The Involvement of DNA-Damage and -Repair Defects in Neurological Dysfunction

Avanti Kulkarni[1] and David M. Wilson III[1,*]

A genetic link between defects in DNA repair and neurological abnormalities has been well established through studies of inherited disorders such as ataxia telangiectasia and xeroderma pigmentosum. In this review, we present a comprehensive summary of the major types of DNA damage, the molecular pathways that function in their repair, and the connection between defective DNA-repair responses and specific neurological disease. Particular attention is given to describing the nature of the repair defect and its relationship to the manifestation of the associated neurological dysfunction. Finally, the review touches upon the role of oxidative stress, a leading precursor to DNA damage, in the development of certain neurodegenerative pathologies, such as Alzheimer's and Parkinson's.

Introduction

DNA damage is an underlying cause of cellular dysfunction and death, carcinogenesis, and the aging process. Every day, cells' genomes endure on average approximately one million lesions, including bulky helix-distorting adducts and modifications or fragmentation of the sugar phosphate backbone. Left unrepaired, DNA damage can drive mutagenesis (e.g., base substitutions, transitions, transversions, frameshifts or chromosomal translocations), disrupt normal gene expression, or create aberrant protein products that are detrimental to cellular function or viability. To cope with such damage, cells are equipped with a myriad of DNA-repair enzymes and pathways. Defects in one or more of the essential components of these pathways can lead to the propagation of genomic instability. In replicating cells, such instability can result in apoptosis (a mechanism for removing abnormal cells) or cellular transformation (a key step in the development of cancer). In nonreplicating terminally differentiated cells, such as neuronal tissue, the loss of genomic integrity often leads to cell death via apoptosis and, consequently, to tissue degeneration and atrophy. In recent years, increasing attention has been paid to the role that DNA damage and repair play in neurological disease.

Several neurodegenerative disorders are directly or indirectly linked to defects in DNA single-strand break (SSB) repair (SSBR) or double-strand break (DSB) repair (DSBR). A number of these disorders adversely affect the operation of the cerebellum, which is the part of the brain responsible for coordination of muscular movement and for the maintenance of posture and balance. In addition, the cerebellum coordinates non-motor functions such as cognition and emotions. Given the central role the cerebellum plays in motor-sensory perception, it follows that damage to this part of the brain manifests itself in the form of in-coordination of speech, thought, and movement; collectively, such damage is termed ataxia. In this review, we will summarize the neurological abnormalities related to SSBR, DSBR, and nucleotide excision repair (NER) defects. Given that the road to genomic stability and cell viability is so intricately linked with DNA damage and repair, we begin with a comment on the predominant sources of DNA damage, the lesions that result, and the repair pathways that function in their removal.

Sources of DNA Damage

DNA damage can be classified into two types: endogenous and exogenous. As defined herein, endogenous damage is that caused by agents within the cell itself (i.e., the products of normal cellular metabolism). Exogenous damage, on the other hand, refers to damage caused by physical or chemical agents in the environment. Spontaneous endogenous DNA damage typically occurs at a higher frequency than exogenous damage, but in developed societies, exogenous factors from accidental or involuntary exposures contribute to 75%–80% of cancer cases.[1–3] Notably, both modes of damage can result in similar types of DNA lesions and genetic outcomes. It is important to emphasize that in most cases there is no direct evidence linking exogenous damage (e.g., that induced by external radiation) to neurological disease, and thus, endogenous damage is considered most relevant in terms of disease manifestation. However, for the sake of completeness, outlined below are the principal agents that contribute to endogenous and exogenous damage and some of the main lesions they create. Collectively, these sources generate a high number of DNA modifications (see Table 1), which if left unrepaired can be detrimental to the cell and, ultimately, to the organism.

1. Endogenous Damage

Reactive Oxygen Species. Reactive oxygen species (ROS), which include species such as singlet oxygen atoms, hydroxyl radicals, superoxide radicals, and hydrogen peroxide, are generated as by-products of normal mitochondrial respiration,[4] during conditions of chronic inflammation,[5,6] or as discussed below, upon exposure to certain

[1]Laboratory of Molecular Gerontology, National Institute of Aging, National Institutes of Health, Baltimore, MD 21224, USA
*Correspondence: wilsonda@grc.nia.nih.gov
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Table 1. Endogenous DNA Damage

Source of Damage	Mechanism of Formation[a]	Type of Damage	Lesions[b]	Measured Levels of Damage or Rates of Formation	Tissue	Reference
ROS (reactive oxygen species)	mitochondrial respiration; glucose metabolism; estrogen metabolism; chronic inflammation; muscle contraction; vascular function; neurotransmission	oxidized bases and phosphodiester backbone products	5′-hydroxy-2′-dC 5′-hydroxy-2′-dU	$1.02/10^6$ nt $0.69/10^6$ nt	leukocytes	[29]
RCS (reactive carbonyl species)	glycation; oxidation of sugars; breakdown of lipid hydroperoxides	bulky base adducts; cross links; base deamination products	MG-3′-dGMP (3-(2′-deoxy-β-D-erythro-pentafuranosyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo[2,3-*b*]purine-9(8*H*)one)	$0.26/10^6$ nt	buccal epithelial cells	[28,24]
			malonaldehyde-dG	5400/cell	liver	
alkylators	choline metabolism; S-adenosyl methionine (SAM)	alkylated bases	O^6 methyl guanine	$0.022–0.13/10^6$ nt	liver	[25,27]
			O^4 alkylthymidines	$0.003–4.24/10^6$ nt	liver	
			7-alkylguanines	$1.31/10^6$ nt	leucocytes	
catechol estrogens	steroid metabolism	bulky depurinating adducts; oxidized bases	4-hydroxy catechol estradiol-1(α,β)-N7 guanine	1.4–2.3 μmol/mol DNA phosphate	rat mammary gland	[14]
aldehydes	lipid peroxidation; glycation; estrogen metablosim; prostaglandin biosynthesis	bulky base adducts	MIG (dG pyrimido[1,2-α]purin-10(3*H*)-one)	$0.14/10^6$ nt	liver	[30,10]
			HNE-dG (4-hydroxynonenal-dG)	$0.0006–0.0018/10^6$ nt		
spontaneous hydrolytic deamination	enhanced by thermal fluctuations	modified bases	modified bases uracil, hypoxanthine, xanthine	100–500 cytosine-uracil/cell/day	in vitro determination on isolated DNA as a function of pH and temperature	[22]
spontaneous depurination	enhanced by thermal fluctuations or base modification	sites of base loss	abasic sites	$\sim 4/10^6$ nt	lymphoblastoid cell line H2EI	[26]
				$8–9/10^6$ nt	liver	

[a] Represent some of the primary endogenous pathways and processes that generate the damaging agent indicated.
[b] Representative lesions. For a more comprehensive list of lesions and their frequencies, refer to the review by Rinne De Bont et al.[1]

environmental agents. These reactive chemicals are usually neutralized by cellular defense systems, which include ROS-scavenging enzymes (superoxide dismutase and catalase), antioxidant enzymes (glutathione reductase and glutathione peroxidase) and vitamins (E and C).[7] However, in situations when the generation of ROS exceeds the cell's antioxidant capacity (i.e., during conditions of oxidative stress), ROS amass and attack macromolecules such as lipids, proteins, and DNA. In the case of DNA, ROS react with both purines and pyrimidines to generate modified bases such as 8-oxoguanine (8-oxo-dG), 5-hydroxyuracil, uracil glycol, 5-hydroxymethylthymidine, 5-hydroxymethyluracil, cytosine glycol, 2,6-diamino-4-oxy-5-formamidopyrimidine (FAPY) adenine, FAPY guanine, and thymine glycol, to name a few.[1] Other frequent lesions arising from ROS attack of the sugar phosphate backbone include abasic sites and DNA strand breaks. Such lesions, as will be discussed later, are also products of enzymatic processing of oxidized bases during repair.[8,9]

Aldehydes, Estrogens, and Reactive Carbonyl Species. ROS-mediated lipid peroxidation, glucose metabolism, and estrogen metabolism are some of the processes that lead to the production of reactive aldehydes. The aldehydic by-products of lipid peroxidation, i.e., crotonaldehyde, acrolein, 4-hydroxynonenal (HNE), and malondialdehyde (MDA), form exocyclic ethano and propane base adducts in DNA that can block base pairing and are highly mutagenic.[10,11] Oxidation of sugars, such as glucose, and sugar metabolism itself creates reactive carbonyl species, e.g., glyoxal and methylglyoxal, which can produce carcinogenic base adducts such as glyoxalated deoxycytidine.[12,13] Estrogen metabolism generates metabolites such as catechol estrogens (CEs). If incompletely inactivated by endogenous processes, such metabolites can undergo oxidation

to form quinones and semiquinones (CE-Q and CE-SQ, respectively). The redox cycling of quinone moieties can generate additional ROS.[14,15] CE-Q moieties are also capable of directly attacking DNA to create bulky depurinating adducts (e.g., estrogen-DNA adducts and estradiol-induced MDA adducts).

Alkylators. Another class of endogenous DNA-damaging agents includes alkylators, such as nitrosated bile salts, which are formed by bile acids that act upon N-nitroso compounds (found in nitrite-treated meat products or food),[16] and S-adenosyl methionine (SAM). SAM is a natural reactive methyl-group donor for a variety of enzymatic reactions involving a range of acceptor molecules.[17,18] SAM can inappropriately alkylate DNA bases to generate products such as 7-methylguanine, 3-methyladenine, and N^7-carboxymethylguanine, which can be mutagenic or interfere with DNA replication or transcription.[19,20] On the basis of in vitro studies, it has been estimated that SAM-mediated alkylation accounts for approximately 4000 7-methylguanine, 600 3-methyladenine, and 10–30 O^6-methylguanine residues per mammalian genome per day. Abasic sites are also prominent intermediates of alkylation base damage; these result from enzymatic base removal during repair (see below) or from spontaneous depurination of the hyper-labile glycosidic bond between the modified base and the sugar moiety.[21–23]

Intrinsic Instability of DNA. In addition to being under constant attack from endogenous sources, the chemical structure of DNA is susceptible to spontaneous decay. For instance, pyrimidines and purines endure spontaneous hydrolytic deamination; cytosines and 5-methylcytosines are particularly susceptible (100–500 cytosines are spontaneously deaminated/cell/day to form uracil).[22] Purine deamination is less frequent; adenine is deaminated to hypoxanthine and guanine to xanthine at about 2%–3% the rate of cytosine deamination.[1,22] Such deamination events eventually lead to the creation of abasic sites after the enzymatic removal of the nonconventional base moiety via a repair response (see below). Apurinic/apyrimidinic (AP) sites are also frequent products of spontaneous hydrolysis of the N-glycosidic bond that links the base to the sugar phosphate backbone; such hydrolysis arises an estimated 10,000 times per human genome per day under normal physiological conditions.[22] An overview of the main sources of endogenous DNA damage, examples of the lesions produced, and the measured frequencies of occurrence are presented in Table 1.[1,10,14,22,24–30]

2. Exogenous Damage

Physical agents. Ionizing and non-ionizing radiation is the most prominent environmental physical agent that damages DNA. Exposure to radiation can occur from both natural (cosmic rays from the sun, radioactive elements in the environment, atmosphere, soil, plants and trees) and artificial (diagnostic X-rays, smoke detectors) sources. Most ionizing radiation (IR)-induced DNA damage results from reactions with hydroxyl radicals that are generated from radiolysis of water, although some damage arises from direct ionization of nucleic acid. IR-dependent DNA lesions include "simple" oxidative damage, such as modified bases, SSBs or abasic sites, and more "complex" clustered lesions (defined as those damages within a helical turn of one another) or DSBs.[31,32] Non-IR-induced DNA damage primarily involves ultraviolet (UV) radiation from the sun. The ring-structure content and the conjugated bonds in DNA bases make them prominent absorbers of both short- and long-wave UV radiation. The most important targets of UV light are adjacent pyrimidines that dimerize to form helix-distorting photoproducts, e.g., thymidine dimers.[33,34] Again, we note that radiation-induced DNA damage is not considered a major factor in the manifestation of neurological disease.

Chemical Agents. An array of genotoxic compounds are found within the environment in air, soil, water, and food. These include polluting heavy metals such as mercury, arsenic, and cadmium, which can produce ROS that create base modifications or SSBs;[35] food-based carcinogens such as aflatoxin, found in oilseeds, nuts, and cereals that react with DNA bases to form bulky adducts;[36] and air pollutants such as radon, air-borne fine particulate matter, and secondhand smoke, which can produce both oxidative and alkylative stress.[37] Exposure to these and other environmental hazards has been linked epidemiologically to increased incidence of cancer and, in some cases, neurological defects.[35,38–41] In addition to inadvertent environmental exposure, occupational exposure to DNA-damaging agents such as mutagenic chemicals and drugs can occur in certain industrial, laboratory, and clinical settings. Finally, crosslinking agents such as cisplatin and base analogs such as 5′-fluoro-deoxyuridine and 5′-fluorouracil are routinely used in chemotherapy to "selectively" induce DNA damage and apoptosis in rapidly dividing cancer cells.[42–44]

Mechanisms of DNA Repair

As is evident from the above information, endogenous and exogenous DNA-damaging agents can create modified bases, abasic sites, SSBs, helix-distorting adducts, intra- and inter-strand crosslinks, and/or DSBs. If left unrepaired, these lesions can cause base transitions, transversions, frameshift mutations, or gross chromosomal aberrations and can induce cell death. Cells are equipped with enzymes that recognize and remove damaged bases or more complex DNA lesions to prevent mutagenesis and the cellular dysfunction that underlie cancer, neurodegeneration, and other disease states. Discussed next are the major cellular repair pathways and the related enzymatic machinery that operate to correct certain forms of DNA damage.

1. Excision Repair and SSB Processing

Three major pathways function in the removal of modified bases, mismatches, and bulky adducts. These include base excision repair (BER), mismatch repair (MMR), and NER, respectively. Each of these processes proceeds via (1)

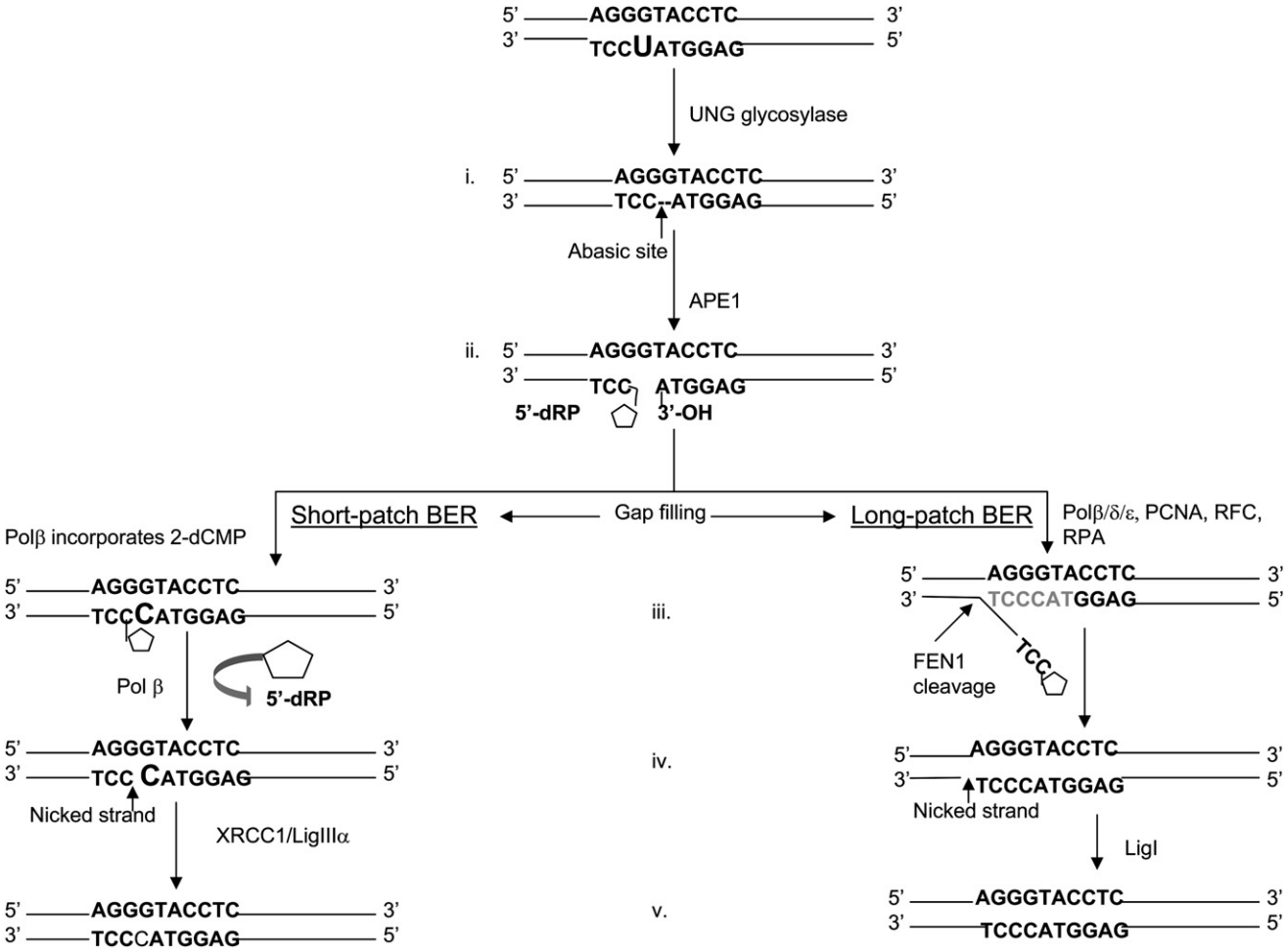

Figure 1. Base Excision Repair

(i) Recognition and removal of a modified base by a DNA glycosylase, leaving behind an abasic site (shown is removal of deaminated cytosine (uracil) by UNG).

(ii) Cleavage at the abasic site by APE1, creating a SSB with a 5′ dRP and 3′ hydroxyl (OH) end.

(iii) Gap filling at the strand break via either short-patch (left) or long-patch repair synthesis (right). In short-patch base excision repair (BER), Pol β replaces the missing nucleotide, whereas in long-patch BER, Pol β, δ, or ε incorporates 2–10 nucleotides via strand displacement (newly synthesized sequence in gray). PCNA and RPA assist in the process.

(iv) Excision of the 5′ dRP to create ligatable ends is performed by the lyase activity of Pol β (short-patch BER) or the flap endonuclease activity of FEN1 (long-patch BER).

(v) The final nick is sealed by Ligase IIIα in complex with XRCC1 (short patch BER) or by Ligase I (long patch BER) to regenerate the intact strand.

removal of the substrate base, (2) formation of a SSB gap at the excision site, and (3) re-establishment of the native DNA content by pathway-specific polymerases and ligases. Because all three repair mechanisms proceed via a SSB intermediate, we discuss them together.

BER. BER functions during all stages of the cell cycle to repair non-helix-distorting base modifications, abasic sites, and various types of SSBs. Typically, the first step in BER is recognition and removal of an inappropriate base, such as one created by ROS attack, alkylation, spontaneous hydrolysis (e.g., deamination of cytosine to uracil), or nucleotide misincorporation (i.e., certain mismatches). Such removal is performed by a class of enzymes called DNA glycosylases (Figure 1). These proteins hydrolyze the N-glycosidic bond to release the substrate base and create an abasic-site intermediate.[21,45,46] Glycosylases, such as uracil DNA glycosylase (UNG [MIM 191525]) and 8-oxo-dG DNA glycosylase (OGG1 [MIM 601982]), generally target specific kinds of base damage, although these enzymes as a whole maintain an overlapping complement of base substrates.[46–50]

Once an AP site has been generated, some DNA glycosylases (e.g., OGG1) possess an AP lyase activity that cleaves 3′ to the abasic residue to produce a SSB with a normal 5′ phosphate group and an atypical 3′ α, β unsaturated aldehyde.[51] More commonly, however, the AP site is incised by the action of a hydrolytic endonuclease, specifically the major mammalian AP endonuclease APE1 (APEX [MIM 107748]) (also called Hap1 or Ref1) (Figure 1). Such

enzymes incise the phosphodiester bond 5′ to an abasic lesion and create a SSB with an abnormal 5′–2′-deoxyribose phosphate (dRP) residue and a conventional 3′-OH terminus that can be extended by the major gap-filling polymerase, DNA polymerase β (Pol β) (POLB [MIM 174760]).[52] APE1 also functions to remove the 3′ α,β-unsaturated aldehydic blocking groups left behind by bifunctional DNA glycosylases (see above) to create 3′-OH priming ends.

