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Journal
DNA Repair, 12(8)

ISSN
1568-7864

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Publication Date
2013-08-01

DOI
10.1016/j.dnarep.2013.04.015

Peer reviewed

Published in final edited form as:
DNA Repair (Amst). 2013 August ; 12(8): 620–636. doi:10.1016/j.dnarep.2013.04.015.

DNA repair mechanisms in dividing and non-dividing cells

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Abstract

DNA damage created by endogenous or exogenous genotoxic agents can exist in multiple forms, and if allowed to persist, can promote genome instability and directly lead to various human diseases, particularly cancer, neurological abnormalities, immunodeficiency and premature aging. To avoid such deleterious outcomes, cells have evolved an array of DNA repair pathways, which carry out what is typically a multiple-step process to resolve specific DNA lesions and maintain genome integrity. To fully appreciate the biological contributions of the different DNA repair systems, one must keep in mind the cellular context within they operate. For example, the human body is composed of non-dividing and dividing cell types, including, in the brain, neurons and glial cells. We describe herein the molecular mechanisms of the different DNA repair pathways, and review their roles in non-dividing and dividing cells, with an eye towards how these pathways may regulate the development of neurological disease.

Keywords

DNA repair; Neural cells; Neurological disorder; Dividing and non-dividing; Endogenous DNA damage

1. Introduction

The genomes of all organisms are constantly being modified by reactive molecules that are produced endogenously, primarily via mitochondrial respiration, or by environmental/exogenous physical, chemical and biological agents, which include ultraviolet (UV) light, ionizing radiation (IR), heavy metals, air pollutants, chemotherapeutic drugs and inflammatory responses [1]. In fact, it is estimated that ~10^5 DNA lesions are produced in a mammalian genome each day as a result of spontaneous decay, replication errors and cellular metabolism [2]. Among the range of lesions formed, which consists of modified bases (bulky and non-bulky), abasic sites, various strand breaks, intra- and interstrand crosslinks and protein-DNA adducts (Figure 1), ~10^4 are oxidized bases and DNA single strand breaks (SSBs) [1]. Persistent DNA damage can induce mutagenesis, such as base substitutions and small insertions/deletions, as well as gross chromosomal rearrangements. Such genome instability is an essential step in the development of cancer, and likely contributes to aging and age-related disease. In addition, DNA damage can promote cell death responses that presumably underlie pathologies that involve tissue atrophy, such as neurodegeneration. Thus, constant genome maintenance is essential for the viability and

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longevity of a healthy organism. Cells have therefore evolved multiple DNA repair pathways to preserve genome integrity when damage arises.

The tissues and organs of mammals consist of various cell types, including those that are dividing and those that are non-dividing. In adults, most cells, such as myocytes, adipocytes, skin cells and neurons, are in the non-dividing state, *i.e.* terminally-differentiated. Terminal differentiation is the process by which cells during the course of development become specialized, taking on specific structural, functional, and biochemical properties and roles. The brain is composed of both non-dividing and dividing cells. Specifically, differentiated neurons are in a post-mitotic state and cannot re-enter the cell cycle. Glial cells (*e.g.*, astrocytes, oligodendrocytes, and microglia) are in either a proliferative or non-proliferative state, depending on their differentiation status and possible re-entry into the cell cycle. Because both neurons and glial cells are required to establish functional clusters and to carry out the various higher-order brain functions, such as language, thinking, learning and memory, it is critically important to maintain the different cell types in an appropriate number and configuration. In recent years, increasing findings have demonstrated that DNA repair plays an important role in preserving brain cell viability and nervous system function [2-7]. Indeed, several neurodegenerative disorders are linked to defects in DNA SSB repair (SSBR) or double strand break repair (DSBR) [2-4,6,8,9]. In this review, we provide an overview of the major DNA repair mechanisms and discuss their roles in safeguarding the proper operation of dividing and non-dividing cell populations.

2. DNA repair pathways

2.1 O^6-methylguanine-DNA methyltransferase (MGMT)

O^6-methylguanine (O^6-meG) is a major mutagenic and cytotoxic DNA lesion, produced by various endogenous and exogenous methylating agents [10-14]. During DNA replication, O^6-meG efficiently blocks elongation *in vitro* [15-17], although this outcome is not generally thought to occur *in vivo*. Biochemical studies have instead revealed that O^6-meG can be bypassed by translesion synthesis DNA polymerases, such as polη, polκ and polζ, often pairing with thymine to drive G-C to A-T transition mutations [18]. During transcription, O^6-meG partially blocks human RNA polymerase II (RNAPII) elongation. In situations where full-length RNA is obtained by bypass, cytosine and uracil are incorporated opposite the lesion at a 3:1 ratio, producing a fraction of mutant RNA molecules [19]. Depending on the location of the base substitution, resulting transcripts could alter the primary amino acid sequence content of the encoded protein and lead to defective protein function in cells [20]. While primarily considered a mutagenic lesion, O^6-MeG can be cytotoxic in certain circumstances, such as when paired with thymine, as this abnormal base pair can be detected and processed in a futile and ultimately lethal cycle via the mismatch repair (MMR) pathway that will be described later [21,22].

To prevent the above deleterious outcomes, most organisms are equipped with a specific repair protein termed *MGMT*. MGMT directly removes, for instance, O^6-alkylation adducts in a one-step reaction that transfers the alkyl group from the O^6-position of guanine to a cysteine residue within its active site pocket, thereby restoring guanosine to its undamaged state and in turn inactivating the MGMT “suicide” protein. The inactivated alkylated MGMT is subsequently ubiquitinated [23] and degraded by the proteasome [24]. As would be expected for a repair protein, MGMT is localized in the nucleus. Here, MGMT is often observed in small foci, termed nuclear speckles or embedded structures, which are presumed to be sites of active transcription [25]. O^6-meG produced by treatment of cells with low doses of N-methylnitrosourea (MNU) rapidly disappeared in these speckles [25], leading to the speculation that MGMT is tightly coupled to the transcription machinery.

The expression and activity of MGMT are quite variable among the different tissues and tumor types [26]. For example, the human MGMT protein is highly expressed in the liver and colon, yet is expressed at comparatively low levels in the brain [26]. The lower MGMT activity in the brain was inferred from the observation that O^6-meG, produced by administration of N-ethyl-N-nitrosourea or MNU, is removed from rat brain genomic DNA more slowly than from the liver or kidney genome [27-29]. The expression of *MGMT* seems to be dictated mainly by the methylation status of the promoter and also by different transcription factors that can activate expression upon exposure to alkylating agents, X-rays or glucocorticoid hormone treatment (review in [26]). In addition, analysis of MGMT activity using cell extracts from human cell lines and mouse embryo cells suggested that there is a significant reduction in MGMT prior to or early in S-phase, followed by a recovery during the G2/S phase [30,31]. However, the mRNA level during the cell cycle has been reported not to change in normal human fibroblasts, indicating that there is perhaps not a strict cell cycle regulation of *MGMT* expression [32].

