these proteins are involved in all homologous recombination events or only in certain subpathways.

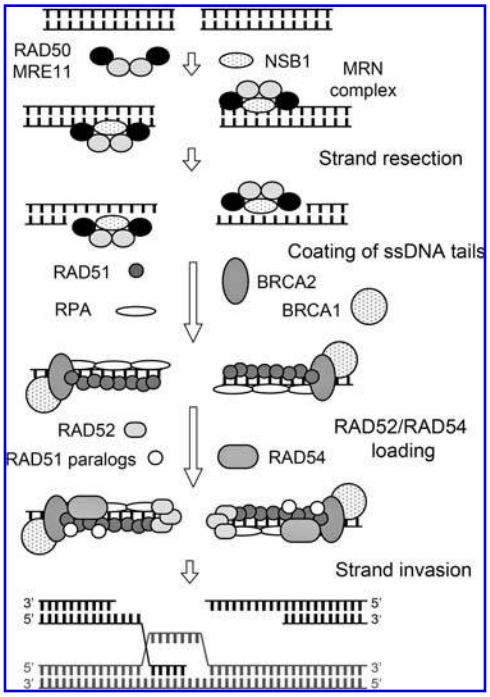

FIG. 14. Schematic representation of DNA double-strand break repair through homologous recombination (HR). The first step in HR repair is the processing of double-strand break (DBS) ends by a nuclease activity to generate 3′ single-stranded DNA tails that are coated with RPA protein. The MRN complex is a candidate for this activity, although other nucleases are likely to be involved. Recruitment of MRN complex to DSB sites is promoted by the binding of NBS1 to phosphorylated-histone-H2AX. After DNA strand resection and protein binding, the resulting nucleoprotein filament invades the complementary sequence of the sister chromatid, forming heteroduplex DNA. This process requires the activity of the breast cancer susceptibility protein BRCA2 and RAD51. BRCA2 is involved in controlling the recombinase activity of RAD51 and its loading onto single-stranded DNA. RAD51 is assisted by a number of protein factors including BRCA1, RAD52, RAD54, and RAD51 paralogues.

5. Synthesis-dependent strand annealing. If the Holliday junction is transported in the same direction as replication, it will release the newly synthesized strand. Once repair synthesis is complete, the next step in this pathway is to release the newly synthesized end, which can be accomplished simply by sliding the Holliday junction toward the 3′-end. The next step is to reconnect the two broken ends, and this process is promoted by the annealing of complementary sequences and is facilitated by RAD52. This process may generate flaps or gaps, depending on the degree to which the 3′-end was extended during repair synthesis. Flaps can be removed by structure-specific endonucleases, such as the XPF/ERCC1 complex, whereas remaining gaps are filled and sealed by PCNA-dependent DNA polymerase δ/ε and DNA ligase I (Fig. 15).

6. Double Holliday-junction model for DSB repair. The double Holliday-junction model was initially designed to explain gene conversion and crossover events occurring simul-

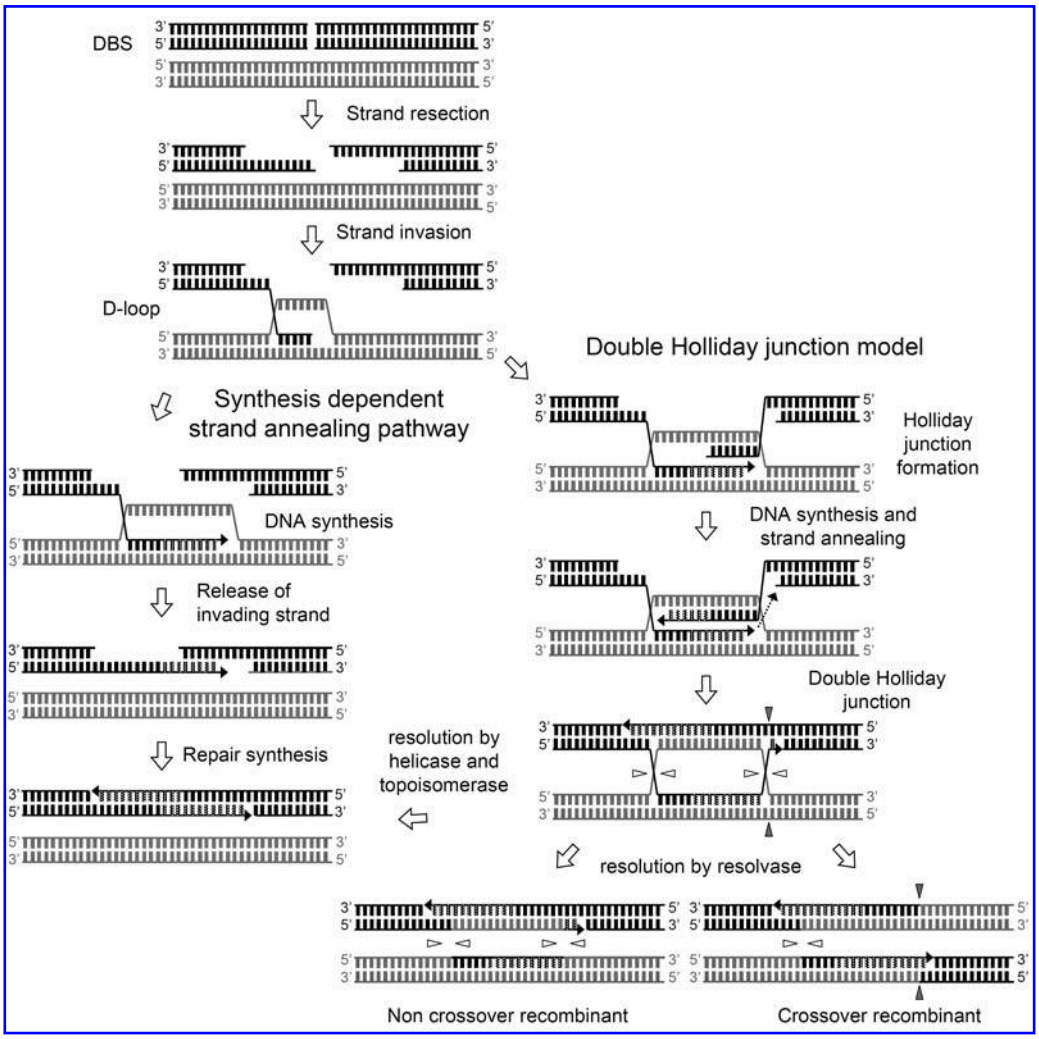

FIG. 15. Schematic representation of DNA double-strand break repair through homologous recombination (HR). HR repair can be performed by the synthesis-dependent strand-annealing pathway or the double Holliday junction model. In the synthesis-dependent strand-annealing pathway (*left*) the 3′ single-stranded tail generated at the double-strand break invades the sister chromatid, generating a D-loop structure. The annealed 3′ DNA end is then extended by repair synthesis beyond the original break site to restore the missing sequence information at the break point. In the next step, the newly synthesized DNA strand is released by sliding the Holliday junction toward the 3′ end. Finally, reconnection of the two broken ends is promoted by the annealing of complementary sequences and is facilitated by RAD52. This process may generate flaps or gaps, depending on the degree to which the 3′ end was extended during repair synthesis. Flaps can be removed by structure-specific endonucleases while remaining gaps are filled and sealed by PCNA-dependent DNA polymerase δ/ε and DNA ligase I. In the double Holliday junction model (*right*), valid only for two-ended double-strand breaks, both 3′ DNA ends invade the homologous DNA template and form a double Holliday junction. To complete DNA end joining after repair synthesis, the invading DNA strands have to be uncrossed. Resolution of Holliday junctions can be performed by structure-specific nucleases called resolvases. These may either preserve the flanking sequence continuity or result in a crossover event, depending on the orientation of cleavage at both junction sites. Alternatively, the crossed DNA strands resulting from the double Holliday junction can be separated by the combined activity of helicases and topoisomerases, avoiding crossover product formation.

taneously after a DSB during meiosis, but it can also explain repair of DSBs during mitosis. In this model, both DNA ends invade the homologous DNA template and form a double Holliday junction. To complete the repair after recombination, the DNA strands have to be uncrossed or cut by structure-specific nucleases called Holliday-junction resolvases. In eukaryotes, two RAD51 paralogues, XRCC3 and RAD51C, appear to be associated with resolvase activity (154). Alternatively, the crossed DNA strands resulting from the double junction can be separated by the combined activity of BLM helicase and a topoisomerase (203). Resolution of Holliday junctions by resolvases always results in gene conversion at the DBS site and may either preserve flanking sequence continuity or result in a crossover event, depending on the orientation of the cleavage at both junction sites. In contrast, strands' displacement by helicase/topoisomerase avoids crossover-product formation (see Fig. 15).

7. Other activities involved in DSB repair. Eukaryotic cells respond to DSBs to maintain genomic integrity through a variety of pathways such as cell-cycle checkpoints, the induction of apoptosis, and direct DNA-repair reactions (15, 179, 226, 227, 241). Unrepaired or improperly repaired DSBs lead to severe consequences for the cell. The initial step of DSB detection involves ATM, as well as the related kinase ATR, and the MRN complex, which functions both as damage sensor and as inducer of strand recombination. ATM and MRE11 interact through the C-terminus of NBS1 in response to the generation of DSBs and are all implicated in checkpoint regulation and DSB repair. NBS1 is phosphorylated by ATM on Ser278 and Ser343 when cells are irradiated, and NBS1 phosphorylation is critical for intra-S-phase checkpoint activation as a downstream event of ATM. BRCA1, the other breast cancer susceptibility protein, plays an important role in DNA repair interacting, directly or indirectly, with other tumor suppressors (such as p53 and BRCA2), DNA damage sensors (such as RAD51 and MRN complex), and signal transducers (such as p21 and cyclin B) to form multisubunit complexes (98). These multisubunit protein complexes are involved in detecting DNA damage, causing cell-cycle arrest, and allowing DNA repair, especially the repair of DNA interstrand crosslinks and double-strand breaks. BRCA1 is involved in DSBs repair through the interaction with MRN complex, facilitating its accumulation and retention at the DSB site, and is required for ATM-dependent phosphorylation of NBS1 after exposure to IR. In addition, BRCA1 binds to DNA, with a preference for branched structures, and it has been proposed that it forms a stable complex with BRCA2 and RAD51 during DSB repair. DSBs are associated with changes in the nearby chromatin. The remarkable phosphorylation of histone variant H2AX was the first chromatin alteration to be described that follows a DNA break. This histone mark may have a role in activating DNA damage-response cell-cycle checkpoints and recruiting chromatin remodeling and repair factors (242), although the lack of dramatic DNA repair-defective phenotype of H2AX-deficient cells in mice suggests its role might be nonessential. The chromatin-remodeling complexes INO80 and SWR1 are also specifically implicated in making broken DNA ends accessible to repair factors (63). The possibility that certain chromatin changes are associated with either homologous recombination or nonhomologous DNA end joining is still unclear.

E. Translesional DNA synthesis

The replicative bypass of base damage in DNA (translesional DNA synthesis or TLS) is a ubiquitous mechanism for relieving arrested DNA replication. TLS mechanism is affected by a recently discovered class of specialized DNA polymerases, also called translesional synthesis polymerases, which can be accurate (error free) or mutagenic (error prone) during TLS (153). Bacteria such as *E. coli* possess three specialized DNA polymerases (POL II, POL IV, and POL V), whereas, to date, 10 such enzymes have been identified in mammalian cells (Rev1 and DNA polymerases ζ, η, κ, ι, λ, μ, β, θ, and υ). Most of these polymerases bypass damage in the DNA, fall off after inserting a few nucleotides, and then allow an accurate replicative polymerase to continue. All prokaryotic and eukaryotic specialized polymerases lack the exonucleolytic proofreading function, which augments the intrinsic high fidelity of their replicative counterparts.

In higher eukaryotes, the multiplicity of specialized DNA polymerases reflects the evolution of different enzymes accurately to negotiate different types of naturally occurring base damage. In any model of TLS, the notion exists that at different stages of the process, different DNA polymerases occupy the primer terminus at or near to sites of arrested replication (polymerase switching) (85, 196). Some of the eukaryotic specialized polymerases are able to replicate past one or more template lesions with surprising accuracy. For example, human DNA polymerase η preferentially incorporates the correct nucleotide dAMP opposite thymine-thymine cyclobutane pyrimidine dimers (CPD) generated in DNA by exposure to UV radiation. Additional observation suggests that, in the absence of the correct polymerase for a particular lesion or class of lesions, other polymerases can be used, thus promoting cell survival but with an increased probability of generating mutations. Several specialized DNA polymerases have been implicated in somatic hypermutation and/or class switching in the immune system, during which they are believed to generate mutations while copying short stretches of undamaged DNA in immunoglobulin genes (17).