After strand breakage, and appropriate 3′-terminal clean up if needed, BER proceeds via either a short-patch or a long-patch repair reaction. In short-patch BER (Figure 1, left), DNA Pol β adds a single nucleotide and removes the 5′-dRP group, and DNA Ligase IIIα (LIG3 [MIM 600940]) (in complex with the X-ray cross-complementing 1 (XRCC1 [MIM 194360]) protein) seals the nick to complete the process.[51–58] In situations where the 5′ terminus is not a substrate for Pol β, such as with certain oxidized or reduced AP-site fragments,[59,60] long-patch BER takes place to promote strand displacement and synthesize 2–10 nucleotides (Figure 1, right). This repair reaction is performed by Pol β or Pol δ/ε (POLD1 [MIM 174761])/POLE [MIM 174762]) in concert with the 5′-flap endonuclease (FEN1 [MIM 600393]), proliferating cell nuclear antigen (PCNA [MIM 176740]) and DNA Ligase I (LIG1 [MIM 126391]).[54,58,61–63]

Recent studies on BER have shown that certain 5′ or 3′ obstructive termini at SSBs are prepared for Pol β polymerization and/or nick ligation by proteins such as polynucleotide kinase/phosphatase (PNKP [MIM 605610]),[64] Aprataxin (APTX [MIM 606350]),[65,66] and tyrosyl-DNA phosphodiesterase (TDP1 [MIM 607198])[67,68] (see more below). Finally, a critical component of BER and its sub-pathway SSBR is the scaffold protein XRCC1, which recruits several key enzymatic factors, including Pol β, Ligase IIIα, TDP1, and Aprataxin, to the site of the damage.[68–75] Figure 1 summarizes the major steps of the BER pathway.

MMR. MMR functions to remove mismatches and small insertions or deletions that arise as replication errors or during recombination.[76] Mammalian MMR is initiated by MutSα (comprised of MSH2 [MIM 609309] and MSH6 [MIM 600678]) or by MutSβ (a MSH2-MSH3 [MIM 600887] heterodimer), which recognize single base mismatches and 1–2 nucleotide insertion-deletion (ID) mismatches (MutSα) and 2–10 nucleotide IDs (MutSβ).[76,77] MutLα (MLH1 [MIM 120436]:PMS2 [MIM 600259] heterodimer) is then recruited and apparently serves to coordinate other proteins, such as PCNA, at the damage site.[78,79] Subsequent steps of MMR are facilitated by the concerted actions of PCNA (strand discrimination), Exo1 (MIM 606063) (excision), replication protein A (RPA2 [MIM 179836]), Pol δ, and a DNA ligase.[76] Because none of the components of MMR have been conclusively linked to neurodegenerative disease to date, this repair system will not be discussed in further detail. For a comprehensive review of MMR, the reader is directed to reviews by Buermeyer et al. and Modrich.[76,80]

NER. NER functions largely independently of the cell cycle to remove bulky DNA adducts, such as UV-induced cyclobutane pyrimidine dimers, DNA crosslinks, and certain oxidative base modifications, and is divided into two sub-pathways: global genome repair (GGR) and transcription-coupled repair (TCR) (Figure 2). As the names suggest, GGR deals with lesions present throughout the genome, whereas TCR handles DNA damage that blocks RNA polymerase elongation during gene transcription. As described in greater detail later, many of the key factors operating in NER have been associated with the cancer-prone disorder xeroderma pigmentosum (XP) and the segmental progeria cockayne syndrome (CS), which both display elements of neurological dysfunction in a sub-set of individuals.

GGR (Figure 2, left) is initiated by recognition of the helix-distorting adduct by an XPC (MIM 278720)-HR23B (MIM 600063) complex.[81] This is followed by recruitment of XPA (MIM 611153), RPA, and the other components of the general transcription factor TFIIH to the lesion site. This multi-subunit assembly establishes the pre-incision complex.[82–85] TFIIH is comprised of two helicases, XPB (MIM 610651) and XPD (MIM 278730), Cdk-activating kinases (Cdk7 [MIM 601955] and cyclin H [MIM 601953]/Mat1), and DNA-binding proteins (p34 and p44), all of which serve to unwind the duplex at the lesion site and create a small bubble structure.[83] Unwinding is followed by dual incision on either side of the damage by XPG (MIM 278780) (incision at the 3′ bubble-duplex junction) and the XPF (MIM 278760)-ERCC1 (MIM 126380) complex (incision 5′) to release a short single-stranded DNA segment (27–39 nucleotides) that contains the lesion.[86–89] Repair synthesis at the resulting gap is carried out by Pol δ/ε in coordination with RPA/PCNA/RFC (replication factor C) and is followed by DNA Ligase I-mediated nick ligation.[58,83,90] Recent evidence also implicates XRCC1-Ligase IIIα in the sealing of nicks during NER, particularly in quiescent cells.[91]

With the exception of the initiating mechanism, TCR proceeds in a manner similar to GGR and involves many of the same protein components.[92] Damage recognition and processing in TCR is initiated when an RNA polymerase stalls at a lesion site and thus initiates recruitment of CS proteins CSA (MIM 216400) and CSB (MIM 133540). The exact function of CSA is not known, but it is reported to translocate to the nuclear matrix in a CSB-dependent manner after UV irradiation or exposure to hydrogen peroxide. This translocation is independent of XPA and XPC, indicating that it is specific to TCR.[93,94] CSA is reportedly linked to a ubiquitin ligase complex and might play a role in ubiquitylation of the RNA polymerase during TCR by tagging the polymerase for degradation to allow for unimpeded repair. Recent evidence also suggests that CSB might be a substrate for the ubiquitin ligase complex and that it is degraded in a CSA-dependent manner during a late stage of the repair response, presumably to permit resumption of transcription.[95,96] CSB is a member of the SWI2/SNF2 family and has DNA-dependent ATPase and ATP-dependent chromatin remodeling activities. It is part

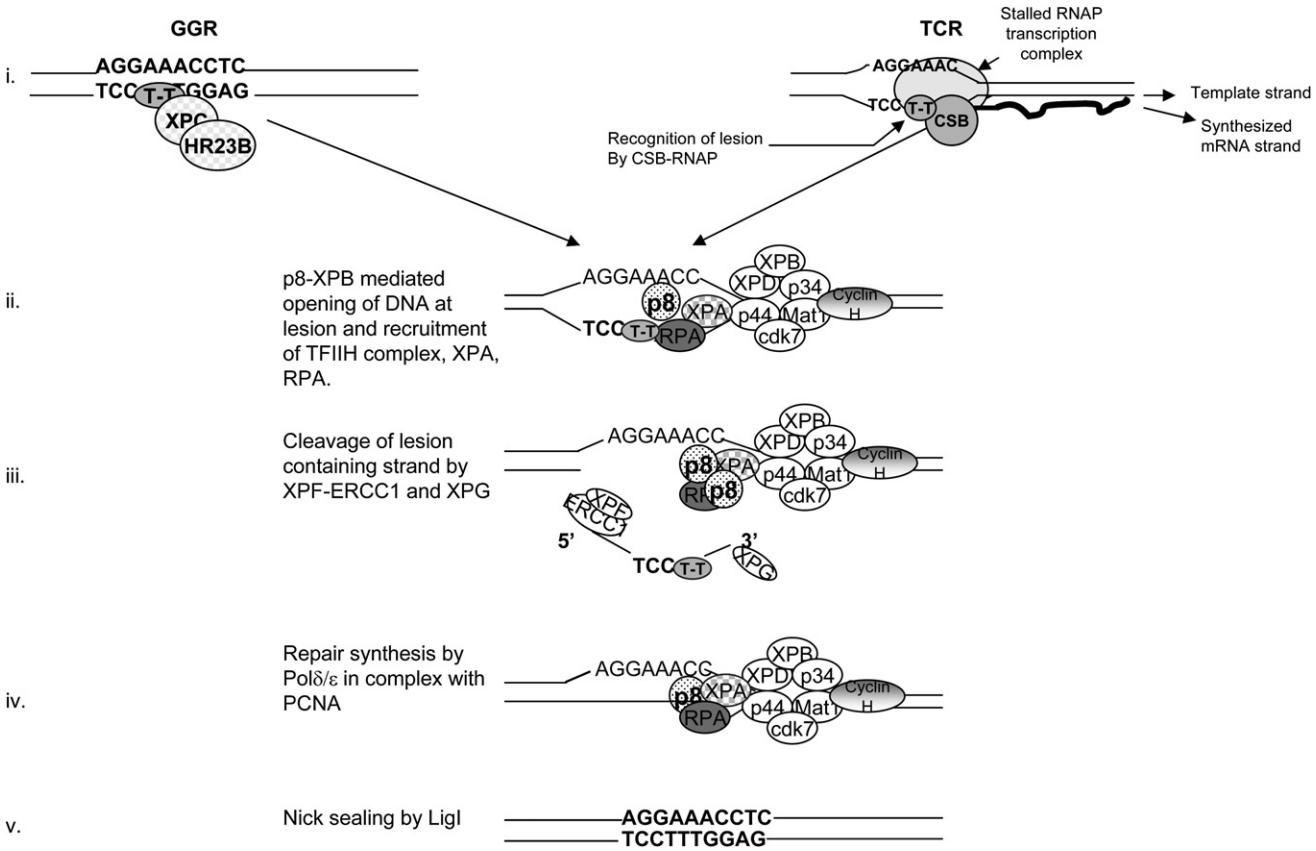

Figure 2. Nucleotide Excision Repair

(i) Recognition and removal of helix-distorting adducts (e.g., thymine dimer, shown) is mediated by the XPC-HR23B complex in global genomic repair (left) or by a stalled RNAP II-CSB complex during transcription-coupled repair (right). Subsequent repair steps are similar for both GGR and TCR.

(ii) XPA, RPA, and the TFIIH complex are recruited to the damage site after p8 stimulation of XPB ATPase and XPB-mediated unwinding; XPB–XPD unwind DNA to create a bubble.

(iii) ERCC1-XPF and XPG are then recruited and incise 5′ and 3′, respectively, to the bubble junction, releasing an approximately 30 nucleotide stretch of DNA bearing the lesion.

(iv) Repair synthesis is carried out by the PCNA-dependent Pol δ/ε.

(v) The remaining nick is sealed by Ligase I or the XRCC1-Ligase IIIα complex (not shown).

of the RNA polymerase II (RNAPII) transcription machinery and recruits various NER components to the stalled RNAPII during TCR, although its precise biochemical function in the process remains unclear.[97–99] CSA and CSB have been speculated to play a role in the removal of oxidative lesions during TCR as well; however, this is currently controversial. More recent evidence implicates these proteins in global repair of oxidative DNA damage.[100–102] The detailed biochemistry of the early steps of TCR are presently being refined, but for a more complete perspective refer to De Laat et al., Mellon et al., and Van Hoffen et al.[83,92,103]

2. DSBR

DNA DSBs take on two forms: two-ended breaks, generated primarily by direct attack on DNA by a physical or chemical mutagen such as IR, or one-ended breaks, created upon replication-fork collapse at sites of DNA damage (e.g., SSBs). Three major pathways function to repair two-ended DSBs: homologous recombination (HR), which entails faithful exchange between regions of homology on sister chromatids and engages the DNA-replication machinery; single-strand annealing (SSA), which involves annealing between complementary single-stranded segments of the same chromosome on either side of the DSB; and nonhomologous end-joining (NHEJ), which involves direct ligation of processed DSB ends. The one-ended, replication-derived DSBs appear to be resolved strictly by classical HR involving sister chromatids. Discussed next are the mechanistic details of these pathways. It is important to keep in mind that the process of DSBR involves dynamic interplay between phases of recognition, cell signaling (the response), and finally, break resolution.

HR and SSA. The process of HR involves exchange of genetic information between complementary regions of two homologous chromosomes (typically sister chromatids) that are aligned for crossover (Figure 3A). It is largely an error-free form of repair and hence integral to the

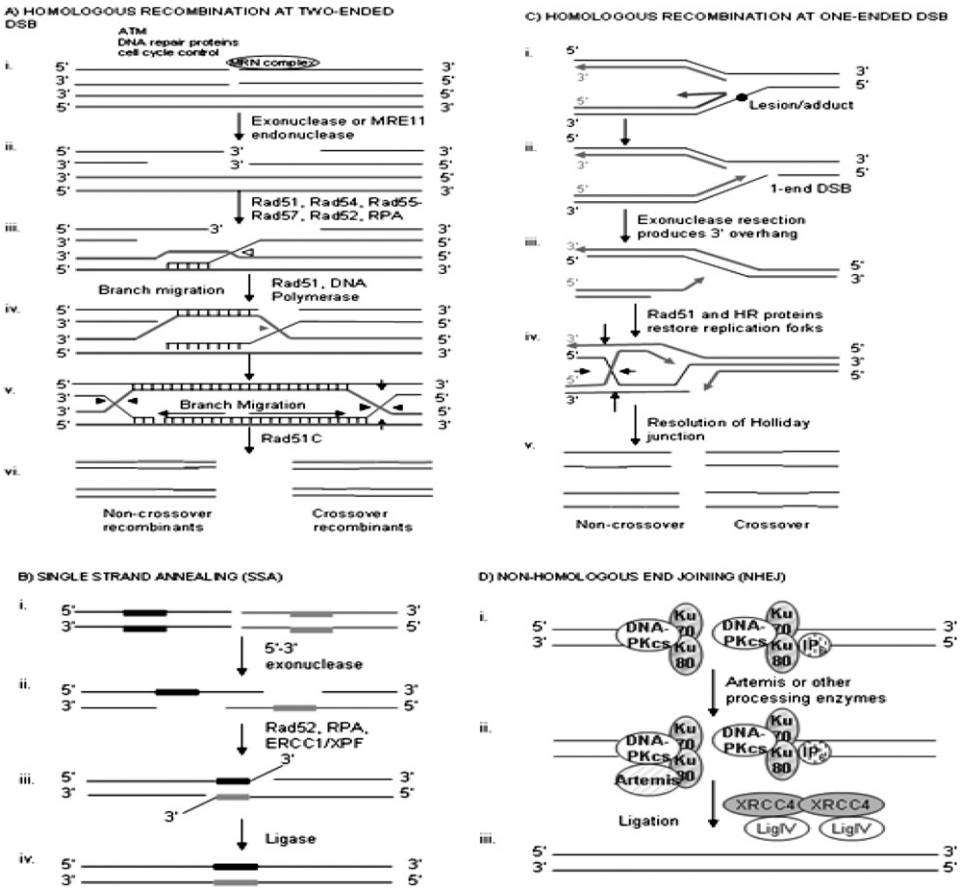

Figure 3. Repair of DSBs

(A) Homologous recombination at two-ended DSBs: (i) Detection of DSB by the MRN complex and recruitment of ATM. Other repair and cell-cycle checkpoint proteins are activated by ATM. (ii) 5′–3′ exonuclease resection of the DSB to generate a 3′ single-stranded overhang. MRE11 endonuclease may play a role in this process. (iii) Rad51-directed homology search, followed by strand invasion, displaces the complementary region of the homolog (typically a sister chromatid) and creates a D-loop (open arrowhead). Rad51 is probably assisted by RPA and other Rad family members, such as Rad52 and Rad54. (iv) Upon formation of a Holliday junction (gray arrowhead), the invading strand can extend in both directions (note long arrows in v). (v) Extension of invading strand by a DNA polymerase can lead to invasion of the homolog by the second end of the original DSB to form a double Holliday junction intermediate. (vi) Rad51C promotes the resolution of the Holliday junctions to yield either crossover (vertical arrows) or non-crossover (horizontal arrowheads) recombination products.

(B) Single-strand annealing (SSA): (i) Formation of a two-ended DSB between homologous repeat sequences (black and gray bars). (ii) Exonuclease resects the ends to generate a 3′ single-strand overhang, exposing the complementary regions. (iii) Alignment and Rad52-dependent annealing of the repeat sequences leads to displacement of the 3′ tail between the repeats or creation of a gap (not shown). ERCC1/XPF is thought to digest 3′-displaced tails. (iv) Ligation of ends regenerates the intact duplex and deletes the sequence between the repeats.

(C) Homologous recombination at a one-ended DSB: (i) Reversal of a stalled replication fork on encountering an obstacle such as a lesion or adduct in the template strand leads to formation of an intermediate. (ii) Endonuclease action on the intermediate can result in a collapsed replication fork with a one-ended DSB. (iii) A 5′–3′ exonuclease resects the DSB to generate a 3′ overhang capable of stand invasion. (iv) Rad51 directs strand invasion into the fully copied complementary duplex, producing a D loop structure and Holliday junction (see panel [A]) necessary to ultimately restore the replication fork. (v) Resolution of the recombination intermediate can occur either via crossover (vertical arrows) or non-crossover (horizontal arrows) events to yield recombinant products.

(D) Non-homologous end-joining (NHEJ): (i) The Ku70-80 heterodimer binds each end of a two-ended DSB, aligns them, and recruits DNA-PK and its cofactor inositol-6 phosphate (IP_6) to form a bridging and signaling complex. (ii) Noncomplementary ends at the DSB may be processed by Artemis exonuclease, MRN complex, or the FEN-1 nuclease to reveal microhomology or to create ligatable ends. Gaps can be filled by Pol μ to generate ligatable nicks, and other repair enzymes such as PNKP can function to generate conventional 3′ hydroxyl or 5′ phosphate termini. (iii) The processed DSB is then sealed by the XRCC4-LigaseIV tetramer to create an intact duplex.

preservation of genetic integrity.[104] Because accessibility of the sister chromatid is crucial for HR (strand exchange during HR is 100-fold more frequent between sister chromatids than between disparate homologous chromosome regions), this process takes place after DNA has been replicated, i.e., in the S or G2 phase of the cell cycle.

Conventional two-ended HR is initiated by recognition of the DSB by the MRN complex comprised of the 3′–5′ exonuclease MRE11A (MIM 600814), the Rad50 (MIM 604040) ATPase, and the regulatory protein defective in Nijmegen breakage syndrome (NBS1 [MIM 602667]).[105,106] This complex acts as a break sensor and recruits the protein kinase ataxia telangiectasia mutated (ATM [MIM 607585]) to the site of the damage.[105,107,108] Once recruited and activated, ATM signals to cell-cycle-checkpoint proteins, chromatin-remodeling factors, and other DNA-repair components to halt ongoing replication and execute break resolution. We emphasize that there is distinction between components that function to elicit a DNA-damage response (e.g., ATM) and those components that function in DSB resolution.

The first step in the repair of DSBs by HR is a 5′–3′ exonuclease-directed resection to produce a 3′ single-stranded DNA overhang. The exact identity of the nuclease is unknown, although studies suggest that the MRN nuclease complex might be involved as a 5′ endonuclease.[105,109] Once generated, the single-stranded overhang is resolved either by genetic exchange with a sister chromatid or by SSA (see below), both of which are HR events (Figures 3A and 3B). Strand invasion, as the name suggests, involves invasion of the 3′ end of the single-stranded DNA overhang into the region of complementarity in the intact sister chromatid (Figure 3A). This is directed by Rad51 (MIM 179617)—the human homolog of the *E. coli* RecA protein—which forms a nucleoprotein filament that directs homology search, strand pairing, and ultimately, invasion of the homologous chromosome.[110–112] Several Rad family members, such as Rad51B (MIM 602940), Rad51C (MIM 602774), Rad51D (MIM 602954), XRCC2 (MIM 600375), XRCC3 (MIM 600675), Rad54 (MIM 603615), and Rad52 (MIM 60392), along with RPA, are thought to play roles in assisting Rad51 in the process.[113] During strand invasion (see details in Figure 3A), Rad51 promotes the formation of the classic D-loop structure (step iii) and creates a four-stranded Holliday junction intermediate,[114] i.e., the site where the strands crossover (step iv). Subsequently, the invading strand is extended by a DNA polymerase, most likely polymerase η (POLH [MIM 603968]).[115] Resolution of the Holliday junction is reported to occur via a Rad51C- and XRCC3 directed mechanism, leading to the formation of a crossover or non-crossover recombination product (Figure 3A, step vi).[104,116–118] A number of nucleases and helicases, such as the RecQ family members WRN (MIM 604611) and BLM (MIM 604610), which are mutated in the premature aging syndromes Werner and Bloom, respectively, are also reportedly involved in resolving Holliday junctions during the final steps.[119,120]

Another form of two-ended DSB recombination is SSA. This pathway resolves DSBs positioned between repeat sequences as shown in Figure 3B and does not require a sister chromatid. SSA is directed by Rad52 and RPA binding to the 3′ end of the nuclease-derived single-stranded 3′ overhang. If present, exposed repeat sequences upstream and downstream of the DSB are aligned, potentially creating 3′ flap or short-gap intermediates. The nonhomologous displaced 3′ tails are most likely removed by the action of ERCC1/XPF, which is homologous to the yeast RAD1 (MIM 603153)/RAD10 complex.[121,122] After appropriate nuclease and/or polymerase processing to create ligatable nicks, the break is sealed by an as-yet-unidentified DNA ligase. SSA leads to the deletion of the genetic information between the repeats, making it error-prone.