As can be concluded from the above discussion, MGMT plays a protective role against the harmful effects of DNA alkylating agents in mammalian cells and tissues. In particular, while MGMT null mice ($Mgmt^{-/-}$) are viable, fertile, outwardly normal and have a normal lifespan [33], these animals display an increased level of cell death in rapidly proliferating tissues, such as the bone marrow, intestine, thymus and spleen, as well as a tremendous loss of leukocytes and platelets in the hematopoietic stem cell compartment, following treatment with a high dose of the alkylating agent MNU [33]. When $Mgmt^{-/-}$ mice were exposed to MNU at a low dose, a large number of thymic lymphomas as well as lung adenomas were detected, likely due to errant replicative bypass of unrepaired O^6-meG adducts [34]. While the role of MGMT in the prevention of O^6-meG-induced carcinogenesis is well established in actively replicating cells [34-37], one might predict an important role for this protein in non-dividing cells given that spontaneous DNA alkylation products are common due to reactions with the endogenous co-substrate, S-adenosylmethionine.

Evidence indicates that unrepaired O^6-meG adducts can promote cell death in non-dividing cells [38]. For example, neuronal development and motor function are severely disrupted in $Mgmt^{-/-}$ mice following alkylating agent treatment, a phenotype that is not observed in the wild-type counterparts. Moreover, primary cultured neurons from $Mgmt^{-/-}$ mice are more sensitive than wild-type neurons to the alkylating agents methylazoxymethanol (MAM) and nitrogen mustard (HN2), suggesting a requirement for O^6-meG repair in non-dividing cell survival [39]. It seems plausible that accumulated O^6-meG lesions will lead to interrupted transcription events or would be recognized by the MMR system, resulting in activation of a cell death response, although the exact mechanism for O^6-meG-induced killing in non-dividing cells needs to be resolved.

2.2 Nucleotide *excision* repair (NER)

The NER pathway resolves numerous DNA lesions, particularly base modifications that distort the normal helical structure of duplex DNA [40]. Examples of NER substrates include: cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6,4)-pyrimidone photoproducts (6-4PPs) generated by UV radiation; base adducts created by exogenous chemical agents such as cisplatin and benzopyrene; base lesions produced by reactions with endogenous lipid peroxidation products, *e.g.* the malendialdehyde-related pyrimidopurinone adduct (M1G); and reactive oxygen species (ROS)-induced base modifications such as the cyclopurines. These “bulky” DNA alterations typically impede progression of a replicating or transcribing polymerase, resulting in replication fork collapse or stalled transcription, but in some circumstances can be bypassed in an error-prone manner. The NER response involves four primary steps: i) recognition of the damage, ii) incision on both sides of the lesion and removal of the damage-containing oligonucleotide fragment, iii) gap-filling

synthesis to restore a damage-free DNA duplex, and iv) ligation to seal the remaining nick. The classic NER pathway involves roughly thirty proteins operating in a coordinated manner. In addition to functioning in global genome repair (GG-NER) [41], NER maintains a specialized pathway, termed transcription-coupled NER (TC-NER), which specifically deals with lesions on the transcribed strand of DNA that block RNA polymerase (RNAP) progression [8]. These two pathways of NER are thought to differ only at the step of recognition, but utilize common machinery to execute the final steps of the repair response (Figure 2). Defects in NER are genetically linked to a group of associated autosomal recessive human diseases (discussed in greater detail below): xeroderma pigmentosum (XP), Cockayne syndrome (CS) and a photosensitive form of trichothiodystrophy (TTD). Each of these disorders is characterized by extreme UV radiation sensitivity, and in some cases, neurological dysfunction is observed [8,42]. We summarize the molecular mechanisms of GG-NER and TC-NER, and discuss the role of the pathways in dividing and non-dividing cells, particularly as it relates to the pathology of the three human disorders.

2.2.1 Global genome NER (GG-NER)—As the name implies, GG-NER removes helix-distorting “blocking” lesions located throughout the genome, presumably in a cell cycle-independent manner (reviewed in [41]). XPC–RAD23B initiates the repair response by recognizing a damage-induced structural change in DNA, binding the strand opposite the lesion and not the chemical adduct itself [43-45]. Once bound, XPC–RAD23B mediates the recruitment of the transcription factor II H (TFIIH) complex, which contains ten subunits, including two helicases XPB (3’-5’) [46] and XPD (5’-3’) [47,48]. Through the activity of the helicase subunits, TFIIH promotes opening of the DNA duplex around the lesion, creating a “bubble” platform for recruitment of XPA and replication protein A (RPA), and assembly of the pre-incision complex. XPA promotes the release of the TFIIH component, the cyclin-dependent kinase (CDK)-activating kinase (CAK) subcomplex [49], and the association of RPA with the single-stranded damaged DNA [50,51]. The dissociation of CAK is thought to facilitate the recruitment of the XPF–excision repair cross complementing 1 (ERCC1) complex and XPG, as well as the release of XPC–RAD23B. The XPF–ERCC1 complex is recruited to the lesion via a direct interaction with XPA [52,53], while XPG is specifically engaged via an interaction with TFIIH and stabilization of the pre-incision complex [54]. The two endonucleases, XPF–ERCC1 and XPG, are then responsible for carrying out incision 5’ and 3’, respectively, to the DNA damage. After the dual incision event and removal of the damage-containing oligonucleotide fragment, DNA polymerases δ, ε or κ carry out gap-filling repair synthesis in cooperation with replication factor C (RFC) and proliferating cellular nuclear antigen (PCNA) [55,56]. Finally, the nick is sealed in dividing cells by either a X-ray repair cross-complementing protein 1 (XRCC1)–DNA ligase III (LIG3) or a flap endonuclease 1 (FEN1)–DNA ligase I (LIG1) complex [55,57], or in non-dividing cells by XRCC1–LIG3α [58].

2.2.2 Transcription-coupled nucleotide excision repair (TC-NER)—Except for the initial damage recognition step, TC-NER engages many of the same protein components as GG-NER. Although not as well understood mechanistically, the current model proposes that TC-NER is initiated by stalling of an elongating RNAP at a lesion on the transcribed strand within an active gene. This arrested RNAP serves as a critical signal via an unknown mechanism to engage the CS proteins, CSA and CSB, which facilitate the eventual removal of the damage and restart of transcription. After recruitment of the TFIIH complex, the same protein machinery as described for GG-NER is presumably called upon for incision, excision of the lesion-containing strand, gap-filling and nick ligation. As with GG-NER, TC-NER appears to function independent of the cell cycle, but as will be discussed later, may have a more prominent role in non-dividing cells.