In conclusion, it is apparent that the multiple polymerase-switching events that occur during TLS involve several contributing mechanisms that regulate the access of low-fidelity, error-prone enzymes to DNA. In eukaryotes, they include ubiquitination of PCNA and possibly of other proteins, including the polymerases themselves.

IV. DNA DAMAGE AND DNA SYSTEM REPAIR–RELATED DISEASES

Biologic evolution is marked by mutations that escape the vigilant DNA-repair process. The cellular sophisticated system apparatus responsible for DNA damage recognition and repair can itself sustain irreversible mutations that are transmitted to the next generation. A defect in these repair systems will either be expressed as a disease phenotype or remain dormant. In the higher organisms, the number of pathways through which damage can be repaired have increased enormously through evolution (*i.e.*, BER, NER, MMR, NHEJ, and HR). Some down-

stream effectors trigger cellular events such as DNA repair, cell-cycle checkpoint, telomere stability maintenance, transcription control, and apoptosis. All these processes are generally known as DNA-damage responses.

A. Human disorders related to defects in DNA-repair pathways

A number of disorders or syndromes are linked to an inherited or acquired defect in one of the DNA-repair pathways, and some DNA repair–linked disorders show a mixed phenotype, including neurologic symptoms (*i.e*., xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy, ataxia–telangiectasia, Nijmegen breakage syndrome and ataxia-like disorder, Alzheimer disease), cancer predisposition (*i.e*., HNPCC, Fanconi anemia, breast cancer, lung cancer, and prostate cancer), and accelerated aging (*i.e*., Bloom syndrome, Rothmund–Thomson syndrome, Werner syndrome), underlining the fundamental importance of the DNA-repair machinery in health and disease (Table 6) (22, 192, 228). The current state of knowledge regarding the diseases linked to mutations in crucial components of the DNA repair pathways is reported later.

1. Xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy. Patients with the rare autosomal recessive genetic disorders xeroderma pigmentosum (XP), trichothiodystrophy (TTD), and Cockayne syndrome (CS) have defects in the NER pathway, which involves more than 28 genes. Mutations in at least 11 of these genes have been associated with clinical disease, with about eight overlapping phenotypes (22, 52, 135). The clinical features of these patients show some similarities as well as marked differences. Patients have complex neurologic abnormalities such as progressive sensorineural deafness, developmental delay, progressive neurologic degeneration, premature aging, or a combination of these. These features are caused by primary neuronal degeneration (XP) or reduced myelination of the brain (CS and TTD) due to developmental defects as well as DNA damage in neuronal cells. In addition, XP individuals are hyperphotosensitive and have a marked risk of skin cancer (192).

In the case of XP, seven complementation groups (XPA, ERCC3/XPB, XPC, ERCC2/XPD, DDB1/XPE, ERCC4/XPF, and ERCC5/XPG) correspond to mutations in seven genes that have a role in the NER pathway (42, 187, 228). XP proteins A–G are crucial in the processes of DNA damage recognition and incision, and patients with XP can carry mutations in any of the genes that specify these proteins. XP proteins are key players in several steps of the NER process, including DNA strand discrimination (XPA, in complex with RPA), repair complex formation (XPC, in complex with hHR23B; XPF, in complex with ERCC1), and repair-factor recruitment (transcription factor IIH, in complex with XPG). A variant of xeroderma pigmentosum (XP-V) is caused by a mutation in a novel DNA polymerase η belonging to the Y family of DNA polymerases, which supports translesional synthesis of thymine dimers CPD (42).

Most XP patients with severe neurodegeneration are found to have mutations in components of the transcription-coupled repair system (TC-NER), such as the Cockayne syndrome proteins CSA (ERCC8) and CSB (ERCC6), and common genes such as XPA, XPD (ERCC2), XPB (ERCC3), XPF (ERCC4), and XPG (ERCC5). In addition, XP is associated with a 1,000-fold increase in skin cancer as well as a 20-fold increase in other internal tumors (42). In this case, the main symptoms of cancer predisposition are seen when mutations are found in genes that are unique to global genome repair (GC-NER) and have no role in TC-NER, for example XPC, DDB1 (XPE), and replication polymerase η (228).

The symptoms of Cockayne syndrome include growth retardation, deafness, dysmyelination in white matter, and retinal and Purkinje's cell degeneration. Patients generally have a postnatal growth defect (leading to cachectic dwarfism) and skeletal abnormalities such as a birdlike face (sunken eyes and a beaked nose), kyphosis, and, in older patients, osteoporosis development and cataract develop. In reported cases, the mean age at death is 12.5 years (although patients as old as 55 years have been described), with the most common cause of death being pneumonia as a result of general atrophy and cachexia. CS is not associated with any proneness for increased UV-induced skin cancer (135).

The two proteins found to be mutated in this syndrome, CSA (ERCC8) and CSB (ERCC6), have been shown to be required for transcription-coupled repair. CSA is a subunit of E3 ubiquitin ligase complex, whereas CSB plays a role recruiting all the factors needed for transcription-coupled repair at stalled RNA polymerase II (83). A syndrome closely related to CS, known as cerebro-oculo-facio-skeletal syndrome, involves mutations in XPD (ERCC2), XPG (ERCC5), and CSB (ERCC6), and is associated with more severe eye defects such as microcornea (97).

Recently, Jaspers *et al*. (121) reported the first case of human ERCC1 deficiency, resulting in severe embryonic and postnatal growth failure and cerebro-oculo-facio-skeletal syndrome. This case represents the most clinically severe NER deficiency published. Patient cells showed moderate hypersensitivity to UV radiation and mitomycin C. This case is consistent with mouse models of ERCC1-XPF deficiency and the phenotype is also distinct from and more severe than that of NER deficiency alone. On the basis of this discovery, a new complementation group of patients with defective NER was proposed, revealing the importance of ERCC1-XPF during human fetal development, in particular for the CNS, and suggesting novel functions for ERCC1 (121).

Individuals with trichothiodystrophy (TTD) have characteristically sulfur-deficient brittle hair and nails and may also have physical and mental retardation (192). Mutations in three genes, *XPD* (*ERCC2*), *XPB* (*ERCC3*), and *XPG* (*ERCC5*), can result in combined symptoms of either XP and TTD or XP and Cockayne syndrome, depending on the type of mutation present (42).

Recently, Gorgels *et al*. (95) demonstrated that the *CSB* defect in mouse not only causes corneal UV sensitivity and cancer susceptibility but also predisposes for spontaneous retinal degeneration. Retina in *CSB*-defective mouse is hypersensitive to ionizing radiation, which suggests that oxidative lesions form the basis of this prematurely aging phenotype. This finding highlights the importance of DNA repair and control of oxidative stress levels for long-term survival of photoreceptor cells in the retina and supports Harman's "free-radical theory of aging."

Another key gene that is associated with all three disorders is the XPB helicase, which is part of the general transcription

Table 6. Syndromes Characterized by Defects in DNA-repair Pathways

Syndrome	*Affected gene*	*Protein function*	*Cellular mechanisms and functions involved*	*Related cancer or associated symptoms*
Ataxia-telangiectasia MIM: 208900	ATM (Ataxia telangiectasia mutated)	Serine/threonine–protein kinase activity	DSB repair; checkpoint on apoptosis and genotoxic stresses such as ionizing radiation and UVA light	Various solid epithelial tumors, lymphomas, T-cell leukemias
Seckel syndrome MIM:210600	ATR (Ataxia telangiectasia and Rad3-related protein)	Serine/threonine–protein kinase activity	Redistribution to discrete nuclear foci on DNA damage, hypoxia, or replication fork stalling	Growth retardation, microcephaly with mental retardation, and a characteristic "bird-headed" facial appearance
Ataxia–telangiectasia-like disorder MIM:604391	MRE11	Single-strand endonuclease and double-strand-specific 3′-5′ exonuclease activities; component of MRN complex	DSB repair, DNA recombination, maintenance of telomere integrity and meiosis	Lymphomas, leukemias, breast cancer
Nijmegen breakage syndrome MIM: 251260	NBS1 (Nibrin)	Component of MRN complex; recruitment of MRN, ATM, ATR, and probably DNA-PKcs to the DNA damage sites	Strand break repair, telomere length maintenance by generating the 3′ overhang; player in the control of intra–S-phase checkpoint; involved in G_1 and G_2 checkpoints	Lymphomas, breast cancer, aplastic anemia, childhood acute lymphoblastic leukemia
Bloom syndrome MIM: 210900	BLM (RECQL3)	Mg- and ATP-dependent DNA-helicase activity that unwinds single- and double-stranded DNA in a 3′–5′ direction	Strand-break repair and DNA replication	Osteosarcomas, early occurring cancers of various types; 1/3 of patients are dead at an average age of 24 years, and the mean age of the 2/3 remaining living patients is 22 years
Werner syndrome MIM: 277700	WRN (RECQL2)	Mg- and ATP-dependent DNA-helicase activity	Strand-break repair; formation of DNA replication focal centers	Melanomas (especially UV-independent melanomas of mucosal surfaces and acrolentiginous melanomas), soft tissue sarcomas, thyroid cancers, meningiomas, osteosarcomas
Rothmund–Thompson syndrome MIM: 268400	RECQL4	ATP-dependent DNA helicase activity	Strand-break repair; chromosome segregation	Nonmelanocytic skin tumors, osteosarcomas; cause of RAPADILINO syndrome and Baller–Gerold syndrome (BGS)
Fanconi anemia MIM: 227650	FANC-A, B, C, D1, D2, E, F, G, I, J, L, and M	FANC-A, B, C, E, F, G, L, M complex is requried for FANCD2-monoubiquitination; FANCD1 is BRCA2	Strand-break repair; homologous recombination and single-strand annealing; S phase and G_2 phase checkpoint	Squamous cell carcinomas, acute myelogenous leukemia

(*continued*)

TABLE 6. SYNDROMES CHARACTERIZED BY DEFECTS IN DNA-REPAIR PATHWAYS (CONT'D.)

Syndrome	*Affected gene*	*Protein function*	*Cellular mechanisms and functions involved*	*Related cancer or associated symptoms*
Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome MIM: 120435	MSH2, MSH6, MLH1, MLH3, PMS1, PMS2	DNA-mismatches recognition. Components of the BRCA1-associated genome surveillance complex (BASC), containing BRCA1, MSH2, MSH6, MLH1, ATM, BLM, PMS2	MMR	Colon cancer, other visceral tumors such as endometrial, ovarian, stomach, kidney, and small intestinal cancers. Defects in PMS2 are the cause of supratentorial primitive neuroecto-dermal tumors with café-au-lait spots (SNTCL)
Muir–Torre syndrome MIM: 158320	MLH1, MSH2	DNA-mismatches recognition. Components of the BASC complex	MMR	Sebaceous carcinomas, colon cancer, other visceral tumors such as endometrial, ovarian, stomach, kidney, and small intestinal cancers; lobular carcinoma *in situ* (LCIS), a noninvasive neoplastic disease of the breast
Turcot syndrome MIM: 276300	MLH1, PMS2	DNA-mismatches recognition Components of the BASC complex	MMR	Medulloblastomas, gliomas, lymphomas, colon cancer, other visceral tumors such as endometrial, ovarian, stomach, kidney, and small intestinal cancers
Xeroderma pigmentosum XP-A MIM:278700 XP-B MIM:133510 XP-C MIM:278720 XP-D MIM:278730 XP-E MIM:278740 XP-F MIM:278760 XP-G MIM:278870 XP-V MIM:278750	XPA XPB XPC XPD XPE XPF XPG XPV	XPA: recruitment of NER components. XPB and XPD: ATP-dependent 3′- and 5′-DNA helicases; components of the core-TFIIH basal transcription factor. XPC and XPE: DNA damage recognition. XPF and XPG: structure-specific DNA endonucleases responsible for the 5′ and 3′ incision during NER. XPV: DNA pol η	NER XPB and XPD involved in RNA transcription by RNA polymerase II. XPE is required for histone H3 and histone H4 ubiquitination in response to UV light. XPF is also involved in HR removing interstrand cross-links. XPV plays a role in translesional synthesis	Basal cell carcinomas, squamous cell carcinomas, melanomas (UV-induced skin tumors) in childhood. Defects in XPD are the cause of cerebro-oculo-facio-skeletal syndrome type 2 (COFS2) [MIM:610756]
Cockayne syndrome type B MIM:133540 type A MIM:216400	CSA CSB XPG	CSA and CSB interact with stalled RNA pol-II; CSB may have a DNA/RNA unwinding function	TC-NER	Hypersensitivity to ionizing radiation
Trichothiodystrophy MIM: 601675	TTD-A XPB XPD	TTD-A is a component of the TFIIH basal transcription factor	NER	Some patients have photosensitivity

(*continued*)

Table 6. Syndromes Characterized by Defects in DNA-repair Pathways (cont'd.)