HR at a one-ended DSB functions to resolve breaks that are formed at collapsed replication forks (Figure 3C). When a progressing fork encounters an obstacle, such as a bulky adduct or a persisting SSB, on the template strand of DNA, the fork may stall and form an intermediary structure. One such structure, the so called "chicken foot," which arises from reversion of the stalled fork (Figure 3C, step i), has been reported in bacteria, although not in mammalian cells.[123,124] Nevertheless, the intermediate structure can be processed by endonucleases and/or helicases to create a collapsed replication fork with a one-ended DSB[104] (Figure 3C, step ii). The end of this DSB can then undergo exonuclease resection to generate a 3′ single-stranded overhang, which is used by Rad51 and the related HR proteins to execute strand invasion (Figure 3C, steps iii and iv) and the resolution of Holliday junction(s) (Figure 3C, step v) as described above. For a more detailed understanding of the various DSBR processes, refer to the reviews by Helleday[104] and Szostak et al.[125]

NHEJ. In mammalian cells, two-ended DSBs can be resolved not only by HR or SSA (above) but also by NHEJ, which involves direct end-to-end ligation, and at times, limited processing (Figure 3D). This form of "repair" can lead to the restoration of the original sequence or to the addition or removal of anywhere from a few nucleotides to several kilobases of DNA, making this an error-prone response. NHEJ is the major DSBR pathway in the G1 phase of the cell cycle because a sister chromatid is not needed or available for resolution.[104,126] NHEJ is initiated by the Ku70 (G22P1 [MIM 152690])-Ku80 (XRCC5 [MIM 194364]) heterodimer, which recognizes DSB ends, aligns them, protects them from excessive degradation, and ultimately prepares them for ligation. The Ku heterodimer recruits the protein kinase DNA-PKcs (DNAPK1 [MIM 600899]) (and its cofactor inositol-6-phosphate) to establish a bridging and signaling complex that serves to recruit specific repair factors.[127–131] If complementary ends are not present at the break, they can be subject to nucleolytic degradation to create single-strand overhangs with short stretches of micro-homology. This, and other end processing events, are seemingly effected by the Artemis exonuclease in association with DNA-PK, and possibly by the MRN complex, the FEN1 nuclease, or the WRN/BLM helicases.[130] When necessary, based on its interactions with Ku and the XRCC4 (MIM 194363)-LigaseIV (LIG4 [MIM 601837]) complex, polymerization of missing nucleotides is performed by DNA Pol μ (POLM [MIM 606344]).[130–132]

Table 2. Defective DNA Repair in Neurodegenerative Diseases

Neurodegenerative Disorder	Affected Gene	Encoded Protein	Affected Pathway or Mechanism	Phenotype and Pathology
spinocerebellar ataxia with axonal neuropathy I (SCAN1)	TDP1	Tyrosyl phosphodiesterase 1	SSBR	peripheral axonal motor and sensory neuropathy
ataxia with occulomotor apraxia type I (AOA1)	APTX	Aprataxin	SSBR	cerebellar ataxia and atrophy, peripheral neuropathy, occulomotor apraxia
ataxia with occulomotor apraxia type II (AOA2)	SETX	Senataxin	SSBR?	spinocerebellar ataxia, cerebellar atrophy, peripheral neuropathy, oculomotor apraxia
xeroderma pigmentosum	XPA, XPB, XPC, XPD, XPE, XPG, XPF POL η	xeroderma pigmentosa group Polymerase eta	NER	neurological symptoms like microcephaly, progressive mental deficiency and ataxia, seen in a small fraction of XP patients
Cockayne syndrome	CS-A, CS-B XPB, XPD, XPG	Cockayne syndrome A, B xeroderma pigmentosa group	NER	retinal degeneration, partial deafness and facio-skeletol and/or gait abnormalities, neuronal dystrophy
Trichothiodystrophy (TTD)	XPB, XPD TTD-A	xeroderma pigmentosa group an 8 kDa TFIIH complex member	NER	microcephaly, ataxia, mild mental retardation, neurodysmyelination of cerebrum white matter
ataxia telangiectasia (AT) and ataxia telangiectasia-like disorder (ATLD)	ATM and MRE11	Ataxia Telangiectasia Mutated and MRE11	DSBR (signaling)	progressive neurodegeneration, premature aging, ataxia,
Seckel syndrome	ATR	Ataxia Telangiectasia and Rad3-related protein	DSBR (signaling)	growth retardation, dwarfism, microcephaly
Nijmegen breakage syndrome (NBS)	NBS1	Nibrin	DSBR	microcephaly, growth retardation
LigaseIV syndrome	LIGIV	LigaseIV	DSBR	growth retardation
NHEJ1 syndrome	NHEJ1	XRCC4 like factor (XLF), also called Cernunnos	DSBR	microcephaly, growth retardation

When necessary, 3′- or 5′-blocking ends are also removed to generate the 3′ hydroxyl and 5′ phosphate termini required for ligation, for instance, by the 3′-DNA phosphatase/5′-kinase (PNKP [MIM 605610]).[104,127–131,133,134] The XRCC4-LigaseIV tetramer is recruited to seal the DSB.[130,134] A recently identified component of NHEJ is the Cernunnos-XLF (XRCC4-like factor) protein (NHEJ1 [MIM 611290]), which associates with the XRCC4/ligase IV complex and stimulates its ligase activity in an unknown manner.[135]

Inherited DNA-Repair Disorders with Neurological Abnormalities

As is clear from the discussion above, there is considerable crosstalk and overlap between the processes and enzymatic components of repair, replication, recombination, and transcription. In nervous tissue, where replication-dependent recombinational repair is not possible because of the non-dividing status of the cells, BER/SSBR, NER, and possibly NHEJ assume a special significance as gatekeepers of genomic integrity. This is exemplified by the recent findings that some autosomal-recessive ataxias with purely neurodegenerative pathology (i.e., not accompanied by genetic instability or cancer predisposition) arise from defects in SSBR-related proteins. Interestingly, neurological deficiencies arising from defects in NER or DSBR are often accompanied by some form of genetic instability and cancer. Reviewed next are some of the well-studied and recently characterized ataxias and disorders with neurological pathologies stemming from defects in SSBR, NER, or DSBR components (Table 2).

1. SSBR-Associated Disorders

A few spinocerebellar recessive ataxias have recently been linked to defects in the processing of SSB ends. Such disorders include spinocerebellar ataxia with axonal neuropathy (SCAN1 [MIM 607250]) and ataxia with oculomotor apraxia type I (AOA1 [MIM 208920]). As discussed above, SSBs are common endogenous lesions, arising as intermediates of specific metabolic processes or as products of ROS attack of DNA. Ataxia with oculomotor apraxia type II (AOA2 [MIM 606002]) and a specific case of amyotrophic lateral sclerosis (ALS4 [MIM 602433]), which both arise from a mutation in the *SETX* gene (MIM 608465), might also involve a SSBR defect, although this has not been explicitly shown. Spinocerebellar ataxias are characterized by early to late childhood onset of motor in-coordination in speech, gait, balance, and gaze and, in some instances,

mental retardation arising out of spinocerebellar axonal and post-mitotic neuronal degeneration. In this section, we discuss the above genetic disorders and their corresponding gene and repair defects.

SCAN1. SCAN1 was first identified in nine members of a Saudi Arabian family and is characterized by cerebellar ataxia and peripheral axonal motor and sensory neuropathy that resembles Charcot-Marie-Tooth disease.[136] Symptoms and pathology, which include ataxia, dystharia, mild hypoalbuminaemia, mild hypercholesterolaemia, and sensory loss, manifest around the second decade of life.[137] An interesting feature of this autosomal-recessive disorder is that the symptoms are purely neurodegenerative, without any cancer predisposition, suggesting selective assault on nondividing cells.

SCAN1 arises by a homozygous mutation in the *TDP1* gene. The specific mutation (A1478G) results in substitution of the conserved histidine residue 493 with arginine in the active site of the protein.[136] TDP1 normally removes DNA-bound topoisomerase 1 (Top1 [MIM 126420]) molecules that arise during abortive enzymatic reactions and is thought to be part of the SSBR pathway.[138–140] Top1, a protein involved in the regulation of DNA supercoiling during transcription and replication, transiently breaks one strand of DNA and in the process forms a covalent link with the 3′ terminus.[67,141] After strand cleavage to relieve DNA tension, the protein-DNA intermediate is typically released, and the strand break is resealed. In the event of an incomplete reaction, which may occur when the Top1-DNA complex is formed in the vicinity of a DNA lesion, such as a modified base, gap, or nick or when cells are exposed to the chemotherapeutic agent camptothecin, the topoisomerase is irreversibly trapped on DNA, creating an obstacle for processes such as replication and transcription.[41,139] In most situations, TDP1 will resolve the abortive phosphotyrosine-DNA linkage, releasing the Top1 protein (or a degradation product) and generating a SSB that can be processed by components of BER/SSBR.[142] The A1476G mutation leads to an approximately 25-fold reduction in TDP1 enzyme activity and results in the accumulation of Top1–DNA reaction intermediates.[143]

Supporting the role of SSBR in Top1-DNA removal are the following observations: (1) cells defective in XRCC1, a key scaffold protein in BER/SSBR, are sensitive to camptothecin, a Top1 inhibitor,[144,145] and (2) XRCC1's protein partner, Ligase IIIα, associates with TDP1, and together the XRCC1-Ligase IIIα complex stimulates TDP1 activity at 3′-phosphotyrosine SSBs in vitro.[73,146] Recent studies by El Khamisy et al. found that SCAN1 lymphoblastoid cells exhibit a delay in the removal of IR-induced SSBs and accumulation of IR-induced Top1-DNA abortive complexes.[146] Additional evidence supporting a role of TDP1 in neurodegeneration comes from the studies by Katyal et al. on TDP1$^{-/-}$ knockout mice. These animals show age-dependent and progressive cerebellar atrophy, and their cerebellar neurons and primary astrocytes are defective in rapid repair of SSBs associated with Top1–DNA complexes or oxidative DNA damage.[147] This observation, combined with the purely neurological pathology seen with SCAN1 patients, suggests that SSBs are particularly cytotoxic to nondividing cells, perhaps even more so to neurons as a result of their higher energy needs and elevated oxidative stress.

We point out that SCAN1 is a disorder of a larger family of related disorders, collectively termed spinocerebellar ataxias (SCAs), encompassing at least 29 genetic loci.[148] The so-called autosomal-dominant SCAs constitute a group of progressive ataxias with some similarity in their neuropathology, including olivopontocerebellar atrophy and cell loss from the Purkinje layer. Some of the SCAs are known to be caused by expansion of CAG trinucleotide repeats in the coding region of the mutated gene; such repeats lead to abnormally long polyglutamine stretches in the protein. None are conclusively or directly linked to defects in DNA repair, although there has been some indication of a repair defect in Machado-Joseph disease (SCA3 [MIM 109150]). Specifically, the defective gene product, MJD1 (or ataxin-3) (ATXN [MIM 607047]), interacts with the human homologs of the yeast DNA-repair proteins RAD23A (MIM 600061) and Rad23B (MIM 600062), HR23A and HR23B.[149] The dominant SCAs will not be covered further here, and the reader is directed to reviews by Schols et al.[150] and Paulson[151] for additional details.

AOA1. First discovered in 1988 as a disorder with AT-like symptomology, AOA1 is characterized by early-onset cerebellar ataxia and atrophy, marked loss of Purkinje cells and peripheral nerve fibers, degeneration of posterior columns and spinocerebellar tracts, hypoalbuminaemia, and hypercholesterolaemia.[137,149] Unlike AT, yet similar to SCAN1, AOA1 does not involve non-neurological features such as cancer susceptibility and immunodeficiency.

The defective gene locus for AOA1 (*APTX*) was identified in members of Portuguese and Japanese families independently and was shown to map to chromosome 9p13.[152] The gene product Aprataxin is a ubiquitously expressed, novel member of the histidine triad (HIT) superfamily of hydrolases/translocases.[152,153] Two alternatively spliced variants have been observed, the longer and more abundant of which is a 342 aa protein that can be divided into three domains based on sequence homology. First, the N-terminal region shares homology with PNKP, a protein involved in SSBR and known to interact with other SSBR proteins, i.e., XRCC1, Pol β and DNA Ligase IIIα.[64] Aprataxin has itself been shown to interact with XRCC1 (SSBR) and XRCC4 (DSBR/NHEJ), as well as the strand-break sensor poly (ADP-ribose) polymerase 1 (PARP1 [MIM 173870]).[72,154] Second, the central region of Aprataxin harbors the histidine triad motif typical of the HIT family members, which display nucleotide-binding and hydrolase activities.[155] Indeed, recombinant Aprataxin has been shown to exhibit hydrolase activity on the adenosine-5′-monophosphoramidate and diadenosine tetraphosphate substrates of the histidine triad nucleotide-binding protein (HINT) and the fragile-HIT (FHIT)

subfamily of the HIT proteins.[156,157] Aprataxin was demonstrated to hydrolyze obstructive 5′ adenylate, as well as 3′ phosphate and 3′ phosphoglycolate, groups from SSBs and thereby create ligatable DNA ends.[66,158] Supporting a role for Aprataxin in SSBR, AOA1 cells are hypersensitive to agents that generate SSB damage, such as hydrogen peroxide and methymethane sulfonate (MMS), show elevated levels of DNA SSBs, and are defective in the repair of these breaks over time.[159–161] Most of the mutations discovered in AOA1 family members are confined to the catalytic domain, rendering the protein enzymatically inactive. Finally, the carboxy terminus contains a divergent zinc-finger motif that is involved in binding undamaged and nicked adenylated duplex DNA substrates.[152,156,162]

AOA2. AOA2, which shares some neuropathology with AOA1, is an early-onset (10–22 years of age) disorder characterized by spinocerebellar ataxia with cerebellar atrophy, peripheral neuropathy, oculomotor apraxia, loss of Purkinje cells, mild fibrous gliosis, and elevated serum α-fetoprotein. The disease was first identified in members of Japanese, Pakistani, and Israeli families.[163] The gene defective in AOA2 (*SETX*) was localized to chromosome 9q34 and encodes a novel member of the superfamily 1 helicases.[163–165] Homology searches indicate that this protein, Senataxin, is an ortholog of the yeast RNA helicase Sen1P and is similar to the human helicases RENT1 (MIM 604030) and IGMBP2 (MIM 600502), both of which are involved in aspects of RNA processing.[163] Although neither helicase activity nor a specific role in SSBR has been assigned to Senataxin, we have included AOA2 under the SSBR-related disorders on the basis of the similarities in clinical and cellular phenotypes to AOA1. In particular, AOA2 cells show a similar pattern of sensitivity to the SSB-inducing agents hydrogen peroxide and MMS (but not to IR) and a higher basal level of oxidative DNA damage (8-oxo-dG).[166] The presence of a helicase domain suggests a role in some aspect of nucleic acid metabolism, perhaps the processing of oxidative DNA damage. Disease-associated mutations of Senataxin have been mapped in several ethnically diverse families of AOA2 patients and include missense, nonsense, and frameshift mutations, many of which lead to a truncated product.[163]

Senataxin has also been implicated in a slowly progressing form of amyotrophic lateral sclerosis (ALS) called Juvenile ALS or ALS4.[167] ALS (or Lou Gehrig's disease) was first described by Myrianthopoulos et al.[168] as Charcot-Marie-Tooth disease in a family with English ancestry. It is a heterogeneous group of progressive and lethal neurological disorders that are characterized by degeneration and atrophy of motor neurons in the cerebellar cortex, spinal cord, and brain stem; the result is paralysis and eventual death by respiratory failure. Inheritance is autosomal dominant, and symptoms manifest around the second decade of life. Chen et al. identified 3 *SETX* missense mutations in ALS4 individuals from three geographically diverse families. The mutations—one lies in the C-terminal helicase domain of Senataxin and the two others reside in the N-terminal putative protein-protein interaction domain—are speculated to result in a partial-loss-of-function or a toxic gain-of-function protein.[169,170]

2. NER-Associated Disorders

XP, CS, and Trichothiodystrophy (TTD) (TTDN1 [MIM 234050] and TTDP [MIM 601675]) constitute a spectrum of NER-related disorders broadly characterized by a varying level of photosensitivity, neurodevelopmental abnormalities, and predisposition to cancer. An interesting feature of this set of diseases is the overlap in genes involved in the manifestation of the clinical phenotypes, whereby different mutations in the same gene give rise to CS, XP, TTD, or a combination thereof. As will be discussed below, it appears that the neurological dysfunction exhibited by a sub-set of these patients arises from defective repair of specific forms of endogenous DNA damage (possibly oxidative lesions) and/or general failure of the transcriptional machinery.

XP. XP was first described in 1874 as an inherited syndrome largely affecting the skin. It constitutes a group of autosomal-recessive disorders caused by mutations in the XP gene family that participates in NER. The hallmarks of XP include sensitivity to sunlight, severe sunburns upon short exposure to UV light, excessively dry skin, freckles, dark spots, and premature aging of the skin and eyes, along with an increased risk for skin cancer. XP patients are more than 1000-fold more likely to develop cutaneous basal- and squamous-cell carcinomas at sun-exposed areas (such as the face, neck, and head) and are at increased risk for developing internal cancers by 20 years of age.[171,172] Cells from XP patients exhibit extreme sensitivity to UV radiation and poor NER after exposure to UV radiation. Although the typical features of classical XP are not related to neurodysfunction, a small fraction of XP patients (about 20%) develop neurological abnormalities, including microcephaly, progressive mental deficiency, ataxia, choreoathetosis, and areflexia.[173–176] Neuropathology in some patients includes a primary degeneration affecting large neurons in the brain, spinal cord, and peripheral nervous system and resulting in cortical atrophy, axonopathy, and gliosis.[172,175,176]

The mutant genes in XP were identified as seven independent complementation groups (XPA-XPG) by fusion studies that used cells defective in various aspects of repairing UV-induced DNA damage (i.e., NER). A variant XPV group was found to be associated with an NER-proficient form of XP, but this group maintained the characteristic skin sensitivity to UV light. The genetic defect in XPV is linked to Pol η, which is required for efficient and accurate translesion synthesis (TLS) past UV photoproducts.[177] Cells from these patients show enhanced arrest of DNA replication at pyrimidine dimer sites and increased mutagenesis and recombination. Of the several mutations identified in *POL η*, the majority result in severe truncations of the protein, whereas others in the conserved catalytic domain of the protein affect the TLS activity of the polymerase.[178]

The complementation groups XPA, XPB, XPD, and XPG have been associated with some form of neurological pathology. Certain XPB, XPD, and XPG mutations also result in a dual XP/CS phenotype that displays differing degrees of neuronal, developmental, and skin abnormalities and will be discussed in detail in the section on CS below. XPA, in combination with XPC, facilitates lesion recognition and verification during the early steps of NER and might also play a role in conferring processivity to the XPF and XPG nucleases.[179,180] Several *XPA* deletions or splice-site mutations that result in a frameshift have been reported in the DNA-binding domain of the protein and are associated with a neurological form of XP. Almost all of these mutations seriously disrupt protein composition and/or structure and the ability of XPA to recognize lesions and initiate NER.[181] Several missense mutations have also been reported in the C-terminal TFIIH-binding domain of XPA and are associated with a much milder neurological phenotype. *XPF* mutations in humans are generally associated with milder forms of XP, although one mutation has been linked to a progeroid syndrome, which might result from roles of NER proteins, e.g., XPF, in the repair of endogenous DNA crosslinks.[182] It is interesting to note that the *XPF* knockout mouse exhibits a severe phenotype,[183] which might imply that "mild" *XPF* mutations compatible with viability will be more "common" in humans. A recent case of an inherited ERCC1 defect that is associated with severe developmental failure and cerebro-oculo-facio-skeletal syndrome (COFS4 [MIM 610758]) has been reported to mimic the XPF cellular phenotypes in showing reduced levels of ERCC1-XPF and a mild NER deficiency.[84] A more comprehensive analysis of the XP gene mutations is presented by Itin et al.[184] and Cleaver et al.[185] Finally, *XPC* mutations have not directly been linked to a neurological phenotype, yet one case of a splice-site mutation at exon 9 in the *XPC* gene has been reported in connection with autism in a Korean subject.[186]

The defects described above are largely confined to genes that modulate mainly GGR. Evidence indicates that in nondividing cells global DNA repair, particularly GGR, is mostly confined to actively transcribed gene regions, possibly as a result of DNA accessibility and an effort to conserve energy resources (for review see Nouspikel[187]). The consequence of a repair deficit in noncoding regions is that, in the event of an unexpected checkpoint collapse, culminating in reentry into the cell cycle, attempted replication of DNA would be crippled by the accumulated lesions. This has in fact been speculated to be one of the reasons for neuronal loss in certain neurodegenerative diseases[187,188] and might also explain the neuronal phenotypes associated with certain cases of XP. However, it remains unclear how this phenomenon would impact certain brain regions selectively.