As mentioned above, the CS proteins have been proposed to play critical roles in TC-NER. This conclusion is based on considerable biochemical and biological data. For instance, chromatin immunoprecipitation (ChIP) experiments have revealed that CSB can interact with chromatin-bound RNAPII following *in vivo* formaldehyde crosslinking [59]. In this study, UV irradiation enhanced the association between these two proteins, presumably reflective of a DNA damage-induced cooperative response [59]. Furthermore, co-immunoprecipitation experiments using whole cell extracts from CS1AN SV40-transformed CSB patient cells, which had been complemented with HA-/His_6-double–tagged CSB, found CSB to exist in a complex with RNAPII [60]. Gel filtration studies using extracts from CSB-expressing CS1AN or HeLa cells have also suggested that CSB and RNAPII are together in high molecular weight protein complexes [60]. In addition, in an *in vitro* transcription assay, CSB stimulated transcriptional elongation by RNAPII, promoting the addition of one nucleotide to the nascent transcript, implying a functional interaction between the two proteins [60-62]. Finally, kinetic experiments in live cells using a photobleaching technique have shown that CSB transiently interacts with the transcription machinery [63]. It is thought that the association of CSB with a stalled RNAPII is responsible for recruitment of the various NER factors needed to carry out TC-NER. The endogenous DNA lesions that invoke a TC-NER response, however, are still being determined, yet likely include the cyclopurines and other bulky oxidative base modifications noted earlier.

CSB is a member of the SWI2/SNF2 family of DNA-dependent ATPases, and contains the RecA-like helicase motif found in both DNA and RNA helicases [64,65]. While purified recombinant CSB protein has not been shown to possess classic helicase activity, the protein has been reported to display chromatin remodeling [66] and strand annealing [67] activities, although the precise biochemical role of CSB in TC-NER remains unclear. It is worth noting that apparently independent of its functions in NER, CSB can interact with several members of the base excision repair (BER) pathway (see below), including the strand break response protein poly(ADP-ribose) polymerase-1 (PARP1) [68], endonuclease VIII-like 1 (NEIL1) DNA glycosylase [69], and the apurinic/apyrimidinic (AP) endonuclease 1 (APE1) [70]. In addition, emerging evidence indicates that CSB has a role in maintaining mitochondrial DNA stability and mitochondrial function [71,72]. As for CSA, this protein contains five WD-40 repeats that function as scaffolds for its protein interactions, physically associates with DDB1, and forms a complex with cullin 4A (CUL4A) containing E3 ubiquitin ligase [73-75]. In a mechanism that is not well understood, CSA translocates to the stalled RNAPII–CSB complex at the DNA damage site in a CSB-dependent manner [59,76]. The CSA-containing ubiquitin ligase complex has been implicated in ubiquitination and subsequent degradation of CSB, as well as in termination of the TC-NER process and restoration of transcription [77]. Work is still required to determine the most critical biological roles and the precise biochemical activities of the CS proteins.

2.2.3 Xeroderma pigmentosum (XP)—XP is a rare, autosomal recessive disorder characterized by sun sensitivity and markedly increased risk of UV radiation–induced skin and mucous membrane cancers [78]. Moreover, XP patients exhibit an increased risk for spontaneous internal cancers, such as brain tumors, leukemias, gastric carcinomas, and lung cancers, implying the formation of endogenous DNA substrates for the NER pathway [79]. Eight different genetic complementation groups of XP are known, representing the core components of NER (XPA to XPG) and a variant form that is defective in a specialized translesion DNA polymerase (XPV). The loss of these proteins results in bulky base damage accumulation, such as UV-induced photoproducts, and thus, (i) replication fork collapse or mutagenesis in dividing cells and (ii) transcription problems in both dividing and non-dividing cells. It is likely that the cancer predisposition of XP patients results from mutagenic lesion bypass events that take place in the dividing cell population.

Notably, approximately a quarter of XP patients develop neurological symptoms, including microcephaly, mental deterioration, cerebellar ataxia, sensory deafness and peripheral neuropathy, all of which appear to involve global brain atrophy [42,80]. This neuropathology is observed in approximately 25% of XP patients with a mutation in *XPA, XPB, XPD, XPF* or *XPG*, where the genetic mutation appears to adversely affect the TC-NER pathway specifically [78,81-83]. XPC patients, which have a defect in GG-NER, but not TC-NER, show little neurological impairment, although mild brain atrophy [84] and a brain tumor [85] have been reported. Based on the collective data (see next section on CS as well), it has been speculated that the TC-NER pathway is more critical for preserving non-dividing cell and neural function in the face of normal endogenous DNA damage. Finally, specific *XPB, XPD*, and *XPG* mutations are reported to result in a dual XP/CS phenotype that entails differing degrees of neural, developmental, and skin abnormalities [42,86-88].

2.2.4 Cockayne syndrome (CS)—CS is a rare autosomal recessive disease that is associated with mutations in either of two, what have been traditionally considered, TC-NER genes: *CSA* and *CSB*. Recent clinical evaluation has classified CS patients into two major categories: juvenile-onset, with varying degrees of severity (severe, moderate and mild), and adult-onset, a relatively mild form of the disease [89]. Juvenile-onset CS is characterized by photosensitivity, microcephaly, developmental delays, dwarfism, sensorineural hearing loss, contractures, skill loss and gait ataxia, where the severity groups correlate with physical size, milestones met and life expectancy (e.g., 5, 16 and 30 years, respectively). The clinical course of CS typifies premature aging in many ways, and thus, the disorder is considered a segmental progeria. Notably, unlike XP patients, individuals with CS do not display elevated cancer risk, implying that the molecular defects of these two diseases are distinct.

Cells from CS patients show an inability to recover RNA synthesis following UV irradiation, a feature that is characteristic of failed TC-NER and used as a clinical diagnostic for the disorder. It's also important to emphasize the CS mutant cells exhibit normal GG-NER activity. Notably, mouse models for CS diplay a milder phenotype compared with human patients. For instance, $Csa^{-/-}$ mice exhibit UV-sensitivity and an age-dependent loss of retinal photoreceptor cells, but fail to display the severe developmental and neurological abnormalities of the human syndrome [90]. $Csb^{m/m}$ mouse fibroblasts, which harbor a genetic mutation (K337X) found in a CS patient, display UV-sensitivity, impaired transcription recovery after UV irradiation, a complete loss of TC-NER for CPDs, and normal GG-NER. However, $Csb^{m/m}$ mice do not show pronounced symptoms, such as a reduced life span, reproductive problems, or severe neurologic dysfunction [91]. The lack of phenotypic resemblance to the human disease has been proposed to stem from the reduced overall lifespan of mice, such that, the relevant pathologies have insufficient time to develop. Of course, it may simply reflect unknown molecular differences between the two mammals.