Syndrome	*Affected gene*	*Protein function*	*Cellular mechanisms and functions involved*	*Related cancer or associated symptoms*
Lig4 syndrome MIM:606593	LIG4	ATP-dependent ligase activity specific for single-strand breaks in a double-stranded DNA	NHEJ	Unusual facial features, microcephaly, growth and/or developmental delay, pancytopenia, various skin abnormalities; patient's cell lines show radiosensitivity
Severe combined immunodeficiency with sensitivity to ionizing radiation MIM:602450	ARTEMIS	Single-strand–specific $5' > 3'$ exonuclease activity; endonuclease activity on 5′- and 3′-hairpins and overhangs	NHEJ	Persistent diarrhea, candidiasis, lung infections, fever, and opporttunistic infections
OMENN syndrome MIM:603554	RAG-1/RAG-2 ARTEMIS	RAG1/2: heteromultimeric endonucleases	NHEJ	Erythrodermia, hepatosplenomegaly, lymphadenopathy and alopecia
Cernunnos-XLF	Cernunnos-XLF	Bridge component between XRCC4 and the other NHEJ factors located at DSB ends	NHEJ	Microcephaly, growth retardation, increased cellular sensitivity to ionizing radiation; defective V (D)J recombination, impaired DNA-end ligation process; combined immune deficiency (CID)

References: 238, http://www.expasy.org/sprot/; http://www.ncbi.nlm.nih.gov/sites/entrez

factor TFIIH complex and plays a role in NER. The XPB ATPase and helicase activities are essential to promote DNA melting and clearance steps during the initiation of transcription by RNA polymerase II.

The characterization of XPB helicase has allowed some of the key questions to begin to be addressed about the original mechanisms of XPB and TFIIH function in both transcription and NER. A structural study on a homologue of the human XPB, the archea *Archaeoglobus fulgidus* XPB (AfXPB), revealed a conserved central core structure containing a small N-terminal domain attached to the helicase domain 1 (HD1), which displays a structural similarity to the mismatch-recognition domain of the DNA MMR protein MutS (74). This domain was thus termed the damage-recognition domain (DRD) (74), and it probably recognizes distortions in the DNA typically caused by the broad spectrum of NER lesions. Therefore, this region may explain how DNA damage is located and linked to initiation of DNA unwinding during NER steps by XPB/TFIIH. Structural biochemistry studies have also led to a mechanism being suggested for the involvement of XPB in the unwinding of duplex DNA at sites of DNA repair and transcription (192). When XPB is recruited to DNA, it is proposed that the DRD domain recognizes the distorted damaged DNA. The conformation of XPB will decide whether TFIIH functions as a transcription factor or a DNA-repair factor. In other words, XPB acts as a master key, helping TFIIH switch pathway selection for transcription or DNA repair, whenever it is recruited to the DNA. Interestingly, the disease-related mutations occur exclusively in the N- and C-terminal extensions of human XPB, suggesting that mutation to the conserved XPB central region is lethal.

2. Ataxia–telangiectasia, Nijmegen breakage syndrome, and ataxia-like disorder-Seckel syndrome. Ataxia–telangiectasia (AT), Nijmegen breakage syndrome (NBS), and ataxia–telangiectasia-like disorder (ATLD) are generally considered to be chromosome-instability disorders, and the associated defective genes in these diseases are *ATM*, *NBS1* (also known as nibrin), and *MRE11*, respectively. All of these genes are involved in eliciting a response to DNA damage and, in particular, double-strand breaks that result from ionizing radiation. At the clinical level, AT, NBS, and ATLD are quite distinct, although some similarities exist. Patients with any of these disorders display a measurable immunodeficiency, and both AT and NBS patients have an increased risk of developing lymphoid tumors, whereas it is not known whether ATLD patients have a predisposition to cancer (84, 236).

Ataxia–telangiectasia (AT) is a rare neurodegenerative disease that results from defective DNA-damage signaling. This syndrome displays pronounced neurodegeneration of the nervous system coincident with immune deficiency, radiosensitivity, and proneness to cancer (84). On pathologic examination, cerebellar Purkinje and granule cell degeneration is noted, as well as neuronal loss in striatum and substantia nigra in the more advanced stages of the disease. Other phenotypes in this complex disorder include retinal telangiectasia, immunodeficiency, radiosensitivity, infertility, predisposition to malignancies, and progressive neuronal degeneration (84, 264).

The gene for ataxia–telangiectasia (*ATM*, ataxia–telangiectasia mutated) encodes for a protein of 3,056 amino acids with a mass of 370 kDa. More than 300 distinct mutations of the *ATM* gene have been reported, and most of them (>80%) are base substitutions or insertions/deletions that generate premature termination codons or splicing abnormalities. The truncated species are usually unstable and result in absent or severely reduced protein expression. Some missense mutations can cause ataxia–telangiectasia but were found only for ~10% of ATM mutations identified in AT patients (6).

ATM is a protein kinase that belongs to PI3K-related protein kinases (PIKK), a family of serine/threonine kinases, which have the catalytic domain homologous to phosphatidylinositol 3-kinase (Pl3K) and phosphorylate proteins rather than lipids (5). ATM plays a critical role in regulating the response to DNA double-strand breaks by selective phosphorylation of a variety of substrates (84). In normal cells, ATM exists as inert dimers or multimers. In response to DNA double-strand breaks, ATM dissociates to highly active monomers and undergoes autophosphorylation on Ser1981. ATM is recruited to the sites of DNA damage and initiates a signaling cascade through phosphorylation of multiple DNA-damage response and cell-cycle proteins, including p53, BRCA1, and CHK2 (6). It has been proposed that ATM may activate the apoptosis signaling pathway to eliminate neurons with a heavy load of DNA damage, so that the neurologic phenotype in AT disease could be caused by a neuron-specific failure of ATM-dependent cell-cycle control (264). As a final point, ATM mutations appear to be involved in breast cancer predisposition (6).

A key substrate phosphorylated by ATM is nibrin (NBS1), a protein component of the MRN complex in association with MRE11 and RAD50. The MRN protein complex plays multifaceted roles, acting as a DNA-damage sensor, as an enzymatic effector in repairing DNA double-strand breaks through recombination, and as a transducer of critical damage-response signals to the cell-cycle checkpoint apparatus (47, 226). The essential nature of MRN is highlighted by studies demonstrating that null mutations in any of the three proteins in mice lead to embryonic lethality.

Rare defects in the MRN complex also lead to syndromes with neurologic deficits, cancer predisposition, and phenotypes overlapping AT (84). Mutations in *MRE11* give rise to ataxia-telangiectasia-like disorder (ATLD), a very rare disorder with only six known cases up to 2004, four in the United Kingdom and two in Italy (236). The clinical features of patients with ATLD are very similar to those of AT, with additionally progressive cerebellar ataxia, increased radiosensitivity, and an increased level of spontaneously occurring chromosome aberrations. However, ATLD patients have no telangiectasia, a later onset of the neurologic features, and slower progression of the disorder, to give the appearance of a milder condition than AT in the early years (236). It is not known whether ATLD patients have a predisposition to cancer, as too few patients have been described to date.

Hypomorphic mutations in NBS1, another component of MRN complex, cause Nijmegen breakage syndrome, which displays similar symptoms to ATLD but is characterized by microcephaly, radiosensitivity, immunodeficiency, increased cancer risk, particularly lymphoid malignancy, and growth retardation (84). It is surprising that a deficiency in MRE11 gives rise to an AT-like disorder, whereas NBS1 mutations lead to NBS and NBS-like disorders. NBS1 itself has neither DNA-binding/processing nor kinase activities, which are usually required for DNA-damage repair. The FHA/BRCT domain in the N-terminus of NBS1, however, has been shown to bind directly to the phosphorylated histone H2AX (γH2AX). The binding of NBS1 to γH2AX recruits MRN complex to the proximity of DNA damage sites (270).

Seckel syndrome is an autosomal recessive disorder characterized by marked microcephaly and developmental delay (183). It represents a heterogeneous disorder, and clinical features prerequisite for a diagnosis include severe intrauterine growth retardation, marked proportionate short stature, pronounced microcephaly, mental retardation, and characteristic facial features. Mapping studies have localized a defective region encompassing the *ATR* gene (183).

ATR (ataxia–telangiectasia and Rad3-related) is a central player in the signaling response to DNA damage and functions in concert with ATM and the DNA-dependent protein catalytic subunit (DNA-PKcs). They operate as DNA-damage sensors that relay and amplify the damage signals to effectors controlling DNA repair (270). ATR and DNA-PKcs, as well as ATM, belong to the PIKK protein kinase family. Whereas ATM and DNA-PKcs respond to DNA double-strand breaks, ATR responds to regions of single-stranded DNA generated at stalled replication forks and bulky lesions. Current evidence suggests that ATR is essential not only for development but also for somatic cell growth (183). The substrates of ATM and ATR include mainly factors involved in cell-cycle checkpoint control such as CHK1, CHK2, p53, NBS1, SMC1, and BRCA1 (140), whereas the substrates of DNA-PKcs mainly include factors involved in nonhomologous end joining (NHEJ), such as Ku70 and K80, Artemis, DNA ligase IV, and XRCC4 (44).

Similar and distinct abnormalities in phenotype were observed among patients with defects in these genes. Cell-cycle checkpoint failure occurs in all NBS, AT, and ATR-Seckel patients. However, the immunodeficiency, chromosomal aberration and instability, cancer predisposition, cerebellar development defects, ataxia, and increased radiosensitivity are observed both in AT and NBS patients, whereas microcephaly, growth retardation, and dysmorphic facial features are noted in ATR-Seckel and NBS patients. Clinically the phenotypic alterations in NBS patients encompass almost all the phenotypic alterations observed in AT and ATR-Seckel patients, and even the phenotypes found in SCID mice (44). This suggests that NBS1 may work upstream of the PIKKs (ATM, ATR, and PKcs) and the impaired functions of NBS1 will, in turn, impair the functions of these PIKKs. Therefore, NBS patients show a mixture of clinical manifestations observed in these PIKK-defect syn-

dromes. Clinical observations and experimental studies led Zhou *et al*. (270) to propose a model in which NBS1 may not only function in DSB repair as an effector protein, but may also act as an important DNA damage sensor and signal transducer.

3. Werner, Bloom, and Rothmund–Thompson syndromes. A number of diseases have been categorized as progeria or progeroid syndromes. Patients with progeria display relatively similar symptoms that include a variety of aging phenomena such as gray hair, alopecia, cataract, hoarseness, skin atrophy, pigmentation, diabetes mellitus, osteoporosis, osteoarthritis, hypogonadism, brain atrophy, senile dementia, atherosclerosis, and malignancy. Among the premature aging syndromes, most of the features of ordinary aging can be seen in Werner syndrome. This is an autosomal recessive progeroid disease involving the *WRN* gene and characterized by genomic instability. WRN belongs to the RecQ helicase family, which is widely distributed across the life domains and displays ATPase, helicase, exonuclease, and single-stranded DNA-annealing activities (105). The human genome contains four other RecQ helicase family members, RecQ1, BLM, RecQ4L, and RecQ5. Mutations in BLM and RecQ4L cause Bloom syndrome and Rothmund–Thompson syndrome, respectively. Werner (WS), Bloom (BS), and Rothmund–Thompson (RTS) syndromes share a predisposition to cancer, but significant pathologic differences suggest that each disease pathway is functionally distinct. WS patients have increased risk of soft tissue sarcomas, whereas BS patients are predisposed to lymphoma and leukemia. RTS patients have an increased incidence of skin cancer and osteosarcomas (142). It would appear that the helicases involved in these syndromes are unable to complement one another.

Clinical manifestations of WS include retarded growth, gray hair, hoarseness, skin sclerosis, cataract, type II diabetes mellitus, hypogonadism, osteoporosis, immune abnormalities, atherosclerosis, brain atrophy, and malignancy. Two common causes of death among WS patients are malignancy and myocardial infarction, both occurring at a median age of 47 years. Schizophrenia was noted in 10% of WS individuals, and a few cases of senile dementia, not linked to Alzheimer disease, were also reported (142).

WRN has a molecular mass of around 165 kDa, is ubiquitously expressed in tissues, and contains a C-terminal nuclear-localization signal. WRN primarily localizes to the nucleolus and, in response to some kinds of DNA damage, relocates to nucleoplasm. WRN has a modular composition and contains an N-terminal portion with the exonuclease domain, a central core with the helicase domain, and two C-terminal regions that bind protein partners or DNA or both (131, 219). WRN participates in several DNA-repair pathways including HR, NHEJ, and BER/SSB repair. Mutations in WS individuals are nonsense or frameshift mutations, which lead to a truncated protein that cannot localize to the nucleus and is generally degraded in the cytoplasm. Moreover, posttranslational modifications may affect the catalytic activities of WRN, the nature of WRN interactions with its protein partners, and the subcellular localization in response to DNA damage (142).