CS. First reported in 1936 by Edward Alfred Cockayne, a British physician, CS is an early-onset, progressive neurological disorder characterized by dwarfism, microencaphaly, mental retardation, sensitivity to sunlight, retinal degeneration, partial deafness, and facio-skeletal and/or gait abnormalities, but no increased cancer incidence.[189] In terms of its neuropathology, the CS brain shows increased fibrosis, neuronal dystrophy, and an accumulation of senile plaques and/or neurofibrillary tangles along with progressive demyelination or dysmyelination.[190] CS is divided into two types: type A (CSA) and type B (CSB). CSA is caused by mutations in the *ERCC8* (MIM 609412) gene, and CSB is caused by *ERCC6* (MIM 609413) mutations. The genes map to chromosome loci 5q12 (CSA -*ERCC8*) and 10q11 (CSB -*ERCC6*)[93,191] and fully complement the diagnostic UV-related defect in RNA-synthesis recovery in the appropriate CS cell lines.[192] Two clinical variants of type B, the classic severe infantile variant and COFS syndrome, are both reported to be linked to defects in *CSB*.[193] Several different mutations, including missense mutations, nonsense mutations, frameshifts, insertions, splice mutations, and polymorphs have been identified in the *CSA* and *CSB* genes of an ethnically diverse group of CS patients; the vast majority of these mutations lead to a truncated protein product, and approximately 80% affect CSB.[194]

CSA and CSB operate in TCR of UV adducts (reviewed in Laine and Egly[195]), which explains the defect in RNA-synthesis recovery after UV exposure of CS cells; GG-NER is unaffected in CS.[196,197] CSA is a 44 kDa protein that interacts with CSB and other components of the transcription or repair machinery; for example, such components include XAB2, the p44 subunit of TFIIH, and the hyperphosphorylated form of RNAPII. CSA is needed for recruiting the nucleosomal binding protein HMGN1 (MIM 163920), the XPA-binding protein (XAB2 [MIM 610815]), and TFIIH to the stalled RNAPII and is linked to a ubiquitin ligase complex that regulates CSB degradation and RNA polymerase ubiquitylation during TCR.[93,95,96,198,199] CSB is a member of the SWI2/SNF2 superfamily and has DNA-dependent ATPase and ATP-dependent chromatin-remodeling activities.[200] It interacts with RNAPII in vitro and in vivo, stimulates elongation by RNAPII in vitro, and is needed for assembly of the NER proteins and histone acetyltransferase p300 (EP300 [MIM 602700]) at a stalled RNAPII.[97,98,102] Both CSA and CSB mutant cells are also hypersensitive to oxidative stress and are defective in the removal of certain oxidative lesions, such as 8-oxo-dG and (5′S)-8,5′-cyclo 2′-deoxyadenosine, suggesting that these proteins might be involved in the processing of oxidative DNA damage.[100–102,201] Whereas 8-oxo-dG is excised primarily by the classic BER pathway, the (5′S)-8, 5′-cyclo 2′-deoxyadenosine lesion is corrected specifically by NER. Although there is no direct evidence indicating that cyclopurines are causative in neuronal cell loss, these adducts are known to block transcription and could contribute to the manifestation of the neurological disease seen in some XP and possibly some CS patients.[202] In addition, there is in vitro biochemical evidence that suggests that the neurodegenerative phenotype of some XP patients might stem from a role of NER in the removal and

repair of classic oxidative base lesions such as 8-oxo-dG and thymine glycol.[124]

It is likely that the developmental and neurological defects observed in CS (and probably XP) stem in part from the inability to efficiently process endogenous DNA damage from genes undergoing active transcription. However, there is also evidence indicating that CSB plays a direct role in regulating transcription. De Sanctis et al. demonstrated that CSB cells showed an overall defect in the recruitment of RNA Pol II and basal transcription factors after UV exposure.[203] The complexity and variation of traits observed in CS patients might thus arise out of a combination of transcription and DNA-repair defects.

It is noteworthy that traits of CS are observed in a fraction of XP patients that harbor mutations in *XPB*, *XPD*, or *XPG*, which participate in both TCR and GGR. Such individuals are reported as XP/CS and are characterized by clinical phenotypes of both disorders. Specifically, patients with XP/CS show the sun sensitivity and skin and eye defects of XP and CS's neurological and somatic features, such as short stature and developmental abnormalities. Neuropathology of XP/CS includes cerebellar and cerebral atrophy, dysmyelination, calcification of basal ganglia, and Purkinje neuron degeneration.[174]

XPB (or *ERCC3* [MIM 133510]) maps to chromosome 2q21 and encodes an ATP-dependent 3′-5′ helicase,[204] and XPD (or *ERCC2* [MIM 126340]) maps to chromosome 19q13.2 and encodes a 5′-3′ helicase.[205,206] Both XPB and XPD are members of the transcription complex TFIIH, a central member of the transcriptional machinery, and are involved in basal transcription as well as NER. Mutations in *XPB* associated with CS have been reported in four families, and each leads to a truncation of the protein's C-terminal end, which harbors the critical helicase motifs. Cells from these patients have reduced XPB protein and are defective in post-UV RNA synthesis and DNA repair as measured by unscheduled DNA synthesis.[207,208] In vitro studies on two of the mutant XPB proteins immunoprecipitated from cells of XPB-CS patients indicate that defective promoter opening and reduced transcription by TFIIH might be responsible for the observed phenotypes.[207] Additionally, one of the mutations was shown to weaken the interaction of XPB with p52, which is speculated to be a regulator of XPB ATPase activity.[209] A tenth subunit of TFIIH, p8-TTD-A, which is mutated in a subset of TTD cases (see more below), has also been shown to regulate XPB ATPase activity and hence DNA unwinding at the lesion.[82,210] Thus, the complexity and variation of symptoms in XPB patients can probably be attributed to the disruption of one or more of these interactions. The nature of the disruption(s) would lead to variable disassembly of the TFIIH complex and in turn differentially affect transcription and/or NER responses.

XPD interacts with p44, a TFIIH core subunit that stimulates XPD helicase activity,[211] and with the Cdk2 activating kinase (CAK) complex.[212] The latter association suggests that XPD mediates binding of CAK to the core TFIIH. *XPD* mutations at the C-terminal end of the protein, which is the interaction site for p44, have been reported in XP/CS patients. Studies of one such XPD mutant protein revealed a drop in intrinsic helicase and Cdk7-dependent kinase activities in affected cells, leading to the hypothesis that such mutations affect TFIIH stoichiometry and result in decreased transcription and defective NER.[207,211] An N-terminal mutation has also been reported in two new XPD-CS patients. Cells from these individuals exhibit reduced levels of TFIIH, a characteristic of XP/CS,[213] and random DNA breakage at sites distant from the actual damage after UV irradiation, a feature previously observed in two reported XPD/CS cases and one that is presumably responsible for the extreme UV sensitivity observed in these patients.[214]

An additional component reported to support CAK-TFIIH assembly is the *ERCC5* (MIM 133530) gene product, XPG.[215] *XPG*, which maps to chromosome 13q32-33, constitutes another XP/CS complementation group. The gene encodes an endonuclease that facilitates removal of adducts by incising 3′ to the damage during classic NER.[87,216] Several missense and nonsense *XPG* mutations that result in inactive or severely truncated proteins have been found in a diverse set of individuals with XP/CS.[217] The truncation mutants bring about the most severe phenotype, with mortality in infancy or by early childhood. Emmert et al. correlated severity of phenotype with the extent of *XPG* transcript, where a milder phenotype corresponded to a higher level of mRNA. Cells harboring a severely truncated XPG with very low levels of transcript showed exceptionally poor recovery of post-UV RNA synthesis, very low post-UV cell survival, and impaired DNA repair.[217] In addition to its direct role in NER, XPG has been suggested to coordinate recognition of stalled transcription complexes during TCR in concert with CSB and TFIIH.[218] Recent studies by Ito et al. also documented an altered interaction between XPG-TFIIH and an increased disassociation of CAK and XPD from the TFIIH core in cells from either XPG or XPG-CS patients, suggesting that XPG may play a central role in stabilizing these protein complexes.[215] Finally, there is some evidence that XPG might facilitate repair of oxidative DNA damage, possibly by fostering the DNA-binding and AP lyase activities of the glycosylase hNth1 during BER.[219] The severity of phenotype in XPG patients might therefore be a combined result of the loss of excision repair, transcription, and possibly, efficient removal of oxidative DNA lesions.

TTD. TTD completes the trilogy of neurological disorders related to defects in NER. *Tricho* (Greek for hair)-*thio* (sulfur) *dystrophy* (faulty nourishment) aptly describes this autosomal-recessive disorder characterized by low-sulfur content, brittle hair, and variable neurological and somatic abnormalities. TTD defects include photosensitivity, ichthyosiform erythroderma (fish-like scales on skin), and progeria-like faces (faces that look prematurely aged), microcephaly, ataxia, mild mental retardation, neurodysmyelination of cerebrum white matter, and in some cases,

calcification of the basal ganglia. On the basis of its clinical phenotype, TTD can be divided into two groups, a non-photosensitive group without defects in repair and a photosensitive group with defective NER. Cells from the photosensitive group show impaired removal of UV lesions, hypermutability, decreased unscheduled DNA synthesis, and reduced survival after exposure to UV light. The severity of these phenotypes varies with the location of the mutation and the gene affected.[220,221] Within the photosensitive category, three complementation groups, XPD, XPB, and TTD-A, have been reported; 95% of the reported cases belong to the XPD group.[222]

As in XP and XP/CS, a majority of the *XPD* mutations that give rise to TTD are point mutations leading to a single amino acid change located in the C-terminal portion of the protein, where the substitution may affect interactions with the p44 subunit of TFIIH.[207,211] Approximately one third of the *XPD-TTD* mutations are located within an N-terminal hotspot and lead to an R112-to-H substitution in the DNA-RNA helicase motif of the protein. Patients that are homozygous for this mutation are severely defective in DNA repair as measured by removal of cyclo pyrimidine dimers, although the clinical phenotype is reported to be mild.[223–225] As in XP and XP/CS, *XPB* mutations are rare in TTD. A T116P substitution has been reported in a pair of TTD siblings and is associated with defective post-UV survival, unscheduled DNA synthesis, and cyclo pyrimidine dimer removal, yet imparts a mild clinical phenotype.[204] The third complementation group, designated TTD-A (MIM 608780), was first identified in a patient whose cells complemented all known XP groups, yet showed very low levels of TFIIH.[226] The gene product of *TTD-A* was recently identified and encodes p8, an 8 kDa TFIIH sub-unit that contributes to the stability of the complex in vivo.[210] As noted above, p8 also facilitates duplex unwinding at the DNA lesion and recruitment of XPA. All three *TTD-A* mutations reported thus far, as well as the *XPD-TTD* mutations, result in substantially reduced TFIIH, which could originate from disruption of the p8-XPB-XPD interactions. Of the three *TTD-A* alterations, one maps to a region speculated to play a role in protein-protein interactions, and the others result in either the production of a protein lacking a 15 residue conserved N-terminal region or complete loss of protein synthesis (via alteration of the initiator codon ATG to ACG). Recent structure-function studies on p8 suggest that the affected region could be central to its task because deletion mutants lacking 10 or 20 amino-terminal residues were found to be incapable of restoring the NER defects and reduced TFIIH levels associated with *TTD-A* cells.[210,227]

3. DSB-Response-Associated Disorders

Studies have found that defects in several key participants of the DSBR response are associated with various, and often related, forms of neurodegenerative disease. As discussed below, these include inherited genetic disorders arising from mutations in proteins such as MRE11, NBS1, Cernunnos-XLF, Ligase IV, and the signaling phosphatidylinositol 3-kinase-like protein kinases (PIKKs), ATM and AT and Rad3–related (ATR [MIM 601215]). Each of these proteins functions in differing aspects of DSB resolution and/or DNA-damage-checkpoint responses. It is hypothesized that the neurological dysfunction of the associated disorders arises from (i) a defect in the processing of DSBs presumably by the NHEJ pathway and/or (ii) an inappropriate DNA-damage response, quite possibly during neural development.

The circumstances under which DSBs are formed in terminally differentiated post-mitotic, nondividing cells are not clearly understood. One possibility is that DNA metabolic intermediates, oxidative modifications, and/or SSBs are clustered at specific sites within post-mitotic cells, in a fashion similar to the multiply damaged sites created by IR.[31,228,229] This could result in the generation of endogenous DNA DSBs, where even a single unrepaired DSB has the potential to be lethal, as witnessed from studies in yeast.[230] A related thought is that in highly metabolic cells such as neurons, the rates of formation of endogenous DNA damage might be higher than the normal repair capacity, and these higher rates might result in a greater likelihood of the formation of clustered lesions, perhaps more so in euchromatin domains. If DSBs are indeed formed, then defects in neuronal cells in NHEJ would clearly give rise to unwanted accumulation of these deleterious and cytotoxic damages. In neuronal cells lacking DSB-response proteins, such as AT, neuronal cells might be incapable of eliciting a proper genetic response, culminating ultimately in cell death. It would be interesting to know in the future if the disorders described below exhibit global defects in general genome DNA repair, such as BER. Ongoing work on DSB generation and damage responses in post-mitotic cells will ultimately add to our current understanding of the role of DNA strand breaks in the manifestation of neurological disease.

AT and ATLD (MIM 604391). First designated AT by Boder and Sedgwick,[231] the disease is characterized by progressive neurodegeneration; premature aging; predisposition to cancer, particularly lymphomas and leukemias; and immunological and reproductive abnormalities. The most obvious clinical features of AT are motor incoordination (ataxia) and the appearance of clusters of blood vessels or "spider veins" (telangiectasia) on the whites of the eyes. Some of the neurological features and neuropathology include progressive degeneration of cerebellar Purkinje and granule cells and the resulting cerebellar ataxia and dysfunction, distal spinal muscular dystrophy, occulomotor apraxia, dysarthria, dystonia, choreoathetosis, and oculocutaneous telangiectasia.

The gene defective in AT, *ATM*, codes for a 350 kDa member of the PIKKs family of kinases involved in signal transduction and cell-cycle control.[232] The MRN complex recruits ATM to DSBs, where it acts as a sensor to activate various downstream target proteins, such as NBS1 (see below), p53 (MIM 19110), and BRCA1 (MIM 113707), which function to mitigate checkpoint responses, repair, or

apoptosis.[107,233–235] A vast number of mutations, including frameshifts, missense mutations, nonsense mutations, and splicing mutations spanning the entire length of the *ATM* gene have been identified in AT patients; most of these lead to a truncated protein. Cells from AT patients show radio-resistant DNA synthesis (reduced inhibition of DNA synthesis as seen in normal cells after exposure to low doses of radiation)[236–238] and are defective in activation of the G1/S or G2/M checkpoint[239–242] and the p53 pathway after radiation.[243] The cancer predisposition of AT patients, therefore, undoubtedly stems from the failure of a functional, but overwhelmed, repair system to keep up with the unchecked DNA replication; this failure then leads to genomic instability in dividing cells. The neurological features of AT presumably arise from the appearance of lethal DNA intermediates, e.g., DSBs (see above), or from an inappropriate DNA-damage response.

ATLD, as the name suggests, is characterized by features similar to those of AT, without being linked to mutations in the *ATM* gene. This disorder was first reported by Stewart et al., who identified mutations in *MRE11* in two families initially classified as AT.[244] Individuals from these families exhibited a clinical phenotype and cellular features typical of AT; such features included increased radiosensitivity and spontaneously occurring chromosome aberrations in peripheral blood lymphocytes. *MRE11* encodes a 3′-to-5′ exonuclease, which in complex with NBS1 and Rad50 initiates many forms of recombination, including NHEJ in certain circumstances, and is involved in the maintenance of telomere length.[245] Studies have shown that MRE11 recruits and activates ATM at DSBs and regulates DNA replication at the S phase checkpoint in response to DNA damage.[107,108,246] A recurring mutation in *MRE11* in ATLD is the 1714C-T missense mutation that generates a premature stop codon. Individuals from an Italian and an English family were found to bear this mutation, and in all cases, the transcript harboring the premature stop codon was subject to "nonsense mediated decay" (a process by which mRNA transcripts with a premature stop codon are marked for degradation to prevent expression of erroneous or truncated proteins that might be lethal to the cell).[244,247] These individuals, not surprisingly, are defective in expression and function of the MRN complex and show impaired ATM activity in response to IR. Interestingly, the clinical phenotype in these individuals is mainly neurological, and there is an absence of malignancy through their fourth decade of life, suggesting that the mutation is selectively detrimental to DNA-repair functions of the nervous system.

Seckel Syndrome. Seckel syndrome (MIM 210600) is an autosomal-recessive disorder caused by mutations in the *ATR* gene. The disorder is characterized by growth retardation, dwarfism, microcephaly with mental retardation, and a "bird-headed" facial appearance.[248] Although there is some heterogeneity reported with respect to the defective locus, studies in two consanguineous Pakistani families in which this locus maps to chromosome 3q22.1–q24 have identified mutations in the *ATR* kinase as the causative defect.[249,250] O'Driscoll et al. reported an *ATR* splice-site mutation, which led to reduced but residual levels of normal transcript and protein.[248] In contrast to ATM, which functions predominantly in the IR-induced DSB response, ATR is thought to be activated by multiple forms of DNA damage, including DSBs arising at stalled replication forks, as well as, possibly, IR-induced lesions. Studies have found that ATR, like ATM, can be recruited to IR-induced DSBs in an MRN-dependent manner, suggesting that there might be crosstalk and overlap between these two kinases.[251,252] Once triggered and recruited to a DSB, ATR can phosphorylate and activate some of the same downstream ATM targets, including p53, Rad17 (MIM 603139), NBS1, and H2AX (MIM 601172), a variant form of the histone H2A. The current data suggests that the neurological phenotypes of Seckel syndrome patients, much like AT patients, in all likelihood arise from an overall defect in the DNA-damage response and the recruitment of essential repair components to the site of the lesion.

NBS. NBS (MIM 251260), first identified in 1981, is an autosomal-recessive chromosomal-instability disorder characterized by microcephaly, growth retardation, immunodeficiency, and predisposition to cancer.[253] It is phenotypically indistinguishable from the Berlin breakage syndrome but involves a separate complementation group. Although cells from NBS and AT patients share many similarities, e.g., hypersensitivity to IR, radio-resistant DNA synthesis, and a deficiency in the S phase checkpoint, as well as similar chromosome breakages and exchanges, the clinical phenotypes of the two diseases are distinct. In particular, NBS patients show characteristic microcephaly rarely seen in AT and do not develop ataxia or telangiectasia. The gene defective in NBS was mapped to chromosome locus 8q21, and the gene product was identified as nibrin/p95 (*NBS1*), which is a critical component of DSBR.[254–256] Specifically, NBS1, as part of the MRN complex, regulates repair by activating and recruiting key enzymes and kinases, such as ATM, to the site of a DSB.[105] The NBS1 protein has an N-terminal forkhead-associated domain (FHA) and a breast cancer C-terminal domain (BRCT), which binds to phosphorylated H2AX at DSB ends.[257] The C-terminal portion of NBS1 contains the MRE11-binding site, and the central region bears consensus sequences for phosphorylation by ATM and ATR.[109,258,259] Thus, through these interactions, NBS1 mediates both cell-cycle control and DSBR (HR and NHEJ) and also participates in telomere maintenance.[260]

It follows that *NBS1*mutations that abrogate important protein-protein interactions can lead to the same systemic breakdown of the DNA-damage signaling mechanism and cell-cycle control seen in AT. A mutation found in a vast majority of NBS patients is the 657 del5 frameshift mutation.[256] A deletion of 5 nucleotides in exon 6, it results in two different truncation products by an alternative translation mechanism.[260] One of these products, NBS1[70], is found to associate with the MRN complex and is thought

to be partially active. Consequently, patients homozygous for this mutation display a milder phenotype than individuals harboring more severe *NBS1* gene alterations. Other mutations in *NBS1* have been reported over diverse geographical groups, and most lead to a truncated, nonfunctional or dominant-negative protein.