Recently, it was observed that $Csa^{-/-}$ and $Csb^{m/m}$ mice accumulate increased numbers of activated microglial cells, which are a common indicator of an inflammatory response, in the areas surrounding oligodendrocytes with myelinated axons [92]. This phenotype somewhat resembles key features of the human disorder, in that patients with CS exhibit demyelination, which is the loss of the myelin sheath that insulates nerve endings and preserves function. Notably, activated microglia were not observed in mouse models of a GG-NER-deficiency, *i.e.* in $Xpc^{-/-}$ animals. Combining defects in NER and TCR resulted in progressive neuronal degeneration, disclosing a functional overlap and functional complementarity between the two repair pathways. While the mechanistic reasons for the disparate phenotypes of the mouse models (and the human patients for that matter) remain largely unsolved, the overall picture supports the hypothesis that oxidative stress, possibly

mediated through a hyperinflammatory response, and the associated oxidative DNA damage gives rise to the CS neuropathologies. The identity of the DNA lesions and the precise molecular mechanisms, however, await further investigation.

2.2.5 Trichothiodystrophy (TTD)—TTD patients show similar phenotypes to XP and CS, as well as a wide variety of additional clinical features, including sulphur-deficient brittle hair, which is often used as a diagnostic for this particular NER disorder (reviewed in [93]). Besides the hair abnormality, other common clinical phenotypes include growth defects, ichthyosis, ocular defects, increased infections, and photosensitivity [8,42,78,82,86,88,93]. Notably, over 80% of TTD patients show neurological abnormalities, namely microcephaly, mental retardation, deafness and ataxia [93]. Based on the neuropathology, TTD mainly leads to dysmyelination, which is characterized by a defective structure and function of the myelin sheaths, presumably stemming from impaired biosynthesis or formation of myelin [42]. Like CS, TTD is associated with defective TC-NER, originating from mutations in *XPB, XPD, TTDA* [78], or *TTDN1*, which is a gene of unknown function [94]. *TTDA* encodes for a small subunit of the TFIIH complex [95], and interacts with p52 and XPD and maintains TFIIH stability [95,96]). Notably, XP patients with mutations in *XPB* or *XPD* exhibit increased risk of skin cancer, whereas TTD patients harboring mutations in the same genes have not been reported to display cancer predisposition. This observation (i) suggests that there exists separation-of-function mutations that impact either GG-NER or TC-NER and (ii) supports the model that defects in GG-NER are related to cancer risk, whereas defects in TC-NER are associated more with neurological complications.

2.2.6 Transcription domains-associated repair (DAR)—It has been reported that NER has a specialized pathway that functions after cellular differentiation. In particular, in differentiated NT2 human neurons, GG-NER is strongly attenuated, with essentially no general genome repair of CPDs and a markedly slower repair of 6-4PPs and bulky base adducts [97]. However, in the same cells, transcribed genes remain proficiently repaired on both the transcribed and non-transcribed strands. This apparently selective and targeted corrective response was initially termed *differentiation-associated repair*, and later renamed *transcription domains-associated repair* (DAR). SiRNA experiments in differentiated monocytic leukemia THP1 cells indicate that XPC, but not CSB or XPG, is involved in this specialized repair pathway [98]. However, the precise molecular mechanisms of DAR and its biological relevance remain poorly defined. For more information regarding DAR, the reader is directed to the following review articles [97,99].

2.3 Base excision repair (BER)

DNA base modifications are common damages caused by oxidation, deamination or alkylation. In fact, there are >100 types of oxidative base modifications that can potentially arise in DNA as the result of attack of ROS, which are mainly generated by normal mitochondrial respiration. Among the base lesions, 8-oxo-dG is one of the most abundant and well-characterized [100]. It has been estimated that about 180 guanines are oxidized to 8-oxo-dG per mammalian genome per day [1], and steady-state measurements using HPLC or LC/MS-MS analysis have detected several thousand 8-oxo-dG residues in nuclear DNA isolated from normal human tissue or cultured cells [101,102]. 8-oxo-dG is a potent premutagenic lesion, because it can pair with adenine (as well as cytosine) during DNA replication and cause G:C to T:A transversion mutations [103,104]. DNA containing 8-oxo-dG can also give rise to mutant RNA transcripts [105]. Indeed, analysis of luciferase expression from an 8-oxo-dG:C-containing DNA construct showed that aberrant mRNAs generated during transcription can lead to the production of mutant proteins, suggesting that

erroneous transcription can give rise to phenotypical changes with the potential to alter the fate of mammalian cells [106].

Deamination is another potentially harmful spontaneous reaction, producing uracil, inosine and xanthosine from cytidine, adenine and guanine, respectively. Uracil and inosine base lesions, which can pair with adenine and cytidine, respectively, can lead to C:G to T:A and A:T to G:C transition mutations. It has been estimated by LC-MS/MS analysis that the levels of endogenous uracil and inosine residues in nuclear DNA are similar to the levels of 8-oxo-dG in human cells [107-110], suggesting that deaminated bases represent a mutagenic threat that is comparable to 8-oxo-dG. In addition to potentially causing mutagenic outcomes, base modifications have the ability to hinder or block DNA or RNA polymerase progression and therefore activate cell death responses. Base lesions that appear to be generally strong blocks to progressing polymerases include thymine glycol and 5-hydroxyuracil.

To protect against the harmful consequences of non-bulky base damage, as well as abasic sites and SSBs (see more below), the BER pathway has evolved to maintain genome integrity. Given the frequent nature of oxidation, deamination and spontaneous hydrolysis, BER expectedly operates during all stages of the cell cycle, and thus, serves a critical function in both dividing and non-dividing cells [6]. The BER pathway engages various enzymes and proteins and involves the following major steps: (i) recognition and excision of an inappropriate base, (ii) incision at the resulting abasic site, (iii) replacement of the excised nucleotide, (iv) processing of the terminal end(s), and (v) sealing of the final nick (Figure 3) [3,6]. Conventional BER is initiated by a lesion-specific DNA glycosylase (mono- or bi-functional), which recognizes and hydrolyzes the N-glycosidic bond of a substrate base, creating an AP site intermediate. Monofunctional DNA glycosylases, such as uracil-DNA glycosylase (UNG) and N-methylpurine-DNA glycosylase (MPG), possess only glycosylase activity. Bifunctional DNA glycosylases, such as 8-oxoguanine DNA glycosylase (OGG1), mutY homolog (MUTYH), endonuclease III-like 1 (NTH1) and NEIL1, exhibit both glycosylase activity and an intrinsic 3′ AP lyase activity. Each glycosylase has its own substrate selectivity [111], and in most cases, exists in multiple isoforms that are directed to either the nucleus or mitochondria [6,112].