A mutation in both copies of the *BLM* gene causes Bloom syndrome (BS), a rare human autosomal recessive disorder. Patients with BS are prone to cancers associated with a marked genomic instability (8). Cells from BS patients have a mutator phenotype and display many cytogenetic abnormalities, including increases of chromosome breaks, symmetric quadriradial chromatid interchanges between homologous chromosomes, and sister chromatid exchanges. *BLM* gene encodes for a protein with a molecular mass of 159 kDa belonging to the RecQ helicase subfamily. The preferred substrates for recombinant BLM are G-quadruplex DNA, D-loop structures, and X-junctions, but the specific function(s) of BLM remain(s) unknown (8). However, *BLM*-deficient mice develop an increased number of intestinal tumors, presumably as a result of somatic loss of heterozygosity mediated by increased mitotic recombination in the absence of *BLM* function.

BLM is part of the BASC (BRCA1-associated genome surveillance complex). This complex includes BRCA1, which is mutated in some familial breast cancers, ATM (defective in ataxia–telangiectasia), NBS1 (defective in Nijmegen syndrome), and MRE11 (defective in ataxia–telangiectasia-like disorder), MLH1, MSH2, and MSH6, which are component of MMR pathway involved in human nonpolyposis colorectal cancer, RAD50, and RFC (255). BLM has also been reported to interact with RAD51, WRN, ATR, FEN1, and the monoubiquitinated FANCD2 form, a protein involved in the Fanconi anemia pathway (see later) (51, 195).

BLM has been found predominantly in the nuclear matrix of exponentially growing cells and undergoes phosphorylation, which affects its nuclear distribution. BLM may be phosphorylated either *via* an ATM-dependent or ATM-independent pathway and accumulates in response to IR, UV-C, or after replication arrest. On this basis, BLM appears to be at the crossroads of the response pathways induced by DNA damage and stalled replication forks (51, 195).

4. Fanconi anemia. Fanconi anemia (FA) is a rare recessive disease, originally described by Guido Fanconi in 1927, and clinically characterized by multiple congenital abnormalities, bone marrow (BM) failure and aplastic anemia, cancer and leukemia susceptibility, chromosome instability, and cellular hypersensitivity to interstrand DNA crosslinking agents, such as cisplatin, mitomycin C, diepoxybutane, and melphalan (234). FA can be divided into at least 12 complementation groups (A, B, C, D1, D2, E, F, G, I, J, L, and M) defined by cell-fusion studies, and 11 of the 12 responsible FA genes have been identified. All are autosomal except for *FANCB*, which is located on the X chromosome. Proteins encoded by FA genes (FA proteins) include a multifunctional E3 monoubiquitin ligase complex of eight FA proteins (FANC A, B, C, E, F, G, L, M), named the FA core complex, a monoubiquitinated protein (FANCD2), a helicase (FANCJ/BACH1/BRIP1), and a well-known breast/ovarian cancer susceptibility protein (FANCD1/BRCA2) (234). FA proteins and another well-known breast/ovarian cancer susceptibility protein, BRCA1, cooperate in DNA-damage response and in a common DNA repair process that is required for cellular resistance to DNA interstrand crosslinks (ICLs) known as the FA-BRCA pathway or network (160, 171, 244).

Exogenous and endogenous DNA damage detected during the DNA-synthesis (S) phase of the cell cycle results in the activation of the FA core complex, promoting through its ubiquitin ligase activity (FANCL) the monoubiquitination of FANCD2 at a specific lysine residue (Lys561). USP1 is likely

to be the enzyme for deubiquitination of FANCD2 *in vivo*. Activated FANCD2 is an effector protein that translocates into chromatin-associated foci, at the sites of DNA damage and repair, where it colocalizes and interacts with BRCA2 and several other DNA-repair proteins including BRCA1, PCNA, RAD51, and RPA. Furthermore, evidence exists of a molecular and functional interaction of FA proteins with proteins involved in DNA-damage response including ATM, MRE11, BLM, NBS1 and ATR responsible for other rare genetic chromosome-instability syndromes. FA core complex, BLM, RPA, and topoisomerase IIIα form a larger complex called BRAFT (for BLM, RPA, FA, and TopoIIIα), whereas ICL-induced nuclear foci formation of BLM depends on FANCC, FANCG, and FANCD2. The MRN complex also interacts with FA proteins, and the FA core complex is required for the phosphorylation of NBS1 as well as nuclear foci formation of MRE11 specifically in response to ICLs. XPF/ERCC1 heterodimer is an endonuclease required for incision of ICLs and colocalizes with FANCA in ICL-induced nuclear foci. A detailed description of the FA genes and their interactions is well reviewed in ref. 234.

Bolgiolo *et al*. (21) proposed that phosphorylated histone H2AX (γH2AX) is required for recruiting FANCD2 to chromatin at stalled replication forks induced by DNA damage. FANCD2 binding to γH2AX is BRCA1 dependent, and H2AX-deficient or -depleted cells show an FA-like phenotype, including chromosomal aberrations and hypersensitivity to mitomycin C (21).

The FA pathway appears to be involved in the regulation of DNA repair; however, critical questions remain unanswered. Although FANCD2 monoubiquitination is a required event in the FA pathway, little is known about the downstream function of this posttranslational modification. The absence of profound sensitivity of FA cells to UVC-induced lesions corroborates the lack of involvement of the FA pathway in classic NER. The observation that BRCA2 is an FA gene and probably acts downstream of FANCD2 monoubiquitination suggests that the FA pathway could potentially regulate BRCA2 functions (171). BRCA2 plays a pivotal role in homologous recombination by facilitating the formation of single-stranded DNA-RAD51 nucleoprotein filaments during DNA strand invasion. In accordance with this hypothesis, monoubiquitinated FANCD2 interacts with BRCA2 in chromatin on cellular exposure to DNA damage (254).

Interactions of the FA core complex with the Bloom's helicase BLM, which is involved in Holliday-junction resolution, and the recent identification of the DNA helicase, FANCM, as a member of this complex (168, 174) support the idea that the FA pathway may function downstream of RAD51 focus formation in the resolution of HR intermediates. It is also plausible that the FA pathway regulates a subset of HR, specifically in the repair of DNA ICLs. DNA double-strand breaks generated *via* gamma radiation or restriction-enzyme cleavage are qualitatively distinct from those generated during ICL repair and could require specialized modes of HR that may, in turn, be facilitated by the FA pathway. Compelling evidence indicates that the incidence of NHEJ is unaltered in FA cells, suggesting that FA proteins are unlikely to be involved in the NHEJ reaction itself. Potential functions of the FA pathway could lie in the protection of broken DNA ends generated during ICL repair. FA proteins might prevent incorrect DNA end processing that could lead to deletions and chromosomal aberrations. It has been proposed that double-strand breaks initially serve as substrates for the NHEJ machinery followed by a hand-off to HR. FA proteins could play a role in this process of repair choice, in the absence of which DNA ends may be preferentially bound by NHEJ factors such as the Ku proteins or erroneously processed by the nuclease activity of Artemis (171).

The functions of the FA pathway in the regulation of multiple DNA repair processes that impinge on ICL repair suggest a model in which FA proteins may reside at the crossroads of ICL repair (Fig. 16). DNA ICLs must be processed in a highly orchestrated manner. The function of the FA pathway in coordinating the unique interplay between multiple repair pathways in ICL resolution could explain the selective sensitivity of FA cells toward this class of DNA lesions (171).

ATR-mediated phosphorylation of FANCD2 on serine 222 may enhance monoubiquitination of FANCD2 and is required for the establishment of the S-phase checkpoint. This phosphorylation also depends on NBS1. Conversely, monoubiquitination of FANCD2 on lysine 561 is required for nuclear foci formation of FANCD2 with BRCA1 and RAD51 and for ICL resistance, but it is not required for the S-phase checkpoint. Therefore, FANCD2 has two independent functions, resulting from independent posttranslational modifications. The ICL-induced ATR-dependent phosphorylation of NBS1 and FANCD2

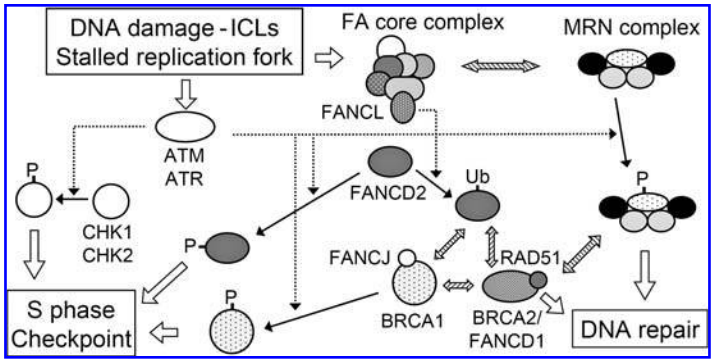

FIG. 16. Schematic representation of the Fanconi anemia/BRCA pathway. DNA damage, as DNA interstrand crosslinks (ICLs), may result in a stalled replication fork, which activates the Fanconi anemia/BRCA pathway. FANCL, a subunit of the Fanconi anemia (FA) core complex provided with ubiquitin ligase activity, modifies FANCD2. Monoubiquitination of FANCD2 is required for nuclear foci formation of FANCD2 and its interaction with other DNA-repair proteins such as BRCA1, BRCA2/FANCD1, and RAD51. The activated FA core complex is also necessary for the formation and recruitment of MRN complex. DNA damage and a stalled replication fork activate ATR-ATM kinases. Their substrates mainly include factors involved in cell-cycle checkpoint control such as CHK1, CHK2, p53, NBS1, and BRCA1, as well as FANCD2, whose phosphorylation is required for the establishment of the S-phase checkpoint. BRCA2/FANCD1, BRCA1, and FANCJ, in addition to MRN complex, contribute to the progress of DNA repair.

seem to be required for the establishment of the ICL-induced S-phase checkpoint (194, 234).

The FA-I complementation group remains uncharacterized at the molecular level. FA-I mutant cells do not ubiquitinate FANCD2, precluding its localization to repair foci. FANCI is an ATM/ATR kinase substrate, is required for resistance to mitomycin C, and shares sequence similarity with FANCD2, probably evolving from a common ancestral gene (221). FANCI is activated by monoubiquitination, and this requires ubiquitinated FANCD2. Thus, these two proteins are interdependent and use a dual-ubiquitination mechanism to affect downstream effector function. Furthermore, phosphorylation of FANCD2 is required for its own efficient ubiquitination and therefore for the efficient ubiquitination of FANCI. This phosphorylation–ubiquitination cascade, culminating in chromatin loading of the ID (FANCI-FANCD2) complex, provides a control at sites of stalled forks. This complex, when correctly placed, can direct DNA-repair pathways to remove ICLs so that replication can resume and cells can survive. Without this key event, cells are prone to genomic instability (221). Recently, mutations in the *PALB2* gene, the "partner and localizer of BRCA2," suggest a new FA complementation group, the FANCN (249, 262). PALB2, originally identified in a screen for proteins present in complexes containing BRCA2, binds to the extreme N terminus of BRCA2 and stabilizes BRCA2 in key nuclear structures. This allows BRCA2 to function in DNA repair and at the S-phase checkpoint. Decrease of PALB2 expression in HeLa cells by siRNAs leads to mitomycin C sensitivity, which causes ICLs and eventually double-strand breaks, and is a hallmark of Fanconi anemia. Reid and colleagues (102, 206) identified biallelic protein-truncating mutations in *PALB2* in seven of 82 individuals with FA not due to known genes. Transfection of wild-type PALB2 in a lymphoblastoid line from one patient with two of such mutations reversed the sensitivity to mitomycin C. Xia and colleagues (262) evaluated one FA patient with a premature truncation in exon 4 of *PALB2* inherited from the mother and a complete deletion of *PALB2* inherited from the father. In this individual, anemia developed at a very early age and the patient died at age 2 years of a kaposiform hemangioendothelioma, a rare and aggressive type of endothelial cancer. These results associate *PALB2* with the new Fanconi anemia subtype N, thus justifying *FANCN* as an alias for this gene.

The only FA core protein exhibiting DNA-binding activity is FANCM (the homologue of an archaeal helicase/nuclease known as HEF). Recently, Ciccia *et al*. (41) described the identification of FAAP24, a component of the FA core complex that associates with the C-terminal region of FANCM and targets FANCM to structures that mimic intermediates formed during the replication/repair of damaged DNA. FAAP24 shares homology with the XPF family of flap/fork endonucleases, and a close similarity exists between FANCM/FAAP24 and ERCC1/XPF complexes. Moreover, FAAP24 is required for normal levels of FANCD2 monoubiquitination after DNA damage, and its depletion by siRNA results in cellular hypersensitivity to DNA crosslinking agents and chromosomal instability. It has been suggested that FAAP24 plays an important role in target recognition by the FANCM/FAAP24 complex because of its specificity for single-stranded or splayed-arm DNA, or both, anchoring the FA core complex to DNA and allowing the monoubiquitination of FANCD2 (41).