LigaseIV Syndrome and NHEJ1 Syndrome. In contrast to AT and Seckel syndrome, which seemingly arise out of defective DNA-damage signaling, LigaseIV and NHEJ1 syndromes presumably stem from an actual defect in the repair of DSBs. These syndromes therefore represent a direct link between DSBR and the manifestation of neurological disease and suggest the possibility of DSBs in post-mitotic cells, even though it is unclear how they might arise. The LigaseIV syndrome is caused by mutations in the *LIG4* gene.[261] As described above, LigaseIV functions to seal DSB ends during NHEJ. Several *LIG4* mutations that disrupt either the ligase domain or its interaction with XRCC4 have been identified.[261,262] The clinical features of this syndrome are very similar to those of NBS and include immunodeficiency and developmental and/or growth delay. Fibroblasts from *LIG4* patients maintain the pronounced radiosensitivity and impaired DSB rejoining expected of defective repair. The NHEJ1 syndrome is caused by mutations in the recently identified *NHEJ1* gene that encodes the 33 kDa XRCC4-like factor (XLF), or Cernunnos. This protein is reported to promote NHEJ via its interaction with XRCC4 and LigaseIV.[135,263] Buck et al. identified mutations in the *NHEJ1* gene from five patients with severe combined immunodeficiency (SCID), microcephaly, growth retardation, and sensitivity to IR. In a separate patient, Ahnesorg et al. reported a frameshift mutation that leads to a truncated protein with severely diminished or almost no expression of XLF. Cells from this patient were found to be radiosensitive and defective in NHEJ, and both of these shortcomings were corrected by reintroduction of the wild-type *XLF*.[135] Thus, on the basis of the LigaseIV and NHEJ1 disorders discussed here, it seems possible that DSBs are formed and can persist in nondividing cells and thus create transcription roadblocks that lead to cell death and neurodegeneration.

Neurodegenerative Disorders Associated with Oxidative Stress

The central nervous system (CNS) is extremely susceptible to oxidative stress on account of its high metabolic rate. Additionally, generation of free radicals during the processing of dead neuronal debris by macrophages adds to the pro-oxidant environment. These facts, coupled with the lack of regenerative capacity of terminally differentiated post-mitotic cells of the CNS, makes neurons a prime target for accumulation of DNA damage and, ultimately, tissue atrophy. Consistently, several major neurodegenerative disorders, such as Lou Gehrig's disease, Alzheimer's disease (AD [MIM 104300]), Parkinson's disease (PD [MIM 168600]), Huntington's disease (HD [MIM 143100]), and Friedreich's ataxia (FRDA [MIM 229300]), display elevated oxidative tension and are probably exacerbated by the accumulation of associated DNA damage. A brief summary of these disorders is presented here, but for a more detailed understanding, the reader is encouraged to access the reviews cited below.

ALS1, an autosomal-dominant form of ALS, arises from mutations in the copper-zinc superoxide dismutase (*SOD-1* [MIM 147450]) gene.[264] *SOD-1* codes for a 153 aa, ubiquitously expressed protein that dismutates superoxide radicals released during oxidative phosphorylation to hydrogen peroxide and that makes up about 0.1%–0.2% of all CNS protein. More than 125 mutations of *SOD-1* have been identified in familial ALS (FALS) patients (20%–25% of all cases); these include missense, nonsense, and deletion mutations, which produce a truncated protein.[265,266] SOD-1, like Alsin (MIM 606352), a 184 kDa GTPase mutated in ALS2 (MIM 205100) (a slowly progressing, autosomal-recessive form of the disorder), functions to maintain oxidative homeostasis. *ALS2* knockout mice exhibit age-dependent deficits in motor coordination and motor learning, and primary cultured neurons from these mice are vulnerable to oxidative stress.[267] Studies have also implied that Alsin might play a neuroprotective role, whereby overexpression of the long-form of the protein counteracts the cell death observed with *SOD1* mutant mouse neuronal cells.[268] Multiple *ALS2* mutations have been associated with FALS, and most give rise to a truncated protein.[264–266] ALS is reviewed in more detail by Lederer and Santama.[269]

Alzheimer's is a progressive neurodegenerative disease characterized by cognitive deterioration, including amnesia, aphasia, apraxia, agnosia, and dementia. The disorder is thought to be caused by misfolding of β-amyloid and Tau proteins, which aggregate and deposit as plaques and neurofibrillary tangles (NFTs) in AD brains.[270–272] Both the NFTs and Aβ plaques are reported to be sites for redox cycling and the generation of damaging free radicals.[273,274] Indeed, brains of AD patients show detectable oxidative damage (e.g., carbonyl- and acyl-modifications) within the NFTs and Aβ plaques and increased levels of oxidative-stress markers such as the DNA base lesions 8-oxo-dG, 5-hydroxycytosine and 5-hydroxy-adenine.[275,276] A protein implicated in the manifestation of AD is apolipoprotein E (ApoE [MIM 10774]), which typically directs the transport of lipoproteins into target cells via receptor-mediated endocytosis.[277] In addition, ApoE has been shown in vitro to chelate redox-active metals such as iron and copper,[273,275] and elevated levels of both these metals have been found in neuropil (i.e., the unmyelinated neuronal processes of the gray matter) of AD patients with ApoE defects.[275] Indeed, inheritance of a mutant ApoE E4 allele confers increased susceptibility in many AD patients. Further discussion of the role of oxidative stress in AD is reviewed by Smith et al.[275]

An increase in ROS and oxidative biomarkers, e.g., 8-oxo-dG, lipid hydroperoxides, malonaldehdyes, and reactive carbonyl species, has been observed in the brains of PD patients.[278,279] PD, which is characterized by muscle rigidity, tremors, and in extreme cases, a loss of physical movement

(akinesia), is caused by degeneration of dopaminergic cells in the brain's susbtantia nigra, the region that controls voluntary movement, produces the neurotransmitter dopamine, and regulates mood. In both AD and PD, it is not clear whether the oxidative stress is the cause of the disease or results because of the disease, as further discussed by Jenner.[280]

FRDA is an autosomal-recessive disorder characterized by progressive ataxia and loss of limb deep-tendon reflexes (contraction of muscles in response to stimuli such as tapping of muscle tendons). Most of the clinical features are the result of degeneration and atrophy of sensory neurons, spinal-cerebellar tracts, and sensory fibers in the peripheral nerves.[281,282] The genetic defect underlying the disease results in reduced expression of a mitochondrial protein called Frataxin (FXN [MIM 606829]), which plays a role in mitochondrial energy activation and oxidative phosphorylation.[282–284] Recent evidence suggests that Frataxin might also function in ROS detoxification via activation of glutathione peroxidase and elevation of thiols, which act as a chaperone for Fe(II) and a storage compartment for excess iron.[284,285] In addition, Frataxin is speculated to play a role in iron loading into Fe-S clusters, for instance into the mitochondrial respiratory-chain complexes I and II/III.[286] A defect in Fe loading is speculated to lead to free iron overload, which promotes free-radical formation and oxidative stress and ultimately leads to impaired oxidative phosphorylation. For a more detailed review of the role of oxidative stress in FRDA, refer to Butterfield et al.[287]

HD is an autosomal-dominant disorder characterized by involuntary choreiform movements, loss of cognitive function, and a massive loss of neurons in the striatum.[286,287] It is caused by CAG trinucleotide expansion in the coding region of the *HD* gene on chromosome 4; this expansion leads to a toxic glutamine-rich protein. Oxidative stress seems to be a feature of the disease; patients harbor elevated levels of oxidative biomarkers, such as malondialdehyde, 3-nitrotyrosine, and heme oxygenase-1 and exhibit oxidative fragmentation of DNA in their cortical and striatal neurons.[287–289] Recent studies have demonstrated a more direct link between oxidative stress and HD by correlating CAG expansion to the activity of the BER enzyme OGG1. In particular, the DNA glycosylase OGG1 is proposed to promote a "toxic oxidation cycle" in which strand displacement and slippage during BER of oxidized bases can result in expansion of CAG triplets during gap-filling synthesis.[288] We emphasize that neurodegeneration is observed in other CAG expansion diseases in which this strand-slippage mechanism does not occur. For a more comprehensive understanding of HD, refer to review by Walker.[289]

Concluding Remarks

We have provided a comprehensive summary of the various forms of DNA damage and the systems that function to repair them. As described above, heritable defects in several components of these DNA-repair pathways, as well as systems that protect against oxidative stress, have been causally linked to cancer or neurological dysfunction. It is presumed that the neuronal cell death in many of these cases stems from the accumulation of DNA damage and the accompanying interference of normal RNA transcription, which leads to cell-death responses. Given the nondividing nature of neurons, it is important to emphasize that persistent DNA lesions cannot be resolved or removed by the more universal pathway of replication-associated HR. Furthermore, the disparate cancer susceptibility of the various DNA-repair disorders (Table 2) probably depends on the nature of the persistent DNA damage and its intrinsic genotoxic potential.

It is interesting that defects in genes involved in SSBR, namely Aprataxin and TDP1, manifest exclusively as neurodegenerative disease, without a predisposition to cancer or genetic instability. Conversely, an NER or DSBR defect appears to affect both dividing and nondividing tissues, largely indiscriminately. These facts suggest that SSBR is crucial to the maintenance of genomic integrity of nondividing cells and that SSBs are not overtly genotoxic in replicating tissue. The results also imply that helix-distorting base adducts and complex DNA damages, such as DSBs, are probably harmful to the genome integrity and functional capacity of both developing and terminally differentiated tissue. The pronounced sensitivity of nondividing neuronal cells to DNA damage probably stems from (i) a reliance on replication-independent repair pathways for lesion removal (e.g., BER/SSBR, NER, and possibly NHEJ) and (ii) their high metabolic needs and the associated oxidative burden.

A noteworthy aspect of the presentation above is the distinct clinical pathologies and cellular phenotypes associated with NER disorders that stem from different, or in some cases the same, mutations within a single gene (as seen with XPD; see the discussion of XP, CS-XP, and TTD-XP). Although it is not completely clear why specific mutations within the same gene give rise to multiple phenotypes, one simple explanation is that the severity and spectrum of pathological features is dictated by the nature of the mutation of the individual. That is to say, individuals harboring dominant-negative or null alleles are likely to exhibit more profound symptoms than individuals possessing partial-function alleles. Furthermore, different mutations within the same gene are likely to lead to disparate effects on enzymatic activities and/or protein-partner interactions that would manifest uniquely in terms of clinical and cellular phenotypes. In situations where distinguishing symptoms have been associated with individuals harboring the same mutation within the same gene, it seems likely that the overall genetic composition of the individual (i.e., modifier genes) would dictate the pathological outcome. Given the complex crosstalk and inter-dependence of repair and the replication and transcriptional machinery, it seems reasonable that slight variations in protein function or interactions might arise from different mutations in the same gene and lead to a broad spectrum of disorders with such distinct yet overlapping characteristics.

Finally, it is worth mentioning that in addition to the diseases above, several premature aging disorders exhibit neurological defects that are linked to impaired DNA repair and/or DNA processing. Some of the well-studied progerias include the Werner, Bloom, and Rothmond Thompson syndromes (RTS [MIM 268400]) caused by mutations in the RecQ family of helicases, which have multiple roles in facilitating DNA transactions.[290,291] In addition, defective DNA repair is associated with other disorders, such as Fanconi Anemia (FA [MIM 227650]), which stems from mutations in the *FANC* gene family.[290] The coming years will prove especially important in unraveling some of the underlying mechanisms and crosstalk between repair pathways that synchronize to maintain genomic integrity in both dividing and nondividing cells.

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Web Resources

The URLs for data presented herein are as follows:

Disorders Discussed in Manuscripts

SCAN1 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=607250
AOA1 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=208920
AOA2 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=606002
TTD http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=601675
ATLD http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=604391
AT http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=208900
Seckel Syndrome http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=210600
NBS http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=251260
LigaseIV Syndrome http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=606593
NHEJ1 Syndrome http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=611291
Alzheimer's Disease http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=104300
Parkinson's Disease http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=168600
Huntington's Disease http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=143100
FRDA http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=229300
ALS1 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=105400
ALS4 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=602433

Genes and Proteins Discussed in Manuscript

ERCC2 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=126340
ERCC3 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=133510
ERCC5 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=133530
ERCC6 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=609413
ERCC8 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=609412
TDP1 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=607198
APTX http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=606350
SOD1 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=147450
XPA http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=278700
XPC http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=278720
XPB http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=610651
XPD http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=278730
XPG http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=278780
CSA http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=216400
CSB http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=133540
TTD-A http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=608780
SETX http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=608465
ATM http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=607585
ATR http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=601215
LIG4 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=601837
MRE11A http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=600814
NHEJ1 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=611290
NBS1 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=602667
ApoE http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=107741
Frataxin http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=606829
OGG1 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=601982

References

1. De Bont, R., and Van Larebeke, N. (2004). Endogenous DNA damage in humans: A review of quantitative data. Mutagenesis *19*, 169–185.

2. Doll, R., and Peto, R. (1981). The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J. Natl. Cancer Inst. *66*, 1191–1308.
3. Trichopoulos, D., and Petridou, E. (1994). Epidemiologic studies and cancer etiology in humans. Med.Exerc. Nutr.Health *3*, 206–225.
4. Chance, B., Sies, H., and Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. Physiol. Rev. *59*, 527–605.
5. Jackson, A.L., and Loeb, L.A. (2001). The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. Mutat. Res. *477*, 7–21.
6. Shen, Z., Wu, W., and Hazen, S.L. (2000). Activated leukocytes oxidatively damage DNA, RNA, and the nucleotide pool through halide-dependent formation of hydroxyl radical. Biochemistry *39*, 5474–5482.
7. Slupphaug, G., Kavli, B., and Krokan, H.E. (2003). The interacting pathways for prevention and repair of oxidative DNA damage. Mutat. Res. *531*, 231–251.
8. Cadet, J., Douki, T., Gasparutto, D., and Ravanat, J.L. (2003). Oxidative damage to DNA: Formation, measurement and biochemical features. Mutat. Res. *531*, 5–23.
9. Dizdaroglu, M., Jaruga, P., Birincioglu, M., and Rodriguez, H. (2002). Free radical-induced damage to DNA: mechanisms and measurement. Free Radic. Biol. Med. *32*, 1102–1115.
10. Chung, F.L., Chen, H.J., and Nath, R.G. (1996). Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts. Carcinogenesis *17*, 2105–2111.
11. Esterbauer, H., Schaur, R.J., and Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. *11*, 81–128.
12. Mistry, N., Podmore, I., Cooke, M., Butler, P., Griffiths, H., Herbert, K., and Lunec, J. (2003). Novel monoclonal antibody recognition of oxidative DNA damage adduct, deoxycytidine-glyoxal. Lab. Invest. *83*, 241–250.
13. Roberts, M.J., Wondrak, G.T., Laurean, D.C., Jacobson, M.K., and Jacobson, E.L. (2003). DNA damage by carbonyl stress in human skin cells. Mutat. Res. *522*, 45–56.
14. Cavalieri, E.L., Stack, D.E., Devanesan, P.D., Todorovic, R., Dwivedy, I., Higginbotham, S., Johansson, S.L., Patil, K.D., Gross, M.L., Gooden, J.K., et al. (1997). Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. Proc. Natl. Acad. Sci. USA *94*, 10937–10942.
15. Liehr, J.G. (2000). Is estradiol a genotoxic mutagenic carcinogen? Endocr. Rev. *21*, 40–54.
16. O'Driscoll, M., Macpherson, P., Xu, Y.Z., and Karran, P. (1999). The cytotoxicity of DNA carboxymethylation and methylation by the model carboxymethylating agent azaserine in human cells. Carcinogenesis *20*, 1855–1862.
17. Rydberg, B., and Lindahl, T. (1982). Nonenzymatic methylation of DNA by the intracellular methyl group donor S-adenosyl-L-methionine is a potentially mutagenic reaction. EMBO J. *1*, 211–216.
18. Sedgwick, B., Bates, P.A., Paik, J., Jacobs, S.C., and Lindahl, T. (2007). Repair of alkylated DNA: recent advances. DNA Repair (Amst.) *6*, 429–442.
19. Barbarella, G., Tugnoli, V., and Zambianchi, M. (1991). Imidazole ring opening of 7-methylguanosine at physiologic pH. Nucleosides Nucleotides Nucleic Acids *10*, 1759–1769.
20. Tudek, B., Boiteux, S., and Laval, J. (1992). Biological properties of imidazole ring opened N7-methylguanine in M13mp18 phage DNA. Nucleic Acids Res. *20*, 3079–3084.
21. Atamna, H., Cheung, I., and Ames, B.N. (2000). A method for detecting abasic sites in living cells: age-dependent changes in base excision repair. Proc. Natl. Acad. Sci. USA *97*, 686–691.
22. Lindahl, T. (1993). Instability and decay of the primary structure of DNA. Nature *362*, 709–715.
23. Ye, N., Holmquist, G.P., and O'Connor, T.R. (1998). Heterogeneous repair of N-methylpurines at the nucleotide level in normal human cells. J. Mol. Biol. *284*, 269–285.
24. Chaudhary, A.K., Nokubo, M., Reddy, G.R., Yeola, S.N., Morrow, J.D., Blair, I.A., and Marnett, L.J. (1994). Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science *265*, 1580–1582.
25. Kang, H., Konishi, C., Kuroki, T., and Huh, N. (1995). Detection of O6-methylguanine, O4-methylthymine and O4-ethylthymine in human liver and peripheral blood leukocyte DNA. Carcinogenesis *16*, 1277–1280.
26. Nakamura, J., and Swenberg, J.A. (1999). Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. Cancer Res. *59*, 2522–2526.
27. Szyfter, K., Hemminki, K., Szyfter, W., Szmeja, Z., Banaszewski, J., and Pabiszczak, M. (1996). Tobacco smoke-associated N7-alkylguanine in DNA of larynx tissue and leucocytes. Carcinogenesis *17*, 501–506.
28. Vaca, C.E., Nilsson, J.A., Fang, J.L., and Grafstrom, R.C. (1998). Formation of DNA adducts in human buccal epithelial cells exposed to acetaldehyde and methylglyoxal in vitro. Chem. Biol. Interact. *108*, 197–208.
29. Wagner, J.R., Hu, C.C., and Ames, B.N. (1992). Endogenous oxidative damage of deoxycytidine in DNA. Proc. Natl. Acad. Sci. USA *89*, 3380–3384.
30. Yi, P., Sun, X., Doerge, D.R., and Fu, P.P. (1998). An improved 32P-postlabeling/high-performance liquid chromatography method for the analysis of the malondialdehye-derived 1, N2-propanodeoxyguanosine DNA adduct in animal and human tissues. Chem. Res. Toxicol. *11*, 1032–1041.
31. Sutherland, B.M., Bennett, P.V., Cintron-Torres, N., Hada, M., Trunk, J., Monteleone, D., Sutherland, J.C., Laval, J., Stanislaus, M., and Gewirtz, A. (2002). Clustered DNA damages induced in human hematopoietic cells by low doses of ionizing radiation. J. Radiat. Res. (Tokyo) Suppl. *43*, S149–S152.
32. Wallace, S.S. (1998). Enzymatic processing of radiation-induced free radical damage in DNA. Radiat. Res. *150* (*Suppl 5*), S60–S79.
33. Cadet, J., Bellon, S., Douki, T., Frelon, S., Gasparutto, D., Muller, E., Pouget, J.P., Ravanat, J.L., Romieu, A., and Sauvaigo, S. (2004). Radiation-induced DNA damage: formation, measurement, and biochemical features. J. Environ. Pathol. Toxicol. Oncol. *23*, 33–43.
34. Cleaver, J.E., and Crowley, E. (2002). UV damage, DNA repair and skin carcinogenesis. Front. Biosci. *7*, d1024–d1043.
35. Calderon, J., Ortiz-Perez, D., Yanez, L., and az-Barriga, F. (2003). Human exposure to metals. Pathways of exposure, biomarkers of effect, and host factors. Ecotoxicol. Environ. Saf. *56*, 93–103.
36. Croy, R.G., Essigmann, J.M., Reinhold, V.N., and Wogan, G.N. (1978). Identification of the principal aflatoxin B1-DNA adduct formed in vivo in rat liver. Proc. Natl. Acad. Sci. USA *75*, 1745–1749.