The monofunctional DNA glycosylases produce a hydrolytic (natural), non-coding AP site by removing the substrate base. Such AP sites, which can also be formed at high frequency by spontaneous or damage-induced hydrolysis of the N-glycosidic bond, are then incised by APE1. APE1 is a class II AP endonuclease that cleaves the DNA backbone immediately 5’ to the abasic lesion, creating a 5’-deoxyribose-5-phosphate (5’-dRP) and 3’-hydroxyl (OH) strand break product [113,114]. DNA polymerase β (Pol β) is the main enzyme responsible for removing the 5’-dRP moiety via an intrinsic lyase activity [115-117]. Following base removal by a bifunctional DNA glycosylase, the protein can incise the DNA backbone immediately 3’ to the AP site product via a β- or β,δ-elimination reaction, producing a SSB with a 3’-phospho-α,β-unsaturated aldehyde (3’-PUA) or 3’-phosphate (3’-P) group, respectively. APE1 removes the 3’-PUA residue generated by β-elimination via its 3’-phosphodiesterase activity, while polynucleotide kinase 3’-phosphatase (PNKP) excises the 3’-P moiety [118,119], establishing a 3’-OH priming group for repair synthesis and ligation.

After generating the necessary 3’-OH and 5’-P termini, BER typically proceeds via the short-patch (SP or single-nucleotide) pathway, which engages Pol β to replace the missing nucleotide and the XRCC1–LIG3α complex to seal the remaining nick [112]. However, in instances where the 5’-terminal moiety is not a substrate for the dRP lyase activity of Pol β, or under circumstances when ATP concentrations are low (resulting in reduced ligation efficiency) or during the S phase of the cell cycle (when replication-associated proteins are

more highly abundant), BER can proceed via a long-patch (LP), strand-displacement synthesis process [120]. LP-BER is most commonly carried out by Pol δ/ε in cooperation with the clamp loading factor RFC and the processivity factor PCNA, and results in synthesis of 2–13 nucleotides. At high concentrations or via stimulation by certain protein-protein interactions, Pol β has the ability to perform strand-displacement synthesis as well [121]. Whatever the case, the resulting 5'-flap structure generated during LP synthesis is removed by the flap endonuclease FEN1 [121,122], and since LIG1 is physically associated with PCNA [123,124], this ligase appears to be the major nick sealing enzyme for completing the LP process [123]. We emphasize that given the involvement of several replication-associated proteins in LP-BER, such as PCNA, FEN1 and LIG1, which are all down-regulated in non-cycling cells, the SP pathway and Pol β likely take on an increased role in non-dividing cells [125].

Despite the documented importance of this pathway in maintaining genome integrity, there are only a few inherited disorders associated with a genetic defect in a classic BER component. These disorders involve cancer predisposition (the MUTYH DNA glycosylase and colorectal cancer [126];, immunological defects (the uracil DNA glycosylase UNG and hyper-IgM syndrome V [127], and neurological abnormalities (see below). The fact that homozygous knockout of the central, core BER participants (APE1, Pol β, XRCC1, LIG1 and LIG3) leads to embryonic or post-natal lethality underscores the frequent nature of relevant endogenous DNA damage and suggests that complete elimination of the pathway is incompatible with life. As such, several investigators are pursuing the hypothesis that more subtle reductions in BER capacity are associated with disease risk, likely in an exposure-dependent manner [111]. In fact, there have been reported associations between reduced BER capacity and neuropathologies, such as Alzheimer disease [128-130]. Moreover, there is emerging evidence that defects in BER give rise to increased susceptibility to stroke-induced complications, presumably due to acute oxidative stress [131-133].

2.3.1 DNA single strand break repair (SSBR)—SSBs are one of the most common DNA lesions, arising at an estimated rate of tens of thousands per cell per day [1,134]. SSBs are formed via a variety of mechanisms, including as (i) direct products of reactions between the deoxyribose sugar of DNA and endogenous ROS, namely the hydroxyl radical, (ii) normal enzymatic intermediates of most repair pathways, including BER, and (iii) catalytic intermediates of proteins, such as topoisomerase 1 (TOP1), which forms a temporary covalent complex with DNA to resolve supercoiling that arises during replication and transcription. Given the different mechanisms for SSB formation, it is not surprising that the chemical composition of SSB ends can be quite diverse, ranging from 3'-P, -phosphoglycolate (PG) and - protein/peptide lesions to 5'-OH and -adenosine monophosphate (AMP) damaged termini. Persistent SSBs can lead to the collapse of replication forks during chromosome duplication and the formation of one-ended DSBs, as well as to blocked transcription events.

To prevent the deleterious consequences of SSB damages, including genomic instability and the activation of cell death responses, cells are equipped with specialized enzymes, such as APE1, PNKP, tyrosyl-DNA phosphodiesterase 1 (TDP1), aprataxin (APTX) and DNA ligases, which work to generate the appropriate 3' and 5' termini and ultimately seal the strand break interruption. Given the frequency with which relevant substrates are formed endogenously, it is assumed that the above proteins are present throughout the cell cycle and important to both dividing and non-dividing cells. Notably, defects in components of the specialized SSBR pathways have been directly associated with maintaining proper brain function. In particular, as will be discussed next, mutations in *TDP1, APTX* and *PNKP* are genetically linked to specific neurological disorders that exhibit no apparent cancer predisposition.

2.3.2 Tyrosyl-DNA phosphodiesterase 1 (TDP1)—TOP1 is responsible for relaxing higher order DNA structures during transcription and replication. As part of the enzymatic mechanism, TOP1 forms a stable protein-DNA cleavage complex (TOP1cc), in which TOP1 becomes temporarily covalently bound to the 3'-terminus of the catalytically-generated DNA strand break. Persistent or trapped TOP1cc are harmful to normal cellular functions, as they block both DNA and RNA polymerases. TDP1, which was initially identified in yeast [135], is responsible for removing stable TOP1cc by hydrolyzing the phosphodiester bond between a 3′ end of the SSB and the catalytic tyrosyl residue of TOP1. This activity of TDP1 leaves behind a 3′-P terminus, which is converted to a 3′-OH group by PNKP. PNKP also phosphorylates the 5′-OH terminus generated by TOP1, thus facilitating gap-filling and ligation by Pol β and the XRCC1–LIG3α complex, respectively. Studies also suggest that TDP1 can remove 3'-PG damages formed by free radical attack of DNA, again creating a 3'-P that requires processing by PNKP [136]. Alternatively, APE1 or APTX can convert 3'-PG residues to 3'-OH termini, with the different proteins likely serving as back-up enzymes for each other, while exhibiting preferential activity on certain DNA arrangements (*e.g.*, SSBs versus DSBs) [137,138]. Immunohistochemical analysis of TDP1 expression in the human brain indicates that the protein is highly expressed in neurons, with very low levels in glia cells.