Recently, in a crystallographic and biologic study, Nookala *et al*. (181) reported the first structure of an FA protein, FANCE, which provides a template for the structural rationalization of other proteins defective in FA. Structural analysis predicted that three of four mutations identified in FANCE affected amino acids important for maintaining the conformation of the polypeptide chain (181). FANCE is essential for FANCC accumulation in the nucleus and assembly of the FA core complex. Moreover, FANCE localizes to constitutive nuclear foci and becomes associated with ubiquitinated FANCD2 and BRCA2 in a chromatin complex (254). FANCE is the only member of the FA core complex for which a direct association with FANCD2 has been demonstrated, and disease-associated mutations in FANCE may disrupt the FANCE–FANCD2 interaction, providing a structural rationale for their pathologic effect in FA patients (181).

Generation of *FANCA, FANCC*, *FANCG*, *FANCD2*, *FANCA-FANCC* double, and *FANCL* knockout mice has been reported and, importantly, in some of these FA mice, tumors develop. The characterization of the FA genes and proteins has allowed new diagnostic approaches to FA and suggests novel treatment options for patients with FA. Mutations in FA genes may become a useful predictor of sensitivity to chemotherapy with widely used anticancer DNA cross-linking agents (cisplatin, mitomycin C, and melphalan) (234).

5. LIG4, Artemis, and Omenn syndromes. Genes encoding two factors, *Artemis* and *DNA Ligase IV (LIG4)*, have been found mutated in rare inherited syndromes (183). Individuals with these syndromes have whole-body and cellular hypersensitivity to ionizing radiation and DNA-damaging chemotherapeutic compounds and are immunocompromised. LIG4 and Artemis are components of the DNA nonhomologous end-joining (NHEJ) machinery, the major process in mammalian cells for the repair of DNA double-strand breaks. Double-strand breaks are the most lethal of the lesions induced by ionizing radiation but also arise endogenously during V(D)J recombination, the essential rejoining process that serves to rearrange the variable, diversity, and joining segments during T- and B-cell development. Defects in V(D)J recombination result in SCID (severe combined immunodeficiency) characterized by the absence of mature B and T cells, but by the presence of NK cells. Mutations in *LIG4* and *Artemis* in humans that produce proteins with low residual activity have been found to be associated with the onset of lymphopoietic malignancies (26, 70, 175). LIG4 syndrome is a rare disorder arising from mutations in the *LIG4* gene (94, 183). LIG4 plays a critical role in both DNA replication and V(D)J recombination. Thus, its deficit might cause the death of the cells with nonfunctional repair or recombination during meiosis or rearrangement of both T- and B-cell receptors, which promotes a progressive combined immunodeficiency. The critical role for LIG4 in NHEJ is highlighted by the embryonic lethality of *LIG4*-deficient mice in contrast to the viability and normal size of *Artemis*-deficient mice (210). The LIG4 syndrome is characterized by chromosomal instability, immunodeficiency, and developmental and growth delay. Some patients show acute T-cell leukemia and NBS-like facial abnormalities. Collectively, LIG4 syndrome patients are likely to be at increased risk for lymphoid malignancies (26, 70).

LIG4 has a conserved ligase domain at its N-terminal and two C-terminal BRCT domains. Interaction with XRCC4, in the final rejoining step of NHEJ, occurs *via* the region that lies between the two BRCT domains. Several mutations have been identified in the *LIG4* gene of patients affected by the LIG4 syndrome, expressing proteins retaining little or no residual function (94, 183). The Arg278His mutation influences LIG4 function by reducing its activity to 5–10% of wild-type levels. In addition, two closely linked N-terminal LIG4 polymorphisms (Ala3Val and Thr9Ile) mildly reduce adenylation and ligation activities (two- to threefold) but no residual activity is detectable when both mutations are coupled with the Arg278His substitution (94). Two other mutations identified in the *LIG4* gene are a Met249Val substitution, located near the ATP binding site, and a five-nucleotide deletion, which results in a frameshift, whereas its product, lacking the C-terminal XRCC4 binding site, could be nonfunctional. Van der Burg *et al*. (243) presented a study on a patient with a new type of radiosensitive $T^-B^-NK^+$ SCID (RS-SCID), with a defect in LIG4 and hypersensitivity to ionizing radiation. The *LIG4* mutation in this patient was a homozygous deletion of three nucleotides, resulting in deletion of glutamine at position 433, within the catalytic domain. This deletion resulted in undetectable levels of LIG4, most probably due to instability of the protein. This study demonstrated that *LIG4* mutations could give rise not only to the LIG4 syndrome, but also to RS-SCID without neurologic defects and pancytopenia (except for lymphopenia). The different clinical forms are most probably due to distinct mutations resulting in normal levels of low-activity protein (as found in LIG4 syndrome) *versus* an undetectable level of LIG4 protein (as found in RS-SCID). Low levels of LIG4 are sufficient for cell survival, but V(D)J recombination clearly requires higher LIG4 protein levels.

Artemis is a component of the NHEJ pathway and is associated with the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Artemis is phosphorylated by DNA-PKcs, and in this activated form possesses overhang endonucleolytic and DNA hairpin-opening activities. A lack of Artemis activity causes a type of severe combined immunodeficiency called radiosensitive SCID (RS-SCID), a disorder originally identified in Athabascan-speaking Navajo and Apache Native Americans, who display a high incidence of SCID (175). Cell lines or lymphocytes derived from these patients showed marked sensitivity to ionizing radiation, and the defective protein was subsequently identified as Artemis.

It has been shown *in vitro* that Artemis is a structure-specific nuclease capable of cleaving the hairpin junction formed at coding ends during V(D)J recombination (159). This is essential for completion of V(D)J recombination, and the pronounced SCID phenotype of Artemis patients and accumulation of hairpin intermediates in both mouse SCID (deficient in DNA-PKcs) and Artemis-deficient cells is consistent with the notion that *in vivo* Artemis cleaves the hairpin generated during V(D)J recombination in a manner dependent on DNA-PKcs (158). *In vivo*, Artemis protein levels may become rate-limiting in V(D)J recombination, and the absolute amount of Artemis protein per cell requires close regulation for the development of a diverse adaptive immune system.

More than 40 years ago, Gilbert Omenn (111) described a rare clinical entity that he called reticuloendotheliosis, with eosinophilia characterizing the microscopic lymph node morphology of the disease. Originating from this report, the term "Omenn syndrome" (OS) was coined in the medical literature for the clinical description of infants with severe combined immunodeficiency (SCID) in combination with failure to thrive, squamous erythrodermia, alopecia, lymphadenopathy, hepatosplenomegaly, and intractable diarrhea (111). Since the first description, ~70 patients have been reported. In patients with Omenn syndrome, B cells are mostly absent, whereas T-cell counts are normal to elevated. Natural killer (NK) cells function and absolute numbers are unaffected, and thus the majority of patients may be classified as having $T^+B^-NK^+$ SCID (68).

Mutations of the recombination activating genes 1 and 2 (RAG1/2) with low residual activity have so far been described in the majority of patients with Omenn syndrome. The RAG proteins are indispensable for V(D)J recombination, and rearrangement of the DNA is guided by the recombination signal sequences (RSSs) that flank the individual V, (D), or J gene segments. The RAG proteins specifically recognize the RSSs and, acting as a heteromultimeric endonuclease, initially introduce a nick in the DNA double-strand between a coding V, (D), or J element and the heptamer of its flanking RSS. After the generation of this initial cut, the resulting free 3′-OH promotes the hydrolysis of the phosphodiester bond in the opposite strand, leading to specific double-strand breaks and the formation of covalently closed hairpins at the coding ends of the V, (D), and J elements (89).

In humans, a severe/null defect in either RAG-1/RAG-2, Artemis, or LIG4 leads to the SCID phenotype owing to impaired V(D)J recombination activity, the lack of the generation of immunoglobulin, and a block of development of lymphocyte precursors. Except for RAG deficiencies, cells of these patients also display an increased radiosensitivity, because of a general deficiency in NHEJ. During V(D)J recombination, NHEJ factors execute the downstream processing of RAG-generated DNA double-strand breaks and the rejoining of hairpin coding ends of V, (D), and J elements. Artemis-deficient patients with Omenn syndrome have been described with one allele carrying a null mutation that disables the catalytic site of Artemis, and the other containing a translation initiation codon substitution (AUG to ACG) that lowers the efficiency of Artemis protein synthesis and thus also the V(D)J recombination. Except for the radiosensitivity of dermal fibroblasts, these patients were phenotypically and clinically indistinguishable from patients with Omenn syndrome who only have RAG mutations (68).

A recent work proposed a mouse model that may be a useful tool to gain further insight into the pathophysiology of this complex syndrome. Marrella *et al*. (164) demonstrated that introduction of a homozygous *RAG2* mutation (Arg229Gln) in mice caused disturbed lymphoid development and phenotypic changes that largely overlap clinical manifestations associated with *RAG* mutations seen in humans with Omenn syndrome (164).

6. Cernunnos-XLF syndrome. Recently, a new syndrome of human combined immunodeficiency (CID) characterized by a profound T+B lymphocytopenia associated with microcephaly, growth retardation, and increased cellular sensitivity to ionizing radiation has been described. A defective

V(D)J recombination and an impaired DNA-end ligation process both *in vivo* and *in vitro* are indicative of a general DNA-repair defect in these patients owing to mutations in a novel NHEJ factor that could not be complemented by any of the known NHEJ genes. This factor has been named both Cernunnos (an enigmatic Celtic god of the hunt, the underworld, fertility, and possibly more) and XLF (a descriptive name for XRCC4-like factor) (7, 25). The clinical phenotype of Cernunnos/XLF-deficient patients shares several characteristics with NBS and LIG4 deficiency. However, Cernunnos deficiency does not lead to impaired cell-cycle checkpoints, as observed in NBS condition, but leads rather to an NHEJ defect as observed in LIG4 deficiency. Whether Cernunnos, similar to LIG4/XRCC4, is an essential factor for viability is currently unknown. The question of the genomic caretaker status of Cernunnos is of importance because it may turn out that Cernunnos deficiencies are linked to a higher risk of developing cancer (25).

To characterize Cernunnos-XLF function, Zha *et al*. (267) used a gene-targeted mutation to delete exons 4 and 5 from both copies of the *Cernunnos-XLF* gene in mouse ES cells. This mutation led to increased spontaneous genomic instability, including translocations, indicating that, in mice, Cernunnos-XLF is essential for normal NHEJ-mediated repair of DNA double-strand breaks and acts as a genomic caretaker to prevent genomic instability. The genomic instability in Cernunnos-XLF mutant mouse cells suggests that, similar to other NHEJ factors, Cernunnos-XLF may also function as a tumor suppressor, perhaps by cooperating with checkpoint factors to suppress tumors that arise from aberrant V(D)J recombination (267).

7. Neurological diseases and DNA-repair defects. A relation has been established between increased DNA damage, defective DNA repair, and several neurodegenerative disorders. In addition to neurologic defects associated with the previously mentioned disorders, recent studies of patients with Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, Friedreich's ataxia, and Huntington's disease suggest that increased oxidative DNA damage and possible defects in DNA repair play a key role in selective neuronal loss associated with aging and neurodegeneration (22, 228).

Alzheimer and Parkinson diseases are devastating neurologic pathologies that have familial and sporadic forms and are attributable to a vulnerable genetic lineage combined with aging and possibly as-yet-unknown lifestyle factors. Two DNA-repair pathways that are most likely to be adversely affected in Alzheimer disease are BER and NHEJ. A diminished BER capacity and a reduction in free 8-oxo-7,8-dihydro-2′-deoxyguanosine, a by-product of the BER process, observed in the brain of patients with Alzheimer disease, are associated with elevated levels of unrepaired DNA (79). However, the exact mechanism(s) by which oxidative DNA damage might cause neurodegeneration or neuronal cell death is poorly understood, and no unequivocal identification has been made of a precise locus or gene (or genes) affected in these pathways (22, 228). Recently, Dogru-Abbasoglu *et al*. (62) investigated the relation between Alzheimer disease and the XRCC1 Arg194Trp polymorphism, and their results suggest that the latter may play a role in development of the disease.

In spinocerebellar ataxia with axonal neuropathy-1 (SCAN1), a progressive degeneration of postmitotic neurons may occur. El-Khamisy and co-workers (69) recently demonstrated that this neurodegenerative disease results from a mutation in the gene encoding tyrosyl-DNA phosphodiesterase 1 (TDP1). TDP1 is required for the repair of chromosomal single-strand breaks, removing topoisomerase I from 3′-trapped protein-DNA intermediates, transiently formed during the reaction, arising from abortive topoisomerase I activity or oxidative stress. This group has also shown that TDP1 directly interacts with DNA ligase IIIα (LIG3α), forming a multiprotein single-strand break–repair complex that is inactive in SCAN1 cells. Normally, single-strand breaks or gaps are repaired in neurons through BER, in which both LIG3α and XRCC1 participate, together with a polynucleotide kinase. These findings indicate the existence of a slightly different mode of single-strand break–repair pathway in differentiated neurons and TDP1-dependent single-strand break–repair could be of considerable importance in brain cells, where it deals with single-strand breaks resulting from a variety of causes.