37. Tovalin, H., Valverde, M., Morandi, M.T., Blanco, S., Whitehead, L., and Rojas, E. (2006). DNA damage in outdoor workers occupationally exposed to environmental air pollutants. Occup. Environ. Med. *63*, 230–236.
38. Epstein, S.S., and Shafner, H. (1968). Chemical mutagens in the human environment. Nature *219*, 385–387.
39. O'connor, J.M. (2001). Trace elements and DNA damage. Biochem. Soc. Trans. *29*, 354–357.
40. Peto, J. (2001). Cancer epidemiology in the last century and the next decade. Nature *411*, 390–395.
41. Pourquier, P., Ueng, L.M., Kohlhagen, G., Mazumder, A., Gupta, M., Kohn, K.W., and Pommier, Y. (1997). Effects of uracil incorporation, DNA mismatches, and abasic sites on cleavage and religation activities of mammalian topoisomerase I. J. Biol. Chem. *272*, 7792–7796.
42. Cohen, S.M., and Lippard, S.J. (2001). Cisplatin: from DNA damage to cancer chemotherapy. Prog. Nucleic Acid Res. Mol. Biol. *67*, 93–130.
43. Cullinane, C., Van Rosmalen, A., and Phillips, D.R. (1994). Does adriamycin induce interstrand cross-links in DNA? Biochemistry *33*, 4632–4638.
44. Hannun, Y.A. (1997). Apoptosis and the dilemma of cancer chemotherapy. Blood *89*, 1845–1853.
45. Barnes, D.E., Lindahl, T., and Sedgwick, B. (1993). DNA repair. Curr. Opin. Cell Biol. *5*, 424–433.
46. Krokan, H.E., Standal, R., and Slupphaug, G. (1997). DNA glycosylases in the base excision repair of DNA. Biochem. J. *325*, 1–16.
47. Bessho, T., Tano, K., Kasai, H., Ohtsuka, E., and Nishimura, S. (1993). Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. J. Biol. Chem. *268*, 19416–19421.
48. Bessho, T., Roy, R., Yamamoto, K., Kasai, H., Nishimura, S., Tano, K., and Mitra, S. (1993). Repair of 8-hydroxyguanine in DNA by mammalian N-methylpurine-DNA glycosylase. Proc. Natl. Acad. Sci. USA *90*, 8901–8904.
49. Friedberg, E., Walker, G.C., and Siede, W. (1995). DNA Repair and Mutagenesis (Washington, DC: ASM Press).
50. Impellizzeri, K.J., Anderson, B., and Burgers, P.M. (1991). The spectrum of spontaneous mutations in a Saccharomyces cerevisiae uracil-DNA-glycosylase mutant limits the function of this enzyme to cytosine deamination repair. J. Bacteriol. *173*, 6807–6810.
51. Hinmura, K., Kasai, H., Sasaki, A., Sugimura, H., and Yokota, J. (1997). 8-Hydroxyguanine (7,8-dihydro-8-oxoguanine) DNA glycosylase and AP lyase activities of hOGG1 protein and their substrate specificity. Mutat. Res. *385*, 75–82.
52. Barzilay, G., and Hickson, I.D. (1995). Structure and function of apurinic/apyrimidinic endonucleases. Bioessays *17*, 713–719.
53. Cappelli, E., Taylor, R., Cevasco, M., Abbondandolo, A., Caldecott, K., and Frosina, G. (1997). Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. J. Biol. Chem. *272*, 23970–23975.
54. Frosina, G., Fortini, P., Rossi, O., Carrozzino, F., Raspaglio, G., Cox, L.S., Lane, D.P., Abbondandolo, A., and Dogliotti, E. (1996). Two pathways for base excision repair in mammalian cells. J. Biol. Chem. *271*, 9573–9578.
55. Kubota, Y., Nash, R.A., Klungland, A., Schar, P., Barnes, D.E., and Lindahl, T. (1996). Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase beta and the XRCC1 protein. EMBO J. *15*, 6662–6670.
56. Matsumoto, Y., and Kim, K. (1995). Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. Science *269*, 699–702.
57. Singhal, R.K., and Wilson, S.H. (1993). Short gap-filling synthesis by DNA polymerase beta is processive. J. Biol. Chem. *268*, 15906–15911.
58. Tomkinson, A.E., and Mackey, Z.B. (1998). Structure and function of mammalian DNA ligases. Mutat. Res. *407*, 1–9.
59. Klungland, A., and Lindahl, T. (1997). Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). EMBO J. *16*, 3341–3348.
60. Podlutsky, A.J., Dianova, I.I., Podust, V.N., Bohr, V.A., and Dianov, G.L. (2001). Human DNA polymerase beta initiates DNA synthesis during long-patch repair of reduced AP sites in DNA. EMBO J. *20*, 1477–1482.
61. Dianov, G.L., Prasad, R., Wilson, S.H., and Bohr, V.A. (1999). Role of DNA polymerase beta in the excision step of long patch mammalian base excision repair. J. Biol. Chem. *274*, 13741–13743.
62. Fortini, P., Pascucci, B., Parlanti, E., Sobol, R.W., Wilson, S.H., and Dogliotti, E. (1998). Different DNA polymerases are involved in the short- and long-patch base excision repair in mammalian cells. Biochemistry *37*, 3575–3580.
63. Prasad, R., Singhal, R.K., Srivastava, D.K., Molina, J.T., Tomkinson, A.E., and Wilson, S.H. (1996). Specific interaction of DNA polymerase beta and DNA ligase I in a multiprotein base excision repair complex from bovine testis. J. Biol. Chem. *271*, 16000–16007.
64. Whitehouse, C.J., Taylor, R., Thistlethwaite, A., Zhang, H., Karimi-Busheri, F., Lasko, D., Weinfeld, M., and Caldecott, K. (2001). XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. Cell *104*, 107–117.
65. Sano, Y., Date, H., Igarashi, S., Onodera, O., Oyake, M., Takahashi, T., Hayashi, S., Morimatsu, M., Takahashi, H., Makifuchi, T., et al. (2004). Aprataxin, the causative protein for EAOH is a nuclear protein with a potential role as a DNA repair protein. Ann. Neurol. *55*, 241–249.
66. Takahashi, T., Tada, M., Igarashi, S., Koyama, A., Date, H., Yokoseki, A., Shiga, A., Yoshida, Y., Tsuji, S., Nishizawa, M., et al. (2007). Aprataxin, causative gene product for EAOH/AOA1, repairs DNA single-strand breaks with damaged 3′-phosphate and 3′-phosphoglycolate ends. Nucleic Acids Res. *35*, 3797–3809.
67. Raymond, A.C., Staker, B.L., and Burgin, A.B. Jr. (2005). Substrate specificity of tyrosyl-DNA phosphodiesterase I (Tdp1). J. Biol. Chem. *280*, 22029–22035.
68. Wilson, D.M. III, Sofinowski, T.M., and McNeill, D.R. (2003). Repair mechanisms for oxidative DNA damage. Front. Biosci. *8*, d963–d981.
69. Caldecott, K.W., Aoufouchi, S., Johnson, P., and Shall, S. (1996). XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' in vitro. Nucleic Acids Res. *24*, 4387–4394.
70. Campalans, A., Marsin, S., Nakabeppu, Y., O'Connor, T.R., Boiteux, S., and Radicella, J.P. (2005). XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair. DNA Repair (Amst.) *4*, 826–835.

71. Fan, J., Otterlei, M., Wong, H.K., Tomkinson, A.E., and Wilson, D.M. III. (2004). XRCC1 co-localizes and physically interacts with PCNA. Nucleic Acids Res. *32*, 2193–2201.
72. Luo, H., Chan, D.W., Yang, T., Rodriguez, M., Chen, B.P., Leng, M., Mu, J.J., Chen, D., Songyang, Z., Wang, Y., et al. (2004). A new XRCC1-containing complex and its role in cellular survival of methyl methanesulfonate treatment. Mol. Cell. Biol. *24*, 8356–8365.
73. Plo, I., Liao, Z.Y., Barcelo, J.M., Kohlhagen, G., Caldecott, K.W., Weinfeld, M., and Pommier, Y. (2003). Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions. DNA Repair (Amst.) *2*, 1087–1100.
74. Tebbs, R.S., Flannery, M.L., Meneses, J.J., Hartmann, A., Tucker, J.D., Thompson, L.H., Cleaver, J.E., and Pedersen, R.A. (1999). Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. Dev. Biol. *208*, 513–529.
75. Vidal, A.E., Boiteux, S., Hickson, I.D., and Radicella, J.P. (2001). XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. EMBO J. *20*, 6530–6539.
76. Modrich, P. (2006). Mechanisms in eukaryotic mismatch repair. J. Biol. Chem. *281*, 30305–30309.
77. Genschel, J., Littman, S.J., Drummond, J.T., and Modrich, P. (1998). Isolation of MutSbeta from human cells and comparison of the mismatch repair specificities of MutSbeta and MutSalpha. J. Biol. Chem. *273*, 19895–19901.
78. Gu, L., Hong, Y., McCulloch, S., Watanabe, H., and Li, G.M. (1998). ATP-dependent interaction of human mismatch repair proteins and dual role of PCNA in mismatch repair. Nucleic Acids Res. *26*, 1173–1178.
79. Umar, A., Buermeyer, A.B., Simon, J.A., Thomas, D.C., Clark, A.B., Liskay, R.M., and Kunkel, T.A. (1996). Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. Cell *87*, 65–73.
80. Buermeyer, A.B., Deschenes, S.M., Baker, S.M., and Liskay, R.M. (1999). Mammalian DNA mismatch repair. Annu. Rev. Genet. *33*, 533–564.
81. Yokoi, M., Masutani, C., Maekawa, T., Sugasawa, K., Ohkuma, Y., and Hanaoka, F. (2000). The xeroderma pigmentosum group C protein complex XPC-HR23B plays an important role in the recruitment of transcription factor IIH to damaged DNA. J. Biol. Chem. *275*, 9870–9875.
82. Coin, F., Proietti De, S.L., Nardo, T., Zlobinskaya, O., Stefanini, M., and Egly, J.M. (2006). p8/TTD-A as a repair-specific TFIIH subunit. Mol. Cell *21*, 215–226.
83. De Laat, W.L., Jaspers, N.G., and Hoeijmakers, J.H. (1999). Molecular mechanism of nucleotide excision repair. Genes Dev. *13*, 768–785.
84. Jaspers, N.G., Raams, A., Silengo, M.C., Wijgers, N., Niedernhofer, L.J., Robinson, A.R., Giglia-Mari, G., Hoogstraten, D., Kleijer, W.J., Hoeijmakers, J.H., et al. (2007). First reported patient with human ERCC1 deficiency has cerebro-oculo-facio-skeletal syndrome with a mild defect in nucleotide excision repair and severe developmental failure. Am. J. Hum. Genet. *80*, 457–466.
85. Park, C.H., Mu, D., Reardon, J.T., and Sancar, A. (1995). The general transcription-repair factor TFIIH is recruited to the excision repair complex by the XPA protein independent of the TFIIE transcription factor. J. Biol. Chem. *270*, 4896–4902.
86. Matsunaga, T., Mu, D., Park, C.H., Reardon, J.T., and Sancar, A. (1995). Human DNA repair excision nuclease. Analysis of the roles of the subunits involved in dual incisions by using anti-XPG and anti-ERCC1 antibodies. J. Biol. Chem. *270*, 20862–20869.
87. O'Donovan, A., Davies, A.A., Moggs, J.G., West, S.C., and Wood, R.D. (1994). XPG endonuclease makes the 3′ incision in human DNA nucleotide excision repair. Nature *371*, 432–435.
88. Sijbers, A.M., van der Spek, P.J., Odijk, H., van den Berg, J., van Duin, M., Westerveld, A., Jaspers, N.G., Bootsma, D., and Hoeijmakers, J.H. (1996). Mutational analysis of the human nucleotide excision repair gene ERCC1. Nucleic Acids Res. *24*, 3370–3380.
89. Sijbers, A.M., de Laat, W.L., Ariza, R.R., Biggerstaff, M., Wei, Y.F., Moggs, J.G., Carter, K.C., Shell, B.K., Evans, E., de Jong, M.C., et al. (1996). Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. Cell *86*, 811–822.
90. Wood, R.D., and Shivji, M.K. (1997). Which DNA polymerases are used for DNA-repair in eukaryotes? Carcinogenesis *18*, 605–610.
91. Moser, J., Kool, H., Giakzidis, I., Caldecott, K., Mullenders, L.H., and Fousteri, M.I. (2007). Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase III alpha in a cell-cycle-specific manner. Mol. Cell *27*, 311–323.
92. Mellon, I. (2005). Transcription-coupled repair: a complex affair. Mutat. Res. *577*, 155–161.
93. Henning, K.A., Li, L., Iyer, N., McDaniel, L.D., Reagan, M.S., Legerski, R., Schultz, R.A., Stefanini, M., Lehmann, A.R., Mayne, L.V., et al. (1995). The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. Cell *82*, 555–564.
94. Kamiuchi, S., Saijo, M., Citterio, E., de Jager, M., Hoeijmakers, J.H., and Tanaka, K. (2002). Translocation of Cockayne syndrome group A protein to the nuclear matrix: possible relevance to transcription-coupled DNA repair. Proc. Natl. Acad. Sci. USA *99*, 201–206.
95. Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., Kisselev, A.F., Tanaka, K., and Nakatani, Y. (2003). The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. Cell *113*, 357–367.
96. Groisman, R., Kuraoka, I., Chevallier, O., Gaye, N., Magnaldo, T., Tanaka, K., Kisselev, A.F., Harel-Bellan, A., and Nakatani, Y. (2006). CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. Genes Dev. *20*, 1429–1434.
97. Fousteri, M., Vermeulen, W., van Zeeland, A.A., and Mullenders, L.H.F. (2006). Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. Mol. Cell *23*, 471–482.
98. Selby, C.P., and Sancar, A. (1997). Cockayne syndrome group B protein enhances elongation by RNA polymerase II. Proc. Natl. Acad. Sci. USA *94*, 11205–11209.
99. Van Gool, A.J., Citterio, E., Rademakers, S., van Os, R., Vermeulen, W., Constantinou, A., Egly, J.M., Bootsma, D., and

Hoeijmakers, J.H. (1997). The Cockayne syndrome B protein, involved in transcription-coupled DNA repair, resides in an RNA polymerase II-containing complex. EMBO J. *16*, 5955–5965.
100. D'Errico, M., Parlanti, E., Teson, M., Degan, P., Lemma, T., Calcagnile, A., Iavarone, I., Jaruga, P., Ropolo, M., Pedrini, A.M., et al. (2007). The role of CSA in the response to oxidative DNA damage in human cells. Oncogene *26*, 4336–4343.
101. De Waard, H., de Wit, J., Gorgels, T.G., van den Aardweg, G., Andressoo, J.O., Vermeij, M., van Steeg, H., Hoeijmakers, J.H., and van der Horst, G.T. (2003). Cell type-specific hypersensitivity to oxidative damage in CSB and XPA mice. DNA Repair (Amst.) *2*, 13–25.
102. Sunesen, M., Stevnsner, T., Brosh, R.M. Jr., Dianov, G.L., and Bohr, V.A. (2002). Global genome repair of 8-oxoG in hamster cells requires a functional CSB gene product. Oncogene *21*, 3571–3578.
103. Van Hoffen, A., Balajee, A.S., van Zeeland, A.A., and Mullenders, L.H. (2003). Nucleotide excision repair and its interplay with transcription. Toxicology *193*, 79–90.
104. Helleday, T. (2003). Pathways for mitotic homologous recombination in mammalian cells. Mutat. Res. *532*, 103–115.
105. Paull, T.T., and Gellert, M. (1999). Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. Genes Dev. *13*, 1276–1288.
106. Van Dyck, E., Stasiak, A.Z., Stasiak, A., and West, S.C. (1999). Binding of double-strand breaks in DNA by human Rad52 protein. Nature *401*, 403.
107. Lee, J.H., and Paull, T.T. (2005). ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science *308*, 551–554.
108. Uziel, T., Lerenthal, Y., Moyal, L., Andegeko, Y., Mittelman, L., and Shiloh, Y. (2003). Requirement of the MRN complex for ATM activation by DNA damage. EMBO J. *22*, 5612–5621.
109. Desai-Mehta, A., Cerosaletti, K.M., and Concannon, P. (2001). Distinct functional domains of nibrin mediate Mre11 binding, focus formation, and nuclear localization. Mol. Cell. Biol. *21*, 2184–2191.
110. Baumann, P., Benson, F.E., and West, S.C. (1996). Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. Cell *87*, 757–766.
111. Baumann, P., and West, S.C. (1998). Role of the human RAD51 protein in homologous recombination and double-stranded-break repair. Trends Biochem. Sci. *23*, 247–251.
112. Ogawa, T., Yu, X., Shinohara, A., and Egelman, E.H. (1993). Similarity of the yeast RAD51 filament to the bacterial RecA filament. Science *259*, 1896–1899.
113. Sung, P., Krejci, L., Van, K.S., and Sehorn, M.G. (2003). Rad51 recombinase and recombination mediators. J. Biol. Chem. *278*, 42729–42732.
114. Holliday, R. (1964). Mechanism for gene conversion in fungi. Genet. Res. *5*, 282–304.
115. McIlwraith, M.J., Vaisman, A., Liu, Y., Fanning, E., Woodgate, R., and West, S.C. (2005). Human DNA polymerase eta promotes DNA synthesis from strand invasion intermediates of homologous recombination. Mol. Cell *20*, 783–792.
116. Constantinou, A., Davies, A.A., and West, S.C. (2001). Branch migration and Holliday junction resolution catalyzed by activities from mammalian cells. Cell *104*, 259–268.
117. Liu, Y., Masson, J.Y., Shah, R., O'Regan, P., and West, S.C. (2004). RAD51C is required for Holliday junction processing in mammalian cells. Science *303*, 243–246.
118. Liu, Y., Tarsounas, M., O'Regan, P., and West, S.C. (2007). Role of RAD51C and XRCC3 in genetic recombination and DNA repair. J. Biol. Chem. *282*, 1973–1979.
119. Constantinou, A., Tarsounas, M., Karow, J.K., Brosh, R.M., Bohr, V.A., Hickson, I.D., and West, S.C. (2000). Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. EMBO Rep. *1*, 80–84.
120. Karow, J.K., Constantinou, A., Li, J.L., West, S.C., and Hickson, I.D. (2000). The Bloom's syndrome gene product promotes branch migration of holliday junctions. Proc. Natl. Acad. Sci. USA *97*, 6504–6508.
121. De Laat, W.L., Appeldoorn, E., Jaspers, N.G., and Hoeijmakers, J.H. (1998). DNA structural elements required for ERCC1-XPF endonuclease activity. J. Biol. Chem. *273*, 7835–7842.
122. Fishman-Lobell, J., and Haber, J.E. (1992). Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene RAD1. Science *258*, 480–484.
123. Cordeiro-Stone, M., Makhov, A.M., Zaritskaya, L.S., and Griffith, J.D. (1999). Analysis of DNA replication forks encountering a pyrimidine dimer in the template to the leading strand. J. Mol. Biol. *289*, 1207–1218.
124. Reardon, J.T., Bessho, T., Kung, H.C., Bolton, P.H., and Sancar, A. (1997). In vitro repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in xeroderma pigmentosum patients. Proc. Natl. Acad. Sci. USA *94*, 9463–9468.
125. Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J., and Stahl, F.W. (1983). The double-strand-break repair model for recombination. Cell *33*, 25–35.
126. Johnson, R.D., and Jasin, M. (2000). Sister chromatid gene conversion is a prominent double-strand break repair pathway in mammalian cells. EMBO J. *19*, 3398–3407.
127. Hanakahi, L.A., Bartlet-Jones, M., Chappell, C., Pappin, D., and West, S.C. (2000). Binding of inositol phosphate to DNA-PK and stimulation of double-strand break repair. Cell *102*, 721–729.
128. Hanakahi, L.A., and West, S.C. (2002). Specific interaction of IP6 with human Ku70/80, the DNA-binding subunit of DNA-PK. EMBO J. *21*, 2038–2044.
129. Jeggo, P.A. (1997). DNA-PK: at the cross-roads of biochemistry and genetics. Mutat. Res. *384*, 1–14.
130. Pastwa, E., and Blasiak, J. (2003). Non-homologous DNA end joining. Acta Biochim. Pol. *50*, 891–908.
131. Walker, J.R., Corpina, R.A., and Goldberg, J. (2001). Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. Nature *412*, 607–614.
132. Mahajan, K.N., Nick McElhinny, S.A., Mitchell, B.S., and Ramsden, D.A. (2002). Association of DNA polymerase mu (pol mu) with Ku and ligase IV: role for pol mu in end-joining double-strand break repair. Mol. Cell. Biol. *22*, 5194–5202.
133. Chappell, C., Hanakahi, L.A., Karimi-Busheri, F., Weinfeld, M., and West, S.C. (2002). Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. EMBO J. *21*, 2827–2832.
134. Grawunder, U., Wilm, M., Wu, X., Kulesza, P., Wilson, T.E., Mann, M., and Lieber, M.R. (1997). Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. Nature *388*, 492–495.