Spinocerebellar ataxia with axonal neuropathy-1 (SCAN1) is an autosomal recessive disorder with a progressive neurodegenerative phenotype that results from mutation of *TDP1* [139]. As detailed above, human TDP1 is required for repair of chromosomal strand breaks that arise from abortive TOP1 reactions, which may be more prominent during periods of oxidative stress and increased oxidative DNA damage [140]. Consistently, human SCAN1 lymphoblastoid cells [140,141] and post-mitotic neurons from *TDP1*$^{-/-}$ mice [142] accumulate topoisomerase-DNA complexes and hydrogen peroxide (H_2O_2)-induced SSBs. The fact that SCAN1 exhibits severe neurological defects, yet displays no cancer predisposition, has prompted the hypothesis that DNA strand break repair processes function primarily to maintain viability of certain cell types, yet do not play a major role in preserving genetic integrity, at least in the absence of exogenous challenges. The tissue-selective nature of SCAN1, as well as ataxia with ocular motor apraxia (AOA1) and microcephaly with early-onset, intractable seizures and developmental delay (MCSZ) (discussed below), has been argued to stem from: (i) cells that are highly metabolically active, *e.g.*, neurons, likely generate a greater number of lethal oxidative DNA strand breaks that require fully active repair mechanisms to avoid transcriptional arrest and apoptotic signaling and (ii) replicating cells, unlike differentiated non-cycling neurons, utilize compensatory pathways, *e.g.*, homologous recombination (HR), to faithfully resolve any unrepaired strand break interruptions.

2.3.3 Aprataxin (APTX)—APTX is a member of the histidine triad superfamily of nucleotide hydrolases and transferases, but is unique from other family members in that it operates on DNA, not nucleotide monophosphates linked to an amino acid or carbohydrate [143]. Specifically, APTX possesses a DNA deadenylation activity, converting 5'-AMP groups, which result from unsuccessful ligation reactions, to normal 5'-P ends at nicks or breaks. Unrepaired 5'-AMP moieties at SSBs could pose a significant block to transcription and replication, driving cellular dysfunction. APTX interacts with the XRCC1–LIG3α SSBR complex via phosphorylated residues in XRCC1, and also appears to interact with phosphorylated XRCC4 [144], suggesting a role in DSBR (see below) as well.

AOA1 is a rare autosomal recessive disorder caused by mutations in the *APTX* gene and is characterized by early-onset slowly progressive ataxia, ocular motor apraxia, peripheral neuropathy and hypoalbuminemia. Neuropathological examination reveals severe loss of Purkinje cells and moderate neuronal loss in the anterior horn and dorsal root ganglia

[145,146]. Moreover, as noted earlier, AOA1 does not show cancer predisposition and genome instability [147]. APTX localizes in both the nucleoplasm and the nucleolus [147,148], and recently, has been found to reside in the cytoplasm of Purkinje cells [149] and the mitochondria of human neuroblastoma SH-SY5Y and primary human skeletal muscle cells [150]. Several reports on the sensitivities of ATPX-deficient cells to genotoxic agents are conflicting. In particular, a few studies have found that AOA1 lymphoblastoma cells or APTX-knockdown HeLa cells are hypersensitive to methyl methanesulfonate (MMS) or H_2O_2 [147,151]. However, in separate studies, cells from AOA1 patients and *Aptx* knockout mice, as well as APTX-knockdown neuroblastoma SH-SY5Y cells, were not found to be profoundly sensitive to MMS, H_2O_2 or menadione relative to comparable controls [150,152,153]. As has been described above for SCAN1, a deficiency in nuclear SSBR may be behind the tissue selectivity of the disorder. However, in light of the new evidence for a role of APTX in mitochondrial DNA repair, a significant contribution of mitochondrial dysfunction must be considered as well.

2.3.4 Polynucleotide kinase 3'-phosphatase (PNKP)—Multiple mutations in the *PNKP* gene were recently identified by genome-wide linkage analysis to be tied to the hereditary disease, MCSZ [154]. Clinical features of this disorder include microcephaly, infantile-onset seizures, developmental delay and variable behavioral problems, especially hyperactivity. Magnetic resonance imaging (MRI) of patient brains revealed microcephaly with preserved brain structures, with no apparent neuronal migration or other structural abnormalities, and no evidence of degeneration. This observation strongly implicates decreased neurogenesis as the major reason for the manifestation of this disorder. Significantly, there have been no observations of cancer or immunodeficiency associated with MCSZ, features that are typical for a deficiency in DNA repair. Lymphoblastoid cells isolated from MCSZ patients are sensitive to radiation and other DNA-damaging agents, such as H_2O_2 and CPT, indicative of a direct involvement of PNKP in DNA damage processing [154,155].

Whereas AOA1 and SCAN1 show a degenerative and progressive phenotype, MCSZ is thought to be primarily a developmental disease. In particular, the pronounced microcephaly of MCSZ syndrome likely stems from an inadequate generation of neurons during development, although the remote possibility remains that the cellular loss occurs after normal development. *In situ* hybridization indicates that human and mouse *PNKP* mRNA is expressed in dividing neuronal precursors and in differentiated neurons, supporting both a role for the protein in development and the maintenance of adult cells. In addition, siRNA knockdown experiments in mouse E13.5 cerebral cortical cells reveals that there is a significant increase in apoptosis in neuronal precursors and differentiated neurons deficient for PNKP, suggesting that the neuropathology of MCSZ patients arises specifically from DNA strand break-induced cell death. Thus, like other strand break repair enzymes, it appears that the DNA processing activity of PKNP plays a critical role in non-dividing cell populations.

2.4 Mismatch repair (MMR)

The architecture of the MMR pathway is well conserved from bacteria to mammals. This system recognizes and repairs base-base mismatches and insertion-deletion loops (IDLs) that arise primarily as errors or intermediates of DNA replication or HR [21,22]. In fact, MMR leads to an ~100-fold increase in DNA replication fidelity by preventing base substitutions or repeat sequence instability. The MMR response is broadly considered to be comprised of two major components: MutS and MutL [156]. In eukaryotes, there exist two functional equivalents of *E. coli* MutS (the so-called MSH proteins), i.e., MutSα or MutSβ. MutSα, which is composed of MSH2 and MSH6, is responsible for recognizing single base-

base mismatches and 1–2 base IDLs. The MutSβ complex, consisting of MSH2 and MSH3, deals primarily with IDLs of two or more bases, with some overlap in substrate specificity among the two MutS complexes.