Yet another hereditary disease with neurologic symptoms and a defect in the repair of DNA single-strand breaks has been recognized. This disease is called triple-A (achalasia–addisonian–alacrima) syndrome, and it was found to be caused by a mutation in a gene called *AAAS* coding for a protein named ALADIN (24). Triple-A syndrome shows considerable genetic heterogeneity. ALADIN is a component of the nuclear pore complex, and mutant ALADIN Ile482Ser fails to target the complex. The consequences of this failure were recently investigated by using fibroblasts from patients with triple-A syndrome (106). Mutant ALADIN was found to decrease the nuclear accumulation of both aprataxin, a repair protein for DNA single-strand breaks (233), and DNA ligase I; this decrease was reversed by wild-type ALADIN.

8. Progeroid syndromes and aging. Aging can be defined as progressive functional decline involving an increased mortality over time. It has been proposed that organisms invest sufficient energy into maintenance of the soma only to survive long enough to reproduce. Aging would occur, at least in part, as a consequence of this imperfect maintenance rather than as a genetically programmed process. Various evidence links aging with DNA lesions: DNA damage accumulates with age; the efficiency of DNA-repair pathways may decline with time; and repair defects can cause phenotypes resembling premature aging (156). Many DNA-damaging agents are known, but ROS, as normal by-products of metabolism, are a potential source of chronic, persistent DNA damage and may contribute to aging (99, 156, 166). As mentioned earlier, a number of diseases can be categorized as progeria or progeroid syndromes, and Werner syndrome has been suggested to be a model system for the study of normal aging (142, 143). Niedernhofer *et al*. (178) proposed a mouse model of the progeroid syndrome, caused by a severe mutation in XPF that can reconcile two apparently disparate hypotheses, in which aging is genetically regulated or is a consequence of the accumulation of stochastic damage. Recently, an *XPD(ERCC2)* mouse model for combined xeroderma pigmentosum and Cockayne syndrome that exhibited both cancer and segmental progeria was reported (10). The fibroblasts from these animals showed defective repair of oxidative DNA lesions. Moreover, Andressoo *et al*. (9) proposed that NER dis-

orders are potentially informative about the connection between cancer and aging. In particular, they focused attention on the Cockayne syndrome (CS) and trichothiodystrophy (TTD). These syndromes are caused by defects in genome maintenance *via* the NER pathway and display severe progeroid symptoms but lack any cancer predisposition. In contrast, xeroderma pigmentosum (XP) syndrome has 1,000-fold elevated sun-induced skin cancer. In rare cases, symptoms of XP are combined with those of CS or TTD, and it is interesting that defects in one NER gene, *XPD*, can lead either to XP (Arg683Trp), TTD (Arg722Trp), or XP-CS (Gly602Asp) diseases. Further insights into the basic biology of DNA-repair proteins, the exact nature of DNA lesions that are most important in causing aging, and their roles in cell checkpoints may also contribute to the research on aging.

B. DNA repair and cancer-associated diseases

The hereditary syndromes are conferred by mutations, including polymorphisms, that have a profound impact on protein function and that may contribute to instances of sporadic cancer. Cancer cells are often defective in one of the major DNA-repair pathways, and inherited mutations that affect DNA-repair genes are strongly associated with a high risk of cancer.

1. Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome.

Failure of mismatch repair (MMR) processing has the potential to allow the incorporation of unrecognized base-pair mismatches into the genome, which may be sufficient to affect deleteriously the expression of genes essential to normal cellular function. An inherited MMR deficiency is mirrored by three diseases: the HNPCC syndrome, the Muir–Torre syndrome, and the Turcot syndrome. The latter two syndromes can be viewed as subtypes of HNPCC in which patients either skin tumors (keratoacanthomas, sebaceous gland tumors) or brain tumors (glioblastomas) develop, in addition to colorectal cancer (238). Microsatellite instability (MSI) caused by expansion or contraction of short nucleotide repeats is a characteristic of MMR deficiency and is detectable in the majority of colorectal cancers arising in carriers of germ-line MMR mutations (1).

HNPCC is an autosomal-dominant inherited disease associated with a marked increase in susceptibility for cancer. It is characterized by a familial predisposition to early onset of colorectal carcinoma and extracolonic cancers of the gastrointestinal, urologic, and female reproductive tracts (269). Mutation rates in tumor cells with MMR deficiency are 100- to 1,000-fold more than those in normal cells. The accumulation of mutations accelerates tumor progression and might explain why, in a majority of HNPCC patients, colon cancer develops during their lifetime, compared with only 5% of the general population. HNPCC patients can have synchronous and metachronous colorectal cancers as well as other primary extracolonic malignancies, the most common of which is endometrial adenocarcinoma, followed by carcinomas of the stomach, small intestine, liver and biliary tract, pancreas, ovary, transitional cell carcinoma of the ureters and renal pelvis, and brain tumors. The term hereditary nonpolyposis colorectal cancer may therefore be too restrictive, and Lynch syndrome has been proposed as a more appropriate name to cover this syndrome. The so-called Bethesda and Amsterdam criteria II are two guidelines for performing MSI testing and identifying patients with HNPCC syndrome (reviewed in ref. 3). Recently, a protocol using a family-based strategy for HNPCC detection was presented (144). The average age at colorectal cancer diagnosis in HNPCC is 44 years (64 years in the general population). HNPCC colon tumors such as microsatellite-unstable colon cancers usually have a favorable prognosis (3). Seven genes have been associated with HNPCC (*MSH2*, *MLH1*, *MSH6*, *PMS1*, *PMS2*, *MLH3*, and *EXO1*), but only mutations in *MSH2*, *MLH1*, *MSH6*, and *PMS2* are currently considered to cause HNPCC (144), with *MLH1* (50%) and *MSH2* (39%) primarily involved (269). *MSH2* and *MLH1* mutations often give rise to classic HNPCC families that fulfill the Amsterdam criteria and have a high degree of MSI in tumors (according to international criteria, a high degree of MSI is defined as instability at $\geq$2/5 loci, or $\geq$30–40% of studied loci, whereas instability at fewer loci is referred to as MSI-low). The risk of colorectal carcinoma is low before 30 years of age in MLH1 and MSH2 families and very low in MSH6 families (3). Recent data suggest that *PMS2* germline mutations in HNPCC are more frequent than previously thought, and *MLH3* gene may also be mutated in the germline in some suspected HNPCC families with a variable degree of MSI in tumor tissue, but little evidence supports its role in predisposition to classic HNPCC (3). In HNPCC families, other internal tumors often develop, such as endometrial carcinoma, the second most common extracolonic cancer in HNPCC. Its cumulative risk is 50–60% in female mutation carriers, and the mean age at diagnosis is $\sim$50 years in MLH1 and MSH2 families and 55 years in MSH6 families (3). Racial or ethnic variations in the HNPCC phenotype have been reported, some of which might represent cultural and social differences, whereas others might reflect genetic variations. Of particular note is the increased incidence of gastric cancer in Japanese, Korean, and Chinese populations compared with that in Western countries.

More than 200 mutations in *MSH2* were found to be associated with HNPCC, including nucleotide substitutions, deletions, and insertions. Pathogenic mutations in *MSH2* were scattered throughout all exons and may account for $\sim$40% of HNPCC cases (176). A novel duplication mutation of four nucleotides in exon 7 of *MSH2* (1216_1219dupCGAC), resulting in a 10-codon frameshift followed by a premature stop in *MSH2* (L407fsX417), was found in a Chinese family. This mutation disrupts the normal open reading frame leading to a predicted premature truncated MSH2 product that is associated with HNPCC and lacks evolutionarily conserved domains required for interaction with either MSH3 or MSH6 and DNA binding (269).

Microsatellite instability (MSI) may also result from epigenetic inactivation of MLH1. Kàmory *et al*. (125) reported a study on a patient with synchronous (cecal and rectal) colorectal cancer, without a family history. The patient had two germline alterations, a Val716Met polymorphism in *MLH1* and a 2210+1G>C mutation in the *MSH2* gene, and both tumors failed to express MLH1 and MSH2 proteins. The Val716Met polymorphism is a rare mutation causing a putative disease, whereas 2210+1G>C is a true pathogenic mutation causing an out-of-frame deletion of exon 13, as the nucleotide change affects a splice site at the exon–intron boundary. The Val716Met

variant may not be a sufficient explanation for the loss of expression of the *MLH1* gene, and the patient may have harbored a somatic regulatory mutation. Thus, it has been hypothesized that the presence of both Val716Met polymorphism and 2210+1G>C mutation may enhance the adverse effects, resulting in causing earlier cancer development (125).

Epidemiologic studies showed that having a first-degree relative with colorectal cancer approximately doubled the risk of colorectal cancer, and if the affected relative was diagnosed before the age of 45 years, the risk was increased approximately fourfold. However, limited data exist on the genetic basis of the excess familial risk associated with MMR-competent and MMR-deficient cancers. To determine the contribution of known genes to the overall disease burden, Aaltonen *et al*. (1) analyzed a large population of patients with colorectal cancer and verified family histories. Approximately 80% of the excess risk associated with MSI-positive patients could be ascribed to the effect of possessing a germline *MLH1*/*MSH2* mutation, but pathogenic changes in introns or in promoter regions, exonic deletions, and a small minority of base substitutions remain undetected. An important conclusion of this study is that one third of the excess risk of colorectal cancer associated with family history is not explained by mutations in known genes (1).

Diagnosis of the Muir–Torre syndrome should be made with the synchronous or metachronous occurrence of at least one sebaceous gland neoplasia and at least one internal neoplasm in a patient, regardless of the family history. Therefore, screening for MSI in sebaceous gland neoplasias might help in the detection of an inherited DNA mismatch repair defect. The majority of Muir–Torre syndrome families are linked to *MSH2* mutations, and the remaining families, to *MLH1* (3, 238).

Turcot syndrome is characterized by the development of colorectal adenomas and carcinomas, and primary central nervous system tumors and Turcot phenotype appear to represent a complex interplay between the characteristics of the germline mutation and additional somatic events in target tissues. The combination of colon cancer and malignant glioma/glioblastoma has been reported to occur in the HNPCC variant of Turcot syndrome in association with homozygous or compound heterozygous germline mutations in *MLH1*, *MSH2*, *PMS2*, or *MSH6*. The familial adenomatous polyposis (FAP) variant of Turcot syndrome is characterized by colon polyposis and medulloblastoma, and is associated with heterozygous mutations of the *APC* gene involved in mitotic spindle checkpoint (3).

2. Inherited breast cancer. Inherited breast cancer is associated with germline mutations in ten different genes, *BRCA1*, *BRCA2*, *p53*, *PTEN*, *CHEK2, ATM*, *NBS1*, *RAD50*, *BRIP1*, and *PALB2*, which share important features in their impact on breast cancer. They act in pathways whose role it is to preserve genomic integrity. One of the major causes of the genetic instability associated with their deficiency is that mutant cells have an impaired ability to undergo homologous recombination and therefore cannot effectively repair DNA damage, such as DNA double-strand breaks and ICLs. A single deleterious mutation in any one of these genes is sufficient to increase breast cancer risk significantly. However, nearly 50% of familial breast cancer remains unresolved by any of these genes, which indicates the involvement of other genes in this disease (102). Inherited *BRCA1* and *BRCA2* mutations, including polymorphisms, truncating mutations, and intronic variants, confer very high risks of breast and ovarian cancer, with lifetime risk estimates ranging from 10% to 60%. Mutations in *p53* and *PTEN* lead to very high breast cancer risks associated with rare cancer syndromes, whereas mutations in *CHEK2*, *ATM*, *NBS1*, *RAD50, BRIP1*, and *PALB2* are associated with doubling of breast cancer risks (102, 250). *BRIP1* and *PALB2*, also known as FANCJ and FANCN, respectively, are also involved in Fanconi anemia.

Recently, it was shown that mutations in *PALB2* are implicated in breast cancer predisposition, and a formidable breast cancer history is associated with the 229delT mutation, whose product (Cys77fs) is a remarkable frameshift fusion protein containing an unusually long "alien" tail (239). Five different truncating mutations of PALB2 were also identified in 10 breast cancer patients with wild-type sequences at *BRCA1* and *BRCA2* (102, 199). *PALB2* mutations are associated with a relative risk for breast cancer >2.3, and *PALB2*-related breast cancers showed tumor characteristics clearly different from *BRCA1*-related tumors and some similarities to *BRCA2*-related cancers (239).

All *ATM* mutations together confer a twofold risk of breast cancer (207). However, indications suggest that factors such as age and the specific mutation may influence breast cancer risk. Epidemiologic analyses suggest that the risk of breast cancer in *ATM* carriers is higher in women younger than 50 years and may be higher in first-degree relatives of ataxia–telangiectasia cases compared with more distantly related *ATM* heterozygotes. One particular mutation in *ATM*, T7271G, has been proposed to be associated with higher risks of breast cancer (6).