135. Ahnesorg, P., Smith, P., and Jackson, S.P. (2006). XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell *124*, 301–313.
136. Takashima, H., Boerkoel, C.F., John, J., Saifi, G.M., Salih, M.A., Armstrong, D., Mao, Y., Quiocho, F.A., Roa, B.B., Nakagawa, M., et al. (2002). Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. Nat. Genet. *32*, 267–272.
137. Taroni, F., and Didonato, S. (2004). Pathways to motor incoordination: the inherited ataxias. Nat. Rev. Neurosci. *5*, 641–655.
138. Davies, D.R., Interthal, H., Champoux, J.J., and Hol, W.G. (2002). The crystal structure of human tyrosyl-DNA phosphodiesterase, Tdp1. Structure *10*, 237–248.
139. El-Khamisy, S.F., Saifi, G.M., Weinfeld, M., Johansson, F., Helleday, T., Lupski, J.R., and Caldecott, K.W. (2005). Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1. Nature *434*, 108–113.
140. Interthal, H., Pouliot, J.J., and Champoux, J.J. (2001). The tyrosyl-DNA phosphodiesterase Tdp1 is a member of the phospholipase D superfamily. Proc. Natl. Acad. Sci. USA *98*, 12009–12014.
141. Larsen, A.K., and Gobert, C. (1999). DNA topoisomerase I in oncology: Dr Jekyll or Mr Hyde? Pathol. Oncol. Res. *5*, 171–178.
142. El-Khamisy, S.F., and Caldecott, K.W. (2006). TDP1-dependent DNA single-strand break repair and neurodegeneration. Mutagenesis *21*, 219–224.
143. Interthal, H., Chen, H.J., Kehl-Fie, T.E., Zotzmann, J., Leppard, J.B., and Champoux, J.J. (2005). SCAN1 mutant Tdp1 accumulates the enzyme–DNA intermediate and causes camptothecin hypersensitivity. EMBO J. *24*, 2224–2233.
144. Liu, L.F., Desai, S.D., Li, T.K., Mao, Y., Sun, M., and Sim, S.P. (2000). Mechanism of action of camptothecin. Ann. N.Y. Acad Sci *922*, 1–10.
145. Park, S.Y., Lam, W., and Cheng, Y.C. (2002). X-ray repair cross-complementing gene I protein plays an important role in camptothecin resistance. Cancer Res. *62*, 459–465.
146. El-Khamisy, S.F., Hartsuiker, E., and Caldecott, K.W. (2007). TDP1 facilitates repair of ionizing radiation-induced DNA single-strand breaks. DNA Repair (Amst.) *6*, 1485–1495.
147. Katyal, S., El-Khamisy, S.F., Russell, H.R., Li, Y., Ju, L., Caldecott, K.W., and McKinnon, P.J. (2007). TDP1 facilitates chromosomal single-strand break repair in neurons and is neuroprotective in vivo. EMBO J. *26*, 4720–4731.
148. Morrison, P.J. (2003). Paediatric and adult ataxias (update). Eur. J. Paediatr. Neurol. *7*, 231–233.
149. Aicardi, J., Barbosa, C., Andermann, E., Andermann, F., Morcos, R., Ghanem, Q., Fukuyama, Y., Awaya, Y., and Moe, P. (1988). Ataxia-ocular motor apraxia: a syndrome mimicking ataxia-telangiectasia. Ann. Neurol. *24*, 497–502.
150. Schols, L., Bauer, P., Schmidt, T., Schulte, T., and Riess, O. (2004). Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol. *3*, 291–304.
151. Paulson, H.L. (2007). Dominantly inherited ataxias: lessons learned from Machado-Joseph disease/spinocerebellar ataxia type 3. Semin. Neurol. *27*, 133–142.
152. Moreira, M.C., Barbot, C., Tachi, N., Kozuka, N., Mendonca, P., Barros, J., Coutinho, P., Sequeiros, J., and Koenig, M. (2001). Homozygosity mapping of Portuguese and Japanese forms of ataxia-oculomotor apraxia to 9p13, and evidence for genetic heterogeneity. Am. J. Hum. Genet. *68*, 501–508.
153. Date, H., Onodera, O., Tanaka, H., Iwabuchi, K., Uekawa, K., Igarashi, S., Koike, R., Hiroi, T., Yuasa, T., Awaya, Y., et al. (2001). Early-onset ataxia with ocular motor apraxia and hypoalbuminemia is caused by mutations in a new HIT superfamily gene. Nat. Genet. *29*, 184–188.
154. Clements, P.M., Breslin, C., Deeks, E.D., Byrd, P.J., Ju, L., Bieganowski, P., Brenner, C., Moreira, M.C., Taylor, A.M., and Caldecott, K.W. (2004). The ataxia-oculomotor apraxia 1 gene product has a role distinct from ATM and interacts with the DNA strand break repair proteins XRCC1 and XRCC4. DNA Repair (Amst.) *3*, 1493–1502.
155. Brenner, C., Bieganowski, P., Pace, H.C., and Huebner, K. (1999). The histidine triad superfamily of nucleotide-binding proteins. J. Cell. Physiol. *181*, 179–187.
156. Kijas, A.W., Harris, J.L., Harris, J.M., and Lavin, M.F. (2006). Aprataxin forms a discrete branch in the HIT (histidine triad) superfamily of proteins with both DNA/RNA binding and nucleotide hydrolase activities. J. Biol. Chem. *281*, 13939–13948.
157. Moreira, M.C., Barbot, C., Tachi, N., Kozuka, N., Uchida, E., Gibson, T., Mendonca, P., Costa, M., Barros, J., Yanagisawa, T., et al. (2001). The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. Nat. Genet. *29*, 189–193.
158. Ahel, I., Rass, U., El-Khamisy, S.F., Katyal, S., Clements, P.M., McKinnon, P.J., Caldecott, K.W., and West, S.C. (2006). The neurodegenerative disease protein aprataxin resolves abortive DNA ligation intermediates. Nature *443*, 713–716.
159. Gueven, N., Becherel, O.J., Kijas, A.W., Chen, P., Howe, O., Rudolph, J.H., Gatti, R., Date, H., Onodera, O., Taucher-Scholz, G., et al. (2004). Aprataxin, a novel protein that protects against genotoxic stress. Hum. Mol. Genet. *13*, 1081–1093.
160. Gueven, N., Chen, P., Nakamura, J., Becherel, O.J., Kijas, A.W., Grattan-Smith, P., and Lavin, M.F. (2007). A subgroup of spinocerebellar ataxias defective in DNA damage responses. Neuroscience *145*, 1418–1425.
161. Mosesso, P., Piane, M., Palitti, F., Pepe, G., Penna, S., and Chessa, L. (2005). The novel human gene aprataxin is directly involved in DNA single-strand-break repair. Cell. Mol. Life Sci. *62*, 485–491.
162. Rass, U., Ahel, I., and West, S.C. (2007). Actions of aprataxin in multiple DNA repair pathways. J. Biol. Chem. *282*, 9469–9474.
163. Moreira, M.C., Klur, S., Watanabe, M., Nemeth, A.H., Le Ber, I., Moniz, J.C., Tranchant, C., Aubourg, P., Tazir, M., Schols, L., et al. (2004). Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. Nat. Genet. *36*, 225–227.
164. Bomont, P., Watanabe, M., Gershoni-Barush, R., Shizuka, M., Tanaka, M., Sugano, J., Guiraud-Chaumeil, C., and Koenig, M. (2000). Homozygosity mapping of spinocerebellar ataxia with cerebellar atrophy and peripheral neuropathy to 9q33–34, and with hearing impairment and optic atrophy to 6p21–23. Eur. J. Hum. Genet. *8*, 986–990.
165. Nemeth, A.H., Bochukova, E., Dunne, E., Huson, S.M., Elston, J., Hannan, M.A., Jackson, M., Chapman, C.J., and Taylor, A.M. (2000). Autosomal recessive cerebellar ataxia with oculomotor apraxia (ataxia-telangiectasia-like syndrome) is linked to chromosome 9q34. Am. J. Hum. Genet. *67*, 1320–1326.

166. Suraweera, A., Becherel, O.J., Chen, P., Rundle, N., Woods, R., Nakamura, J., Gatei, M., Criscuolo, C., Filla, A., Chessa, L., et al. (2007). Senataxin, defective in ataxia oculomotor apraxia type 2, is involved in the defense against oxidative DNA damage. J. Cell Biol. *177*, 969–979.
167. Blair, I.P., Bennett, C.L., Abel, A., Rabin, B.A., Griffin, J.W., Fischbeck, K.H., Cornblath, D.R., and Chance, P.F. (2000). A gene for autosomal dominant juvenile amyotrophic lateral sclerosis (ALS4) localizes to a 500-kb interval on chromosome 9q34. Neurogenetics *3*, 1–6.
168. Myrianthopoulos, N.C., Lane, M.H., Silberberg, D.H., and Vincent, B.L. (1964). Nerve conduction and other studies in families with Charcot-Marie-Tooth disease. Brain *87*, 589–608.
169. Chen, Y.Z., Bennett, C.L., Huynh, H.M., Blair, I.P., Puls, I., Irobi, J., Dierick, I., Abel, A., Kennerson, M.L., Rabin, B.A., et al. (2004). DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). Am. J. Hum. Genet. *74*, 1128–1135.
170. Chen, Y.Z., Hashemi, S.H., Anderson, S.K., Huang, Y., Moreira, M.C., Lynch, D.R., Glass, I.A., Chance, P.F., and Bennett, C.L. (2006). Senataxin, the yeast Sen1p orthologue: characterization of a unique protein in which recessive mutations cause ataxia and dominant mutations cause motor neuron disease. Neurobiol. Dis. *23*, 97–108.
171. Kraemer, K.H., Lee, M.M., and Scotto, J. (1984). DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis *5*, 511–514.
172. Robbins, J.H. (1988). Xeroderma pigmentosum. Defective DNA repair causes skin cancer and neurodegeneration. JAMA *260*, 384–388.
173. DeSanctis, C., and Cacchione, A. (1932). L'idiozia xerodermica. Riv. Sper. Freniatr. *56*, 269–274.
174. Kraemer, K.H., Lee, M.M., and Scotto, J. (1987). Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. Arch. Dermatol. *123*, 241–250.
175. Rapin, I., Lindenbaum, Y., Dickson, D.W., Kraemer, K.H., and Robbins, J.H. (2000). Cockayne syndrome and xeroderma pigmentosum. Neurology *55*, 1442–1449.
176. Robbins, J.H., Brumback, R.A., Mendiones, M., Barrett, S.F., Carl, J.R., Cho, S., Denckla, M.B., Ganges, M.B., Gerber, L.H., Guthrie, R.A., et al. (1991). Neurological disease in xeroderma pigmentosum. Documentation of a late onset type of the juvenile onset form. Brain *114*, 1335–1361.
177. Masutani, C., Kusumoto, R., Yamada, A., Dohmae, N., Yokoi, M., Yuasa, M., Araki, M., Iwai, S., Takio, K., and Hanaoka, F. (1999). The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. Nature *399*, 700–704.
178. Broughton, B.C., Cordonnier, A., Kleijer, W.J., Jaspers, N.G., Fawcett, H., Raams, A., Garritsen, V.H., Stary, A., Avril, M.F., Boudsocq, F., et al. (2002). Molecular analysis of mutations in DNA polymerase eta in xeroderma pigmentosum-variant patients. Proc. Natl. Acad. Sci. USA *99*, 815–820.
179. Bartels, C.L., and Lambert, M.W. (2007). Domains in the XPA protein important in its role as a processivity factor. Biochem. Biophys. Res. Commun. *356*, 219–225.
180. Thoma, B.S., Wakasugi, M., Christensen, J., Reddy, M.C., and Vasquez, K.M. (2005). Human XPC-hHR23B interacts with XPA-RPA in the recognition of triplex-directed psoralen DNA interstrand crosslinks. Nucleic Acids Res. *33*, 2993–3001.
181. States, J.C., McDuffie, E.R., Myrand, S.P., McDowell, M., and Cleaver, J.E. (1998). Distribution of mutations in the human xeroderma pigmentosum group A gene and their relationships to the functional regions of the DNA damage recognition protein. Hum. Mutat. *12*, 103–113.
182. Niedernhofer, L.J., Garinis, G.A., Raams, A., Lalai, A.S., Robinson, A.R., Appeldoorn, E., Odijk, H., Oostendorp, R., Ahmad, A., van Leeuwen, W., et al. (2006). A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature *444*, 1038–1043.
183. Tian, M., Shinkura, R., Shinkura, N., and Alt, F.W. (2004). Growth retardation, early death, and DNA repair defects in mice deficient for the nucleotide excision repair enzyme XPF. Mol. Cell. Biol. *24*, 1200–1205.
184. Itin, P.H., Sarasin, A., and Pittelkow, M.R. (2001). Trichothiodystrophy: update on the sulfur-deficient brittle hair syndromes. J. Am. Acad. Dermatol. *44*, 891–920.
185. Cleaver, J.E., Thompson, L.H., Richardson, A.S., and States, J.C. (1999). A summary of mutations in the UV-sensitive disorders: xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. Hum. Mutat. *14*, 9–22.
186. Khan, S.G., Levy, H.L., Legerski, R., Quackenbush, E., Reardon, J.T., Emmert, S., Sancar, A., Li, L., Schneider, T.D., Cleaver, J.E., et al. (1998). Xeroderma pigmentosum group C splice mutation associated with autism and hypoglycinemia. J. Invest. Dermatol. *111*, 791–796.
187. Nouspikel, T. (2007). DNA repair in differentiated cells: some new answers to old questions. Neuroscience *145*, 1213–1221.
188. Nouspikel, T., and Hanawalt, P.C. (2003). When parsimony backfires: neglecting DNA repair may doom neurons in Alzheimer's disease. Bioessays *25*, 168–173.
189. Soffer, D., Grotsky, H.W., Rapin, I., and Suzuki, K. (1979). Cockayne syndrome: unusual neuropathological findings and review of the literature. Ann. Neurol. *6*, 340–348.
190. Bohr, V.A., Sander, M., and Kraemer, K.H. (2005). Rare diseases provide rare insights into DNA repair pathways, TFIIH, aging and cancer center. DNA Repair (Amst.) *4*, 293–302.
191. Troelstra, C., van Gool, A., de Wit, J., Vermeulen, W., Bootsma, D., and Hoeijmakers, J.H. (1992). ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. Cell *71*, 939–953.
192. Tanaka, K., Kawai, K., Kumahara, Y., Ikenaga, M., and Okada, Y. (1981). Genetic complementation groups in cockayne syndrome. Somatic Cell Genet. *7*, 445–455.
193. Meira, L.B., Graham, J.M. Jr., Greenberg, C.R., Busch, D.B., Doughty, A.T., Ziffer, D.W., Coleman, D.M., Savre-Train, I., and Friedberg, E.C. (2000). Manitoba aboriginal kindred with original cerebro-oculo- facio-skeletal syndrome has a mutation in the Cockayne syndrome group B (CSB) gene. Am. J. Hum. Genet. *66*, 1221–1228.
194. Mallery, D.L., Tanganelli, B., Colella, S., Steingrimsdottir, H., van Gool, A.J., Troelstra, C., Stefanini, M., and Lehmann, A.R. (1998). Molecular analysis of mutations in the CSB (ERCC6) gene in patients with Cockayne syndrome. Am. J. Hum. Genet. *62*, 77–85.
195. Laine, J.P., and Egly, J.M. (2006). When transcription and repair meet: a complex system. Trends Genet. *22*, 430–436.
196. Van Hoffen, A., Natarajan, A.T., Mayne, L.V., van Zeeland, A.A., Mullenders, L.H., and Venema, J. (1993). Deficient

repair of the transcribed strand of active genes in Cockayne's syndrome cells. Nucleic Acids Res. *21*, 5890–5895.