Following substrate recognition by one of the MutS complexes, the eukaryotic MutL homologs (MLH1 and PMS1) are recruited to help organize other proteins, such as PCNA, at the damage site. The MutL equivalents in humans exist in three heterodimeric forms: MutLα (MLH1–PMS2), MutLβ (MLH1–MLH3), and MutLγ (MLH1–PMS1). MutLα and MutLγ have endonuclease activity, with the enzyme active site present in PMS2 and MLH3. MutLα is the major MutL homolog that participates in MMR. MutLγ can contribute to the repair of base-base mismatches and small IDLs to some extent in vitro, yet its in vivo contribution appears to be minimal [157]. MutLβ has not been shown to take part in MMR in vitro, and a role for this complex in the MMR response in vivo seems unlikely [158].

To preserve genome integrity, MMR must occur selectively on the newly synthesized strand of DNA that contains the mispaired nucleotide. Thus, it is critical to discriminate between the nascent and template DNA strands. In prokaryotes, the *E. coli* endonuclease, MutH, recognizes the newly synthesized strand by its unmethylated status. In eukaryotic cells, however, this does not appear to be the mechanism of strand discrimination. Instead, one study found that the MMR machinery is associated with the replication apparatus, suggesting that this interaction facilitates strand discrimination [159]. More recently, two additional reports indicate that, for leading strand synthesis, bound PCNA determines the orientation of MutLα incision, while enhancing its endonuclease activity [160]. For the lagging strand, it was proposed that the 5’ ends of existing Okazaki fragments serve as markers for strand discrimination [161]. After the appropriate strand has been selected and incision has been performed, PCNA coordinates with Exo1, which harbors an intrinsic double-strand 5’-3’ exonuclease activity, to excise the mismatch from the nascent strand, generating a multi-nucleotide gap. The removed DNA segment is then resynthesized by polymerase δ and the nicked sealed by LIG1 [156,162].

Perhaps not surprisingly in light of the discussion above, germ line mutations in the MMR genes, namely *MLH1, MSH2, MSH6* or *PMS2*, predispose to hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome [163-165]. In addition, aberrant *MLH1* promoter methylation, which leads to gene inactivation, appears to underlie some sporadic colorectal cancers [166-168]. A hallmark and common diagnostic of HNPCC or a MMR defect is microsatellite instability, typically involving changes in the length of dinucleotide repeats, which presumably arises due to inefficient resolution of loop structures that form during DNA synthesis. Although the general cancer predisposition phenotype of the MMR-deficient mice is similar to human patients (REFS 168, 169), the neoplasm spectrum differs between the two species for reasons that are not presently clear [169]. Notably, the patients and animal models deficient in MMR show no signs of neurological dysfunction.

It is well recognized that a major responsibility of MMR is in dividing cells, where it functions to suppress genetic instability arising from replication errors and the consequent carcinogenesis. The physical interactions of the MutSα and MutLα complexes with the replication and repair factor PCNA, only further support the notion that MMR plays a more pivotal role in dividing cells relative to non-dividing cells. Nevertheless, there are a few reports indicating expression [170,171] and activity [172] of MMR in the brain. However, whether any of the MMR components function in any significant way in preserving brain function remains unclear. It is noteworthy that MMR proteins play a crucial role in not only removing mispaired nucleotides and IDLs, but in recognizing certain forms of DNA damage and eliciting a cellular response. In particular, MMR-deficient cancer cells and primary or immortalized mouse embryonic fibroblasts (MEFs) have a reduced ability to carry out

apoptosis induced by chemical carcinogens, UV light or oxidative stress [173-178]. It therefore seems plausible that certain MMR factors may function as part of a damage-specific signaling or apoptotic pathway in non-dividing cells, particularly in response to certain forms of oxidative DNA damage, such as 8-oxo-dG [179].

2.5 Double strand break repair (DSBR)

DSBs are one of the most deleterious forms of DNA damage, activating cell death responses if unrepaired and promoting genome instability, such as translocations, if misrepaired [180,181]. DSBs can arise endogenously through the action of ROS that are produced by normal cellular metabolism, or during certain scenarios of failed DNA replication or juxtaposed repair events. DSBs are also formed as natural intermediates of V(D)J recombination, a programmed phenomenon that occurs during antibody diversification, and meiosis, the process employed by eukaryotes to generate genetic diversity within gametes. In addition, DSBs are induced by exogenous sources such as IR or anti-cancer chemotherapeutic agents. DSBR is divided into two major pathways: HR and nonhomologous end joining (NHEJ) (Figure 4). HR operates in dividing cells and in S phase, as it requires a homologous sister chromatid for execution, whereas NHEJ can function in both dividing and non-dividing cells and independent of cell cycle. Several human diseases have been reported to derive from deficiencies in HR or NHEJ, and these exhibit neurological, immunological and developmental defects, as well as radiation sensitivity, premature aging phenotypes and cancer predisposition [182-187].

2.5.1 Homologous recombination (HR)—HR resolves DSBs during the S and G2 phases of the cell cycle. The pathway appears to have mainly evolved to cope with the one-ended DSBs that are formed upon replication fork collapse (we note that only two-ended DSBR is shown in Figure 4), most commonly at a polymerase blocking lesion, during duplication of chromosomal DNA in dividing cells. The repair mechanism is pivotal to maintain replication fidelity and employs an intact sister chromatid as a template for information exchange and faithful repair. HR has been proposed to be initiated by recognition of the DSB by the MRN complex, which is comprised of the MRE11, RAD50, and Nijmegen breakage syndrome 1 (NBS1) proteins [188]. This complex acts as a break sensor and recruits the protein kinase, ataxia telangiectasia mutated (ATM), to DSB sites, facilitating the subsequent steps of the recombination process [189-191]. While the detailed molecular mechanisms of the HR pathway remain somewhat elusive, it was recently reported that NBS1 ubiquitination by the SCF-Skp2 E3 ligase can promote ATM recruitment/activation in response to DSBs induced by IR [192]. ATM – defective in the rare human disease (AT) characterized by progressive cerebellar degeneration, extreme cellular sensitivity to radiation and a predisposition to cancer – then transmits the DNA damage signal to downstream targets, such as cell-cycle-checkpoint proteins, chromatin-remodeling factors and other DNA repair components, which help to arrest ongoing replication and promote execution of DSB resolution.