A recent study evaluated, retrospectively, the association of single-nucleotide polymorphisms (SNPs) in DNA-repair genes, *XRCC1* (Arg399Gln) and *XRCC3* (Thr241Met), and in a cell-cycle control gene, *CCND1* (Ala870Gly), with progression-free survival and breast cancer–specific survival in patients with metastatic breast cancer treated with high-dose chemotherapy. This study showed for the first time that SNPs in DNA repair genes *XRCC1* and *XRCC3*, either alone or in combination, were significantly associated with the survival of patients with metastatic breast cancer (18).

3. Prostate cancer. Prostate cancer is the most commonly diagnosed nonskin cancer. Germline mutations and polymorphisms in DNA-damage response genes, including *BRCA1/2*, *OGG1*, *XRCC1*, *CHEK2*, and *ADPRT*, were associated with prostate cancer risk (155). Sequence variants in *OGG1*, a gene involved in BER, were associated with prostate cancer in both sporadic and familial cases as well as SNPs in *XRCC1* (Arg194Trp and Arg399Gln) and *XPC* (Lys939Gln) were associated with elevated prostate cancer risk (107). The association of defects in MMR proteins with prostate cancer has been controversial, and recently Norris *et al*. (182) published a study on this relation. This work determined alterations in key MMR protein levels in prostate cancer and recognized PMS2 elevation as a prognostic marker in preneoplastic and prostate cancer lesions. Protein increase was already detected in preneoplastic lesions and low-grade tumors, suggesting an early event in tumorigenesis (182).

Many researchers have used a variety of approaches to evaluate the functional significance of polymorphisms within DNA-repair proteins in human cancer-risk assessment, and two computational algorithms (SIFT, http://blocks.fhcrc.org/sift/SIFT.html;

and PolyPhen http://www.bork.embl-heidelberg.de/PolyPhen) were evaluated in predicting functional consequences of nonsynonymous SNPs (155). The NER pathway removes some of the DNA adducts generated by prostate cancer–related carcinogens, as tobacco-related polycyclic aromatic hydrocarbons and heterocyclic aromatic amines from well-done meats and pesticides. Thus, attention was focused on the association between prostate cancer and NER. Results indicated that a lower NER capacity, predicted from genetic profiling and computational modeling, might have great potential for prostate cancer risk assessment (155).

4. Polymorphisms in DNA-repair genes and cancer. A large number of SNPs in different DNA-repair genes have been identified, and some of them have been studied for human cancer susceptibility.

An association has been reported between *XRCC1* Arg399Gln polymorphism and the risk of renal cell carcinoma. The effect of the *XRCC1* Arg194Trp polymorphism differs according to cancer type: the 194Arg allele has been thought to be a risk factor in gastric cancer, whereas the 194Trp allele has been reported to be associated with lung cancer and the head and neck squamous cell carcinoma. The XPC Lys939Gln polymorphism has been found to be associated with an increased risk of bladder and lung cancer, whereas polymorphisms in the *XRCC7* gene have been found to be associated with glioma (107).

A recent study evaluated the relation of polymorphisms of DNA-repair genes with the risk of childhood acute lymphoblastic leukemia. The *XRCC1* 194Trp allele and haplotype B (194Trp-280Arg-399Arg) showed a protective effect against development of childhood acute lymphoblastic leukemia. In contrast, individuals with the *XRCC1* 399Gln allele and haplotype C (194Arg-280Arg-399Gln) were associated with increased risk for this disease (186).

The associations between polymorphisms in DNA-repair genes and oral squamous cell carcinoma was also investigated in a Thai population. Nine known SNPs in five common DNA-repair genes were investigated: *XRCC1* (Arg194Trp and Arg399Gln), XRCC3 (Thr241Met), XPC (PAT and Lys939Gln), XPD (exon 6 and Lys751Gln), and MGMT (Trp65Cys and Leu84Phe). This study indicated that the variant genotypes with XRCC3 241Met and possibly XRCC1 194Trp and XPD exon 6 contribute to oral squamous cell carcinoma (130).

When analyzing genetic variation as an important factor in determining susceptibility to tobacco-induced lung cancer, significant associations were found with polymorphisms in genes involved in DNA damage sensing (ATM) and, interestingly, in four genes encoding proteins involved in mismatch repair (*LIG1*, *LIG3*, *MLH1*, and *MSH6*) (146).

Finally, the combination of smoking and low OGG1 was associated with a highly increased estimated relative risk for the head and neck squamous cell carcinoma (190).

V. DNA-REPAIR PATHWAYS AND ANTITUMOR TREATMENT

Cancer is a complex genetic disease that has posed a formidable challenge to devising successful therapeutic treatments. Tumor resistance to cytotoxic drugs and radiation, which induce DNA damage, limits therapy effectiveness and represents the main obstacle to an anticancer treatment. Usually drug resistance is not the consequence of a tumor transformation, but arises from the selection of tumor cell clones, which may develop protective mechanisms and survive genotoxic treatment. Cellular sensitivity to anticancer drugs depends mainly on various activities in DNA metabolism, particularly in DNA repair. However, DNA repair has received inadequate attention as a determinant of drug sensitivity. It has been reported that HR is disrupted in breast and ovarian cancer, NER in testicular cancer, and MMR is disrupted in sporadic colon cancer. Moreover, a few studies have suggested that translesional DNA synthesis (TLS) may be disrupted in several human cancers. Cancer cells exhibit an elevation in spontaneous and damage-inducible point mutagenesis, compared with nonmalignant cells, suggesting an underlying TLS defect. Recent studies indicate that an elevation in the expression and activity of the error-prone polymerase, POLβ, accounts for the increase in cisplatin resistance and mutagenesis in these cancers. In line with this hypothesis, inhibition of POLβ in these cells results in resensitization to cisplatin (23). Then the specific DNA repair pathway affected is predictive of the kinds of mutations, the tumor drug sensitivity, and the effect of treatment. In fact, recent works have identified several gene families that appear to contribute to the evolution of drug resistance and that are involved in regulating DNA damage, apoptosis, and survival signaling.

A. Influence on drug response

DNA-repair genes encode proteins involved in repairing damage caused by anticancer agents. Consequently, an altered expression as well as SNPs in these genes could affect drug response (4, 67, 161).

*1. Mismatch-repair genes (*MSH2 *and* MLH1*).* Polymorphisms in the *hMSH2* gene have been associated with an increased sensitivity to DNA-damaging agents. However, other mismatch-repair genes (*hMLH1*) have been noted to induce resistance to topoisomerase inhibitors and alkylating agents (19). Expression of MLH1 can sensitize cells to cisplatin, whereas methylation of the *MLH1* gene, which leads to its reduced expression, is associated with a poor survival after carboplatin-based combination chemotherapy (232). Accumulated evidence suggests that MMR not only is involved in DNA repair but also stimulates DNA damage–induced G_2 checkpoint and apoptosis, and two models have been proposed (116, 251). The futile-cycle model considers that the MMR-dependent activation of the G_2 checkpoint or apoptosis is due to the creation of breaks in the newly synthesized strand that result from the futile abortive attempts of the MMR pathway to repair alkylated DNA. The direct-signaling model proposes that MMR complexes (*i.e.*, MutSα complex) can function as sensors to activate the DNA damage–signaling network. Whereas MMR-proficient cells respond to cisplatin and alkylating agents by undergoing G_2 arrest followed by apoptosis, MMR-deficient cells are defective in this response, resulting in an increased resistance to DNA damage–inducing agents. A study using breast cancer cell lines with functional and nonfunctional mismatch-repair capabilities demonstrated that loss of either *MSH2* or *MLH1* function resulted in resistance to topoisomerase II in-

hibitors [*e.g*., doxorubicin and epirubicin (77)]. Furthermore, in MMR-deficient colorectal cancer, the anticancer agents that can be recommended are those that exert their cytotoxicity regardless of the MMR status, including some alkylating drugs, brostacillin, gemcytabine, photodynamic therapy, and taxanes.

2. X-ray repair cross-complementation group 1 (XRCC1). The *XRCC1* gene is involved in the repair of DNA base damages, strand breaks, and crosslinks. Platinum drugs (cisplatin, carboplatin, and oxaliplatin) cause intrastrand crosslinking that results in local denaturation of DNA and leads to the formation of inter- and intrastrand DNA adducts. A study on patients with colorectal cancer receiving treatment with 5-fluorouracil and oxaliplatin showed that XRCC1 Arg399Gln polymorphism is associated with treatment response as a consequence of a reduced DNA-repair capacity (225). In fact, 73% of patients who responded had the Arg/Arg genotype, compared with 66% of nonresponders, who had either the Gln/Gln or Arg/Gln genotype.

3. Excision repair cross-complementation groups 1 and 2 (ERCC1 and ERCC2). NER is the major mechanism used to repair DNA damage induced by platinum exposure. Because the *ERCC1* gene product is considered a rate-limiting component of NER, the hypothesis has been made that interfering with *ERCC1* expression should sensitize cells to cisplatin. A clinical resistance to platinum therapy was observed in patients with elevated expression levels of *ERCC1* in their tumor tissue (ovary, colon, and lung studies). In patients with non–small cell lung cancer treated with cisplatin-combination chemotherapy, survival rate was analyzed according to genotype, and patients homozygous for *ERCC1* 118Cys polymorphism showed improved survival (486 *vs*. 281 days) (212).

The *ERCC2 (XPD)* gene encodes for a helicase that is essential to the nucleotide excision–repair pathway. In a study of patients with metastatic colorectal cancer who received combination chemotherapy with oxaliplatin and 5-fluorouracil, the effect of the XPD polymorphism on response and survival was examined. Analysis of XPD Lys751Gln SNP demonstrated a significant increase in both response rate and median survival in patients with the Lys/Lys genotype (188). However, both decreased and increased DNA-repair capacity has been reported for the lysine variant of ERCC2.

4. BRCA. In the past decade, many studies have revealed that *BRCA1* deficiency results in genetic instability, primarily due to a defective cell-cycle checkpoint and an impaired HR-mediated repair of DNA damage, mostly DSBs. In the absence of *BRCA1*, DSBs can gradually accumulate, and this may result in the activation of carcinogenesis or apoptosis. Thus, a possible way to kill *BRCA1*-deficient cancer cells efficiently is to treat them with DNA-damaging agents, thereby introducing acute DNA damage. *BRCA1*-deficient cancer cells may be more sensitive to damaging agents, such as irradiation, mitomycin C (MMC), and doxorubicin (Adriamycin) (54), which can be used in therapeutic approaches. However, most DNA-damage reagents are not specific and can cause a similar amount of damage in both *BRCA1*-mutant and wild-type cells. Recently, it was shown that PARP-1 inhibitors could kill *BRCA1*/2-deficient cells with high specificity, thus heralding great promise for the use of these drugs in the chemoprevention and therapeutic treatment of *BRCA1*/2-associated breast cancers (75, 103).

5. O^6-methylguanine–DNA methyltransferase (MGMT). DNA repair of alkylated guanines involves MGMT that transfers the O^6 position of guanine to a cysteine acceptor site. An increased expression of this enzyme may lead to resistance to anticancer agents such as dacarbazine, temozolomide, nitrosureas, and streptozotocin, which can alkylate guanine at the O^6 position. Thus, evaluation of MGMT level is a critical determinant for efficacy of therapy. Recently, it was suggested that downregulation of MGMT in glioma cells may depend largely on cellular factors other than promoter-hypermethylation of MGMT genes, which is being used in the clinical setting to nominate patients for temozolomide treatment (215). A functional polymorphism (Gly160Arg), seen in 15% of the Japanese population, dramatically reduced MGMT activity as well as resistance compared with the wild-type allele. The frequency of this SNP is $<1.6\%$ in the total disease-free population, and additional studies indicate the presence of other SNPs that are more common in specific ethnic subgroups (67).

An interesting relation exists between MGMT and the MMR pathway, which controls sensitivity to methylating agents. The expression of MGMT is quite frequently suppressed in transformed cells, apparently by an epigenetic mechanism. MGMT levels vary >100-fold among different tissues and individuals. Some tissues such as liver, exposed to high levels of exogenously and endogenously derived methylating agents, are protected by a high constitutive level of MGMT, which may possibly increase in response to DNA damage. In contrast, MGMT levels in normal human colon, stomach, and pancreas tissues, which develop tumors displaying microsatellite instability, are considerably lower (240).

B. DNA repair and chemotherapeutic drugs

A possible therapeutic strategy for combating cancer is to use drugs that specifically inhibit the DNA-repair pathway (58, 161, 240).