197. Venema, J., Mullenders, L.H., Natarajan, A.T., van Zeeland, A.A., and Mayne, L.V. (1990). The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. Proc. Natl. Acad. Sci. USA *87*, 4707–4711.
198. Nakatsu, Y., Asahina, H., Citterio, E., Rademakers, S., Vermeulen, W., Kamiuchi, S., Yeo, J.P., Khaw, M.C., Saijo, M., Kodo, N., et al. (2000). XAB2, a novel tetratricopeptide repeat protein involved in transcription-coupled DNA repair and transcription. J. Biol. Chem. *275*, 34931–34937.
199. Saijo, M., Hirai, T., Ogawa, A., Kobayashi, A., Kamiuchi, S., and Tanaka, K. (2007). Functional TFIIH is required for UV-induced translocation of CSA to the nuclear matrix. Mol. Cell. Biol. *27*, 2538–2547.
200. Citterio, E., Van Den Boom, V., Schnitzler, G., Kanaar, R., Bonte, E., Kingston, R.E., Hoeijmakers, J.H., and Vermeulen, W. (2000). ATP-dependent chromatin remodeling by the Cockayne syndrome B DNA repair-transcription-coupling factor. Mol. Cell. Biol. *20*, 7643–7653.
201. Wong, H.K., Muftuoglu, M., Beck, G., Imam, S.Z., Bohr, V.A., and Wilson, D.M. III. (2007). Cockayne syndrome B protein stimulates apurinic endonuclease 1 activity and protects against agents that introduce base excision repair intermediates. Nucleic Acids Res. *35*, 4103–4113.
202. Brooks, P.J. (2007). The case for 8,5′-cyclopurine-2′-deoxynucleosides as endogenous DNA lesions that cause neurodegeneration in xeroderma pigmentosum. Neuroscience *145*, 1407–1417.
203. Proietti-De-Santis, L., Drane, P., and Egly, J.M. (2006). Cockayne syndrome B protein regulates the transcriptional program after UV irradiation. EMBO J. *25*, 1915–1923.
204. Weeda, G., van Ham, R.C., Vermeulen, W., Bootsma, D., van der Eb, A.J., and Hoeijmakers, J.H. (1990). A presumed DNA helicase encoded by ERCC-3 is involved in the human repair disorders xeroderma pigmentosum and Cockayne's syndrome. Cell *62*, 777–791.
205. Lamerdin, J.E., Stilwagen, S.A., Ramirez, M.H., Stubbs, L., and Carrano, A.V. (1996). Sequence analysis of the ERCC2 gene regions in human, mouse, and hamster reveals three linked genes. Genomics *34*, 399–409.
206. Sung, P., Bailly, V., Weber, C., Thompson, L.H., Prakash, L., and Prakash, S. (1993). Human xeroderma pigmentosum group D gene encodes a DNA helicase. Nature *365*, 852–855.
207. Coin, F., Bergmann, E., Tremeau-Bravard, A., and Egly, J.M. (1999). Mutations in XPB and XPD helicases found in xeroderma pigmentosum patients impair the transcription function of TFIIH. EMBO J. *18*, 1357–1366.
208. Oh, K.S., Khan, S.G., Jaspers, N.G., Raams, A., Ueda, T., Lehmann, A., Friedmann, P.S., Emmert, S., Gratchev, A., Lachlan, K., et al. (2006). Phenotypic heterogeneity in the XPB DNA helicase gene (ERCC3): Xeroderma pigmentosum without and with Cockayne syndrome. Hum. Mutat. *27*, 1092–1103.
209. Coin, F., Oksenych, V., and Egly, J.M. (2007). Distinct roles for the XPB/p52 and XPD/p44 subcomplexes of TFIIH in damaged DNA opening during nucleotide excision repair. Mol. Cell *26*, 245–256.
210. Giglia-Mari, G., Coin, F., Ranish, J.A., Hoogstraten, D., Theil, A., Wijgers, N., Jaspers, N.G., Raams, A., Argentini, M., van der Spek, P.J., et al. (2004). A new, tenth subunit of TFIIH is responsible for the DNA repair syndrome trichothiodystrophy group A. Nat. Genet. *36*, 714–719.
211. Coin, F., Marinoni, J.C., Rodolfo, C., Fribourg, S., Pedrini, A.M., and Egly, J.M. (1998). Mutations in the XPD helicase gene result in XP and TTD phenotypes, preventing interaction between XPD and the p44 subunit of TFIIH. Nat. Genet. *20*, 184–188.
212. Rossignol, M., Kolb-Cheynel, I., and Egly, J.M. (1997). Substrate specificity of the cdk-activating kinase (CAK) is altered upon association with TFIIH. EMBO J. *16*, 1628–1637.
213. Fujimoto, M., Leech, S.N., Theron, T., Mori, M., Fawcett, H., Botta, E., Nozaki, Y., Yamagata, T., Moriwaki, S., Stefanini, M., et al. (2005). Two new XPD patients compound heterozygous for the same mutation demonstrate diverse clinical features. J. Invest. Dermatol. *125*, 86–92.
214. Berneburg, M., Lowe, J.E., Nardo, T., Araujo, S., Fousteri, M.I., Green, M.H., Krutmann, J., Wood, R.D., Stefanini, M., and Lehmann, A.R. (2000). UV damage causes uncontrolled DNA breakage in cells from patients with combined features of XP-D and Cockayne syndrome. EMBO J. *19*, 1157–1166.
215. Ito, S., Kuraoka, I., Chymkowitch, P., Compe, E., Takedachi, A., Ishigami, C., Coin, F., Egly, J.M., and Tanaka, K. (2007). XPG stabilizes TFIIH, allowing transactivation of nuclear receptors: implications for Cockayne syndrome in XP-G/CS patients. Mol. Cell *26*, 231–243.
216. Thompson, L.H., Carrano, A.V., Sato, K., Salazar, E.P., White, B.F., Stewart, S.A., Minkler, J.L., and Siciliano, M.J. (1987). Identification of nucleotide-excision-repair genes on human chromosomes 2 and 13 by functional complementation in hamster-human hybrids. Somat. Cell Mol. Genet. *13*, 539–551.
217. Emmert, S., Slor, H., Busch, D.B., Batko, S., Albert, R.B., Coleman, D., Khan, S.G., bu-Libdeh, B., DiGiovanna, J.J., Cunningham, B.B., et al. (2002). Relationship of neurologic degeneration to genotype in three xeroderma pigmentosum group G patients. J. Invest. Dermatol. *118*, 972–982.
218. Sarker, A.H., Tsutakawa, S.E., Kostek, S., Ng, C., Shin, D.S., Peris, M., Campeau, E., Tainer, J.A., Nogales, E., and Cooper, P.K. (2005). Recognition of RNA polymerase II and transcription bubbles by XPG, CSB, and TFIIH: insights for transcription-coupled repair and Cockayne Syndrome. Mol. Cell *20*, 187–198.
219. Klungland, A., Hoss, M., Gunz, D., Constantinou, A., Clarkson, S.G., Doetsch, P.W., Bolton, P.H., Wood, R.D., and Lindahl, T. (1999). Base excision repair of oxidative DNA damage activated by XPG protein. Mol. Cell *3*, 33–42.
220. Lehmann, A.R., Arlett, C.F., Broughton, B.C., Harcourt, S.A., Steingrimsdottir, H., Stefanini, M., Malcolm, A., Taylor, R., Natarajan, A.T., Green, S., et al. (1988). Trichothiodystrophy, a human DNA repair disorder with heterogeneity in the cellular response to ultraviolet light. Cancer Res. *48*, 6090–6096.
221. Marionnet, C., Benoit, A., Benhamou, S., Sarasin, A., and Stary, A. (1995). Characteristics of UV-induced mutation spectra in human XP-D/ERCC2 gene-mutated xeroderma pigmentosum and trichothiodystrophy cells. J. Mol. Biol. *252*, 550–562.
222. Nuzzo, F., and Stefanini, M. (1989). The association of xeroderma pigmentosum with trichothiodystrophy: A clue to a better understanding of XP-D? In DNA Damage and Repair, A. Castellani, ed. (New York: Plenum Press), pp. 61–72.

223. Berneburg, M., Clingen, P.H., Harcourt, S.A., Lowe, J.E., Taylor, E.M., Green, M.H., Krutmann, J., Arlett, C.F., and Lehmann, A.R. (2000). The cancer-free phenotype in trichothiodystrophy is unrelated to its repair defect. Cancer Res. *60*, 431–438.
224. Botta, E., Nardo, T., Broughton, B.C., Marinoni, S., Lehmann, A.R., and Stefanini, M. (1998). Analysis of mutations in the XPD gene in Italian patients with trichothiodystrophy: site of mutation correlates with repair deficiency, but gene dosage appears to determine clinical severity. Am. J. Hum. Genet. *63*, 1036–1048.
225. Nishiwaki, Y., Kobayashi, N., Imoto, K., Iwamoto, T.A., Yamamoto, A., Katsumi, S., Shirai, T., Sugiura, S., Nakamura, Y., Sarasin, A., et al. (2004). Trichothiodystrophy fibroblasts are deficient in the repair of ultraviolet-induced cyclobutane pyrimidine dimers and (6–4)photoproducts. J. Invest. Dermatol. *122*, 526–532.
226. Stefanini, M., Vermeulen, W., Weeda, G., Giliani, S., Nardo, T., Mezzina, M., Sarasin, A., Harper, J.I., Arlett, C.F., Hoeijmakers, J.H., et al. (1993). A new nucleotide-excision-repair gene associated with the disorder trichothiodystrophy. Am. J. Hum. Genet. *53*, 817–821.
227. Vitorino, M., Coin, F., Zlobinskaya, O., Atkinson, R.A., Moras, D., Egly, J.M., Poterszman, A., and Kieffer, B. (2007). Solution structure and self-association properties of the p8 TFIIH subunit responsible for trichothiodystrophy. J. Mol. Biol. *368*, 473–480.
228. Sutherland, B.M., Bennett, P.V., Sidorkina, O., and Laval, J. (2000). Clustered damages and total lesions induced in DNA by ionizing radiation: oxidized bases and strand breaks. Biochemistry *39*, 8026–8031.
229. Yang, Y., Geldmacher, D.S., and Herrup, K. (2001). DNA replication precedes neuronal cell death in Alzheimer's disease. J. Neurosci. *21*, 2661–2668.
230. Bennett, C.B., Lewis, A.L., Baldwin, K.K., and Resnick, M.A. (1993). Lethality induced by a single site-specific double-strand break in a dispensable yeast plasmid. Proc. Natl. Acad. Sci. USA *90*, 5613–5617.
231. Boder, E., and Sedgwick, R.P. (1958). Ataxia-telangiectasia; a familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. Pediatrics *21*, 526–554.
232. Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., Tagle, D.A., Smith, S., Uziel, T., Sfez, S., et al. (1995). A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science *268*, 1749–1753.
233. Khanna, K.K., Keating, K.E., Kozlov, S., Scott, S., Gatei, M., Hobson, K., Taya, Y., Gabrielli, B., Chan, D., Lees-Miller, S.P., et al. (1998). ATM associates with and phosphorylates p53: mapping the region of interaction. Nat. Genet. *20*, 398–400.
234. Wang, Y., Cortez, D., Yazdi, P., Neff, N., Elledge, S.J., and Qin, J. (2000). BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev. *14*, 927–939.
235. Zhao, S., Weng, Y.C., Yuan, S.S., Lin, Y.T., Hsu, H.C., Lin, S.C., Gerbino, E., Song, M.H., Zdzienicka, M.Z., Gatti, R.A., et al. (2000). Functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products. Nature *405*, 473–477.
236. Edwards, M.J., and Taylor, A.M. (1980). Unusual levels of (ADP-ribose)n and DNA synthesis in ataxia telangiectasia cells following gamma-ray irradiation. Nature *287*, 745–747.
237. Lavin, M.F., and Schroeder, A.L. (1988). Damage-resistant DNA synthesis in eukaryotes. Mutat. Res. *193*, 193–206.
238. Painter, R.B., and Young, B.R. (1980). Radiosensitivity in ataxia-telangiectasia: a new explanation. Proc. Natl. Acad. Sci. USA *77*, 7315–7317.
239. Khanna, K.K., Beamish, H., Yan, J., Hobson, K., Williams, R., Dunn, I., and Lavin, M.F. (1995). Nature of G1/S cell cycle checkpoint defect in ataxia-telangiectasia. Oncogene *11*, 609–618.
240. Nagasawa, H., and Little, J.B. (1983). Comparison of kinetics of X-ray-induced cell killing in normal, ataxia telangiectasia and hereditary retinoblastoma fibroblasts. Mutat. Res. *109*, 297–308.
241. Scott, D., and Zampetti-Bosseler, F. (1982). Cell cycle dependence of mitotic delay in X-irradiated normal and ataxia-telangiectasia fibroblasts. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. *42*, 679–683.
242. Zampetti-Bosseler, F., and Scott, D. (1981). Cell death, chromosome damage and mitotic delay in normal human, ataxia telangiectasia and retinoblastoma fibroblasts after x-irradiation. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. *39*, 547–558.
243. Lu, X., and Lane, D.P. (1993). Differential induction of transcriptionally active p53 following UV or ionizing radiation: defects in chromosome instability syndromes? Cell *75*, 765–778.
244. Stewart, G.S., Maser, R.S., Stankovic, T., Bressan, D.A., Kaplan, M.I., Jaspers, N.G., Raams, A., Byrd, P.J., Petrini, J.H., and Taylor, A.M. (1999). The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. Cell *99*, 577–587.
245. Bi, X., Wei, S.C., and Rong, Y.S. (2004). Telomere protection without a telomerase; the role of ATM and Mre11 in Drosophila telomere maintenance. Curr. Biol. *14*, 1348–1353.
246. Olson, E., Nievera, C.J., Liu, E., Lee, A.Y., Chen, L., and Wu, X. (2007). The Mre11 complex mediates the S-phase checkpoint through an interaction with replication protein A. Mol. Cell. Biol. *27*, 6053–6067.
247. Delia, D., Piane, M., Buscemi, G., Savio, C., Palmeri, S., Lulli, P., Carlessi, L., Fontanella, E., and Chessa, L. (2004). MRE11 mutations and impaired ATM-dependent responses in an Italian family with ataxia-telangiectasia-like disorder. Hum. Mol. Genet. *13*, 2155–2163.
248. O'Driscoll, M., Ruiz-Perez, V.L., Woods, C.G., Jeggo, P.A., and Goodship, J.A. (2003). A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. Nat. Genet. *33*, 497–501.
249. Faivre, L., Le, M.M., Lyonnet, S., Plauchu, H., Dagoneau, N., Campos-Xavier, A.B., ttia-Sobol, J., Verloes, A., Munnich, A., and Cormier-Daire, V. (2002). Clinical and genetic heterogeneity of Seckel syndrome. Am. J. Med. Genet. *112*, 379–383.
250. Goodship, J., Gill, H., Carter, J., Jackson, A., Splitt, M., and Wright, M. (2000). Autozygosity mapping of a seckel syndrome locus to chromosome 3q22. 1-q24. Am. J. Hum. Genet. *67*, 498–503.
251. Adams, K.E., Medhurst, A.L., Dart, D.A., and Lakin, N.D. (2006). Recruitment of ATR to sites of ionising radiation-induced DNA damage requires ATM and components of the MRN protein complex. Oncogene *25*, 3894–3904.
252. Cuadrado, M., Martinez-Pastor, B., and Fernandez-Capetillo, O. (2006). ATR activation in response to ionizing radiation: still ATM territory. Cell Div. *1*, 7.

253. Weemaes, C.M., Hustinx, T.W., Scheres, J.M., van Munster, P.J., Bakkeren, J.A., and Taalman, R.D. (1981). A new chromosomal instability disorder: the Nijmegen breakage syndrome. Acta Paediatr. Scand. *70*, 557–564.
254. Carney, J.P., Maser, R.S., Olivares, H., Davis, E.M., Le, B.M., Yates, J.R. III, Hays, L., Morgan, W.F., and Petrini, J.H. (1998). The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. Cell *93*, 477–486.
255. Matsuura, S., Weemaes, C., Smeets, D., Takami, H., Kondo, N., Sakamoto, S., Yano, N., Nakamura, A., Tauchi, H., Endo, S., et al. (1997). Genetic mapping using microcell-mediated chromosome transfer suggests a locus for Nijmegen breakage syndrome at chromosome 8q21–24. Am. J. Hum. Genet. *60*, 1487–1494.
256. Varon, R., Vissinga, C., Platzer, M., Cerosaletti, K.M., Chrzanowska, K.H., Saar, K., Beckmann, G., Seemanova, E., Cooper, P.R., Nowak, N.J., et al. (1998). Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. Cell *93*, 467–476.
257. Kobayashi, J., Tauchi, H., Sakamoto, S., Nakamura, A., Morishima, K., Matsuura, S., Kobayashi, T., Tamai, K., Tanimoto, K., and Komatsu, K. (2002). NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. Curr. Biol. *12*, 1846–1851.
258. Buscemi, G., Savio, C., Zannini, L., Micciche, F., Masnada, D., Nakanishi, M., Tauchi, H., Komatsu, K., Mizutani, S., Khanna, K., et al. (2001). Chk2 activation dependence on Nbs1 after DNA damage. Mol. Cell. Biol. *21*, 5214–5222.
259. Stracker, T.H., Morales, M., Couto, S.S., Hussein, H., and Petrini, J.H. (2007). The carboxy terminus of NBS1 is required for induction of apoptosis by the MRE11 complex. Nature *447*, 218–221.
260. Maser, R.S., Zinkel, R., and Petrini, J.H. (2001). An alternative mode of translation permits production of a variant NBS1 protein from the common Nijmegen breakage syndrome allele. Nat. Genet. *27*, 417–421.
261. O'Driscoll, M., Cerosaletti, K.M., Girard, P.M., Dai, Y., Stumm, M., Kysela, B., Hirsch, B., Gennery, A., Palmer, S.E., Seidel, J., et al. (2001). DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. Mol. Cell *8*, 1175–1185.
262. Girard, P.M., Kysela, B., Harer, C.J., Doherty, A.J., and Jeggo, P.A. (2004). Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. Hum. Mol. Genet. *13*, 2369–2376.
263. Buck, D., Malivert, L., de Chasseval, R., Barraud, A., Fondaneche, M.C., Sanal, O., Plebani, A., Stephan, J.L., Hufnagel, M., le Deist, F., et al. (2006). Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. Cell *124*, 287–299.
264. Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J.P., Deng, H.X., et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature *362*, 59–62.
265. Browne, S., Ferrante, R., and Beal, M.F. (1999). Oxidative stress in Huntington's disease. Brain Pathol. *9*, 147–163.
266. Pasinelli, P., and Brown, R.H. (2006). Molecular biology of amyotrophic lateral sclerosis: insights from genetics. Nat. Rev. Neurosci. *7*, 710–723.
267. Cai, H., Lin, X., Xie, C., Laird, F.M., Lai, C., Wen, H., Chiang, H.C., Shim, H., Farah, M.H., Hoke, A., et al. (2005). Loss of ALS2 function is insufficient to trigger motor neuron degeneration in knock-out mice but predisposes neurons to oxidative stress. J. Neurosci. *25*, 7567–7574.
268. Kanekura, K., Hashimoto, Y., Niikura, T., Aiso, S., Matsuoka, M., and Nishimoto, I. (2004). Alsin, the product of ALS2 gene, suppresses SOD1 mutant neurotoxicity through RhoGEF domain by interacting with SOD1 mutants. J. Biol. Chem. *279*, 19247–19256.
269. Lederer, C.W., and Santama, N. (2007). Amyotrophic lateral sclerosis—the tools of the trait. Biotechnol. J. *2*, 608–621.
270. Geldmacher, D.S. (2007). Treatment guidelines for Alzheimer's disease: redefining perceptions in primary care. Prim. Care Companion J. Clin. Psychiatry *9*, 113–121.
271. Hashimoto, M., Rockenstein, E., Crews, L., and Masliah, E. (2003). Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases. Neuromolecular Med. *4*, 21–36.
272. Mudher, A., and Lovestone, S. (2002). Alzheimer's disease-do tauists and baptists finally shake hands? Trends Neurosci. *25*, 22–26.
273. Miyata, M., and Smith, J.D. (1996). Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat. Genet. *14*, 55–61.
274. Montine, T.J., Huang, D.Y., Valentine, W.M., Amarnath, V., Saunders, A., Weisgraber, K.H., Graham, D.G., and Strittmatter, W.J. (1996). Crosslinking of apolipoprotein E by products of lipid peroxidation. J. Neuropathol. Exp. Neurol. *55*, 202–210.
275. Smith, M.A., Rottkamp, C.A., Nunomura, A., Raina, A.K., and Perry, G. (2000). Oxidative stress in Alzheimer's disease. Biochim. Biophys. Acta *1502*, 139–144.
276. Weissman, L., de Souza-Pinto, N.C., Stevnsner, T., and Bohr, V.A. (2007). DNA repair, mitochondria, and neurodegeneration. Neuroscience *145*, 1318–1329.
277. Rebeck, G.W., LaDu, M.J., Estus, S., Bu, G., and Weeber, E.J. (2006). The generation and function of soluble apoE receptors in the CNS. Mol Neurodegener. *1*, 15.
278. Sanchez-Ramos, J., Overvik, E., and Ames, B.N. (1994). A marker of oxyradical-mediated DNA damage (8-hydroxy-2deoxyguanosine) is increased in nidro-striatum of Parkinson's disease brain. Neurodegeneration *3*, 197–204.
279. Yoritaka, A., Hattori, N., Uchida, K., Tanaka, M., Stadtman, E.R., and Mizuno, Y. (1996). Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc. Natl. Acad. Sci. USA *93*, 2696–2701.
280. Jenner, P. (2003). Oxidative stress in Parkinson's disease. Ann. Neurol. *53* (*Suppl 3*), S26–S36.
281. Harding, A.E. (1981). Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. Brain *104*, 589–620.
282. Koutnikova, H., Campuzano, V., Foury, F., Dolle, P., Cazzalini, O., and Koenig, M. (1997). Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. Nat. Genet. *16*, 345–351.
283. Babcock, M., de Silva, D., Oaks, R., vis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M., and Kaplan, J. (1997). Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. Science *276*, 1709–1712.

284. Cavadini, P., O'Neill, H.A., Benada, O., and Isaya, G. (2002). Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. Hum. Mol. Genet. *11*, 217–227.
285. Shoichet, S.A., Baumer, A.T., Stamenkovic, D., Sauer, H., Pfeiffer, A.F., Kahn, C.R., Muller-Wieland, D., Richter, C., and Ristow, M. (2002). Frataxin promotes antioxidant defense in a thiol-dependent manner resulting in diminished malignant transformation in vitro. Hum. Mol. Genet. *11*, 815–821.
286. Albin, R.L., Reiner, A., Anderson, K.D., Penney, J.B., and Young, A.B. (1990). Striatal and nigral neuron subpopulations in rigid Huntington's disease: implications for the functional anatomy of chorea and rigidity-akinesia. Ann. Neurol. *27*, 357–365.
287. Butterfield, D.A., Howard, B.J., and LaFontaine, M.A. (2001). Brain oxidative stress in animal models of accelerated aging and the age-related neurodegenerative disorders, Alzheimer's disease and Huntington's disease. Curr. Med. Chem. *8*, 815–828.
288. Kovtun, I.V., Liu, Y., Bjoras, M., Klungland, A., Wilson, S.H., and McMurray, C.T. (2007). OGG1 initiates age-dependent CAG trinucleotide expansion in somatic cells. Nature *447*, 447–452.
289. Walker, F.O. (2007). Huntington's Disease. Semin. Neurol. *27*, 143–150.
290. Joenje, H., and Patel, K.J. (2001). The emerging genetic and molecular basis of Fanconi anaemia. Nat. Rev. Genet. *2*, 446–457.
291. Yu, C.E., Oshima, J., Fu, Y.H., Wijsman, E.M., Hisama, F., Alisch, R., Matthews, S., Nakura, J., Miki, T., Ouais, S., et al. (1996). Positional cloning of the Werner's syndrome gene. Science *272*, 258–262.