To carry out the key steps of HR, the MRN complex associates with the C-terminal binding protein (CtBP)-interacting protein (CtIP), which is brought in to initiate 5′–3′ end resection and generate the needed 3′ ssDNA overhang for strand exchange [193]. Although the MRN complex participates in both HR and NHEJ (see more below), CtIP-dependent end trimming appears to selectively commence HR, while suppressing NHEJ [194,195]. Following this initial step, further resection is carried out by exonucleases (possibly EXO1 in cooperation with Bloom syndrome (BLM) helicase) [196,197], and the resulting ssDNA is stabilized by binding of RPA, which in turn activates ATR via an interaction with the ATR-interacting protein (ATRIP) to signal the full checkpoint response. Subsequently, RAD52 promotes replacement of RPA with Rad51 [198-200], assisting in the formation of the necessary

Rad51 nucleoprotein filaments. Since BRCA2 stimulates the assembly of RAD51 onto the RPA-ssDNA complex, BRCA2 seems to be a key mediator of HR [201]. RAD51-coated ssDNA then enables strand invasion of the intact homologous DNA region, which provides the genetic instruction for accurate repair [202-205].

Following exchange and invasion, the DNA strand is extended by a polymerase, most likely polη [206], to create a D-loop. After D-loop formation, there are two predominant models proposed for HR in mammalian cells. The first model involves the formation of a Holliday junction, a complex DNA structure that needs to be resolved by DNA nucleases, such as GEN1 [207] and SLX1/SLX4 [208,209], and DNA helicases, such as the RecQ family members Werner syndrome (WRN) and BLM, two proteins that are genetically linked to rare premature aging and cancer predisposition disorders [210,211]. The second, alternative model, designated the synthesis-dependent strand annealing (SDSA) pathway, completes DSBR via noncrossover products, without Holliday junction formation. These two distinct mechanisms of DSB resolution after formation of a D-loop are reviewed more extensively in these articles [212,213]. Since most studies have focused on the role of HR in dividing cells, little information is available regarding the functions (if any) of HR-related proteins in non-dividing cell populations. However, the existence of some HR factors, such as MRE11, which is defective in a rare variant form of AT (*i.e.*, the so-called AT-like disorder, ATLD), has been documented in post-mitotic cells [214]. The replication-independent roles of HR-related proteins (assuming found) in non-dividing cells would need to be elucidated. Lastly, it is worth pointing out that like BER, core HR components appear to be essential for animal viability [7,215].

A distinct form of DSB recombination is single-strand annealing (SSA), which has been most intensely investigated in yeast. This pathway resolves two-ended DSBs positioned between highly repetitive regions, such as rDNA loci or *Alu* elements, where two homologous nucleotide stretches are on both sides of the break. As a result, this process does not involve sister chromatid exchange. SSA is facilitated by RPA and RAD52, in a RAD51-independent manner [203,216-218]. In particular, the procedure is initiated by the binding of RPA to the 3′ end of the nuclease-derived single-stranded 3′ overhang and interaction of the ssDNA-RPA complex with RAD52 [217,218]. Exposed complementary sequences upstream and downstream of the DSB are then aligned and annealed by the RAD52-RPA-ssDNA ternary complex, potentially creating flap or short-gap DNA intermediates. Nonhomologous displaced 3′ flap tails are most likely removed by the action of the NER-associated nuclease complex, ERCC1–XPF, which is equivalent to the yeast RAD1–RAD10 complex. After further nuclease and/or polymerase processing to create ligatable ends, the break is sealed by a DNA ligase. In the end, the SSA process leads to the deletion of the genetic material between the repeat sequences, and is therefore error-prone. Since SSA does not require a complementary sister chromatid, it is presumably functional in both dividing and non-dividing cells, although a role for the pathway in non-dividing cells has not been explicitly investigated to our knowledge.

2.5.2 Nonhomologous end joining (NHEJ)—NHEJ is the major DSBR system in higher eukaryotes [219], particularly during phases of the cell cycle when a homologous sister chromatid is absent. NHEJ proteins are also involved in introducing *antibody diversity* via V(D)J recombination [220]. Some reports have recently described how NHEJ contributes to the maintenance of telomere integrity as well [221-224]. NHEJ entails three main steps, which ultimately culminate in the direct ligation of two DNA ends in close spatial proximity: i) recognition of the two-ended DSB, ii) processing to remove non-ligatable termini or other forms of DNA damage at the break and to reveal short stretches of microhomology, and iii) joining of two suitable ends. In general, there is competition between the recognition complexes of HR and NHEJ for DSB termini, with pathway

selection mostly being influenced by the stage of the cell cycle. While NHEJ can operate during all phases of the cell cycle, it is most active during G1 [225,226]. Due to the end processing step, NHEJ often results in an error-prone outcome, with partial loss of genome information at the site of the DSB.

To initiate NHEJ, the Ku70/Ku80 (Ku) heterodimer binds directly to the two DSB ends and recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). This multiprotein complex both stabilizes and aligns the DNA ends [227-229]. The interaction between two DNA-PKcs positioned at each DSB terminus activates its intrinsic protein kinase activity, leading to DNA-PKcs autophosphorylation and dissociation. Depending on the complexity of the DSB and the nature of the ends, different processing factors are then recruited. In one example, DNA-PKcs activates the endonuclease, Artemis, which trims the 3' and 5' single-stranded overhangs at the DNA ends to reveal complementary nucleotide stretches [230]. After generation of terminal overhangs by Artemis, and prior to ligation, PNKP is recruited via an interaction with the XRCC4–Ligase IV (LIG4) protein complex to remove any existing 3′-P groups or add 5′-P residues [231,232]. In instances where short nucleotide gaps remain after microhomology mediated annealing, DNA polymerases μ and λ function to fill in the small gap segments [233,234]. Finally, once appropriate termini have been generated, XLF-Cernunnos (XLF) can interact with the XRCC4–LIG4 complex to stimulate end joining, the final step of the repair process [235]. In recent work, ATM has been implicated in NHEJ as a signaling factor and may play a critical role in the repair of DSBs within regions of heterochromatin [236].

In addition to the canonical NHEJ pathway described above, cells maintain an alternative end-joining pathway that utilizes larger stretches of microhomology and engages various factors that also function in HR or SSBR, such as the MRN complex, PARP-1, WRN, and LIG1 [237,238]. The possible role of XRCC1 and LIG3 in alternative end-joining is currently controversial. In particular, in one study, XRCC1 deficiency in either wild-type or XRCC4-deficient (canonical NHEJ) activated B cells did not alter either class switch recombination (CSR) or IgH/c-myc translocations, suggesting that XRCC1 is not involved in the alternative end-joining pathway [239]. However, in a separate study, heterozygous *xrcc1*$^{+/-}$ B cells showed a decreased length of microhomology at the switch junctions and also a reduction in IgH/c-myc translocations during CSR, implying that XRCC1 does participate in alternative NHEJ [240]. Additionally, XRCC1 was originally identified as part of a biochemical alternative end-joining complex [241,242]. While further studies are necessary to resolve these apparently conflicting results, the alternative