1. Antimetabolites. 5-Fluorouracil-based chemotherapy exerts its anticancer effects through the inhibition of thymidylate synthase (TS), and subsequent incorporation of 5-fluorouracil into DNA. The inhibition of TS leads to a depletion of the intracellular dTTP pool and a perturbation in the levels of the other 2′-deoxyribonucleotides. This increases the error rate of DNA polymerase and promotes the incorporation of 5-fluorouracil, once converted in 2′-deoxyribonucleoside triphosphate, into DNA, deforming the double-strand helix enough to be recognized and bound by the MMR proteins. *In vitro* studies have demonstrated that the response to 5-fluorouracil–based chemotherapy in MMR-proficient cells occurs rapidly, as indicated by a pronounced G_2 arrest. This suggests direct detection by MMR of 5-fluorouracil lesions in DNA and a direct signaling from MMR to the cell-cycle machinery (170).

After incorporation into DNA, 6-thioguanine can be chemically methylated by *S*-adenosylmethionine to form S^6-methylthioguanine. During DNA replication, S^6-methylthioguanine can pair with cytosine as well as with thymine, and the resultant S^6-

methylthioguanine–thymine pairs are also identified by an efficient MMR system as replication errors. MMR recognizes the mismatches and removes drug-induced DNA lesions only in the daughter strand, not in the parental one, leading to repetitive strand breaks causing G_2 cell-cycle arrest and hence cell death (263). If the detector function of the MMR system is disabled, cytotoxicity is markedly reduced, although adducts may persist in the DNA. The MMR-deficient cells, which have inactivating mutations in the MMR genes, are five-fold to 10-fold more resistant to 6-thioguanine (19).

2. Alkylating agents. *N*-methyl-*N*-nitrosourea (MNU), *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG), procarbazine, and temozolomide (an activated form of procarbazine) belong to a class of alkylating agents that carry out their biologic effects, cell-cycle arrest and apoptosis, principally by methylating the O^6 position of guanine to form O^6-methylguanine (O^6-meG). O^6-meG does not form a good match with either cytosine or thymine, and this is considered the initiating event in carcinogenesis by methylating agents. The first defense against such lesions is the MGMT activity, which repairs O^6-methylguanine to guanine. In the absence of MGMT, O^6-meG cytotoxicity is mediated mainly by the MMR system *via* the recognition of O^6-meG:C and O^6-meG:T mismatches, the latter originating from misincorporation of thymine opposite O^6-meG during DNA synthesis. Because no perfect complementary match for O^6-meG exists on the template strand, competent MMR cells make futile abortive repair attempts, and the persistent damage creates breaks in the newly synthesized strand, causing cell death. The inactivation of MGMT by a potent inhibitor, O^6-benzylguanine, sensitizes cells to killing by temozolomide only in normal MMR cell lines, whereas in MMR-deficient cell lines, methylation damage accumulates but does not trigger cell death. Transfer of the *MLH1* gene into HCT116 cells (human colorectal adenocarcinoma cell line MLH1-deficient) corrects the MMR defect, reverses the mutator phenotype, and sensitizes the cells to the methylating agent, indicating that the restoration of functional MMR abolishes methylation tolerance (76).

Mitomycin C and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) are agents more reactive against MMR-deficient cells (246). Whereas MMR-proficient cells retain the ability to remove some clastogenic DNA lesions, caused by mitomycin C and CCNU, MMR-deficient cells, rather than showing classic methylation tolerance, are generally more sensitive to killing by these drugs, probably because of an increased level of chromosomal damage responsible for the initiation of the cell-death pathway.

3. Platinum compounds. Cisplatin and carboplatin, by virtue of their high reactivity, are capable of covalent binding to DNA bases and can form platinum–DNA adducts including intrastrand and interstrand dipurinyl-crosslinks, DNA–protein crosslinks, and monoadducts with purines. The most abundant lesions produced in DNA are intrastrand crosslinks between the N^7 atoms of adjacent purines, with 65% of adducts in GpG sequences, 25% in ApG sequences, and 6% in GpNpG sequences (198). The cisplatin adducts arrest the progress of DNA polymerases, and in their presence, eukaryotic cells respond by activating signal-transduction pathways that result in cell-cycle arrest and apoptosis. The cellular defense against platinating agents is the removal of platinum–DNA adducts by the NER pathway, and thus an increase in the efficacy of NER may reduce the sensitivity of tumors to cisplatin (198).

A number of different observations have suggested that MMR is also involved in the toxicity of cisplatin (76, 128). Purified human MSH2 protein, either alone or in complex with MSH6, can bind to oligonucleotides carrying cisplatin adducts. Most probably the MMR complex recognizes the adduct in the template strand and attempts to repair the newly synthesized nonadducted strand. As long as the adduct persists, insertion of new bases in the nonadducted strand fails to resolve the apparent mismatch, resulting in the generation of a signal that normally causes apoptosis.

Drug resistance is thought to result from failure of the cell to recognize cisplatin adducts and to activate signaling pathways that trigger apoptosis. When the MMR system is defective or disabled, this signal is presumably not generated, and the cell seems to be resistant to the drugs. Tumor cell lines defective for either *MLH1* or *MSH2* showed a twofold resistance to cisplatin and carboplatin compared with the corresponding MMR-proficient cells.

Oxaliplatin is a novel antineoplastic platinum derivative and is more potent than cisplatin *in vitro*, requiring a smaller amount of DNA adducts to achieve an equal level of cytotoxicity (202). It has shown efficacy in many tumor cell lines resistant to cisplatin and carboplatin. When combined with 5-fluorouracil and leucovirin, oxaliplatin is among the most effective chemotherapies for metastatic colorectal cancer. Oxaliplatin shows the same cytotoxicity both in MMR-proficient and MMR-deficient tumor cell lines, suggesting that its adducts are not recognized by the MMR system (218). Thus, components of MMR are able to discriminate closely related DNA adducts generated by oxaliplatin and other platinum-containing analogues that do not have substitutions on the amine groups. This may be the result of a difference in the distortion introduced in DNA by oxaliplatin, or it could be due to a steric hindrance in the binding of MSH2/MSH6 to the diaminocyclohexane ring present in oxaliplatin adducts. Because the loss of MMR does not confer resistance to oxaliplatin, it might be recommended for use in tumors with a deficient MMR system.

4. Topoisomerase inhibitors. Topoisomerases are nuclear enzymes that catalyze the relaxation of supercoiled DNA through the transient cleavage of DNA strands and the formation of a covalently bound protein–DNA intermediate, followed by religation of the cleaved DNA and dissociation of the protein. Because topoisomerases play an essential role in many fundamental cellular processes, such as replication, transcription, repair, recombination, and chromosome segregation, they have been the targets for many anticancer drugs (93, 118). Camptothecin (CPT) and etoposide (ETP) can convert topoisomerases into a cell poison by blocking the religation step, thereby enhancing the formation of persistent DNA breaks responsible for cell death (118).

Topoisomerase I, the cellular target of CPT, cleaves only one strand of DNA; thus, stabilization of topoisomerase I–containing cleavage complexes primarily creates SSBs. The toxicity of CPT is mainly due to the conversion of SSBs to DSBs during S phase. The SSBs induced by topoisomerase I are considered

nontoxic to the cells because they can be efficiently and rapidly repaired. However, their conversion into DSBs, formed when a moving replication fork encounters the cleaved complex, generates potentially lethal lesions. Because DSBs are lethal lesions if not repaired before mitosis, a defect in the G_2 checkpoint in response to CPT-induced damage may also contribute to increase CTP cytotoxicity. CPT-11 (a semisynthetic water-soluble CPT derivative) has received approval by the Food and Drug Administration (FDA) for use in 5-fluorouracil–refractory stage IV colorectal cancer. CPT-11 is metabolized to an active compound, 7-ethyl-10-hydroxycamptothecin β-glucuronide, by a carboxylesterase.

Topoisomerase II acts as a dimer and catalyzes the cleavage of both strands of DNA. By selectively targeting DNA-bound topoisomerase II, ETP lengthens the cleavage complex half-life, which increases the number of double-strand breaks (82).

MMR deficiency is linked to a marked increase in sensitivity to both CPT and ETP drugs, and a defect in either *MSH2* or *MLH1*, the two key components of MMR, most severely affects the cellular resistance to either drug. Although endogenous topoisomerase I levels could not predict the cellular sensitivity to CPT, a molecular profile encompassing p53 wild-type and MMR deficiency is a critical determinant for the chemosensitivity of colorectal cell lines and is predictive of a complete response to treatment with CPT-11, 5-fluorouracil, and radiation (37).

VI. CONCLUSIONS

The relevance of DNA-repair pathways to cancer development and treatment has been ascertained by numerous studies. Individuals with deficiencies in DNA repair have an increased cancer incidence, as observed for the rare syndrome xeroderma pigmentosum and the more common diseases, early-onset breast cancer and hereditary nonpolyposis colon cancer. Moreover, most cancer cells have acquired several mutations in key regulatory genes, such as those involved in cell-growth control, apoptosis, and DNA-damage response, and therefore genomic instability phenotypes can occur from acquired defects in these pathways. Basic mechanisms for DNA repair are now well established, but recent research has revealed an unexpected complexity, with overlapping specificity within the same pathway, as well as extensive functional interaction between proteins involved in repair pathways. The emerging knowledge of mutations and polymorphisms in key human DNA-repair genes may provide a rational basis for improved strategies for interventions into some related tumors and degenerative disorders (4, 161, 192).

The development of biomarkers for the function of DNA-repair pathways may result in better targeting of conventional agents or the use of monotherapies designed to inhibit specific repair pathways. Human terminal transferases appear to be promising targets for anticancer chemotherapy, because of their pleiotropic roles in generating DNA sequence variability at specific DNA target sites, as well as having a more general function during error-prone NHEJ repair of DSBs (57). PARP inhibitors are another promising tool in cancer therapy (119). PARP1 is involved in both SSB and DSB repair, and it has been observed that cancer cells defective in HR genes may be highly sensitive to inhibition of PARP1/2, whereas cells lacking Ku70 could display an increase in resistance (109). Recently, a chemotherapeutic selectivity conferred by selenium was reported (78). Experiments have shown that a selenium-inducible DNA-repair response protects cells from DNA damage and elevates DNA-repair capacity. This effect seems to be p53 dependent, as selenium treatment did not protect or increase DNA repair in p53-deficient cells, and it provides an exciting insight into the future of anticancer development.

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ABBREVIATIONS

6-4PPs, pyrimidine-(6-4)-pyrimidone photoproducts; 8-oxoA, 8-oxo-7,8-dihydroadenine; 8-oxoG, 8-oxo-7,8-dihydroguanine; AD, Alzheimer disease; AGT, O^6-alkylguanine-DNA methyltransferase; AP, apurinic/apyrimidinic or abasic; AT, ataxia–telangiectasia; ATLD, ataxia–telangiectasia-like disorder; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia Rad3-related; BER, base excision repair; BS, Bloom syndrome; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; CID, combined immunodeficiency; cisplatin, *cis*-diamminedichloro-platinum; CPDs, cyclobutane pyrimidine dimers; CPT, camptothecin; CS, Cockayne syndrome; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; dRP, 2-deoxyribose 5-phosphate; DSB, double-strand break; ERCC, excision cross-complementing protein; ETP, etoposide; FA, Fanconi anemia; FAPγA, 4,6-diamino-5-formamidopyrimidine; FAPγG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; FEN1, flap endonuclease 1; GG-NER, global genome repair; HNPCC, hereditary nonpolyposis colorectal cancer; HR, homologous recombination; ICL, intrastrand crosslink; IDL, insertion/deletion loop; LIG1, DNA ligase 1; LIG3, DNA ligase 3; LIG4, DNA ligase IV; LP-BER, long-patch BER; MGMT, O^6-methylguanine–DNA methyltransferase; MMC, mitomycin C; MMR, mismatch repair; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea; MSI, microsatellite instability; NBS, Nijmegen breakage syndrome; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; O6-meG, O^6-methylguanine; OS, Omenn syndrome; PCNA, proliferating cell nuclear antigen; PI3K, phosphatidylinositol 3-kinase; PIKK, PI3K-related protein kinases; PNK, polynucleotide kinase/phosphatase; POLβ, DNA polymerase β; POLδ, DNA polymerase δ; POLε, DNA polymerase ε; POLμ, DNA polymerase μ; POLλ, DNA polymerase λ; RFC, replication factor C; ROS, reactive oxygen species; RPA, replication protein A; RS-SCID, radiosensitive SCID; RTS, Rothmund–Thompson syndrome; SCAN1, spinocerebellar ataxia with axonal neuropathy-1; SCID, severe combined immunodeficiency; SNP, single-nucleotide polymorphism; SP-BER,

short-patch BER; SSB, single-strand break; TdT, terminal deoxynucleotidyl transferase; TC-NER, transcription-coupled repair; TDP1, tyrosyl-DNA phosphodiesterase 1; TFIIH, transcription factor IIH complex; TLS, translesional DNA synthesis; TTD, trichothiodystrophy; WS, Werner syndrome; XP, xeroderma pigmentosum; XRCC, x-ray repair cross-complementation group; γH2AX, phosphorylated histone H2AX.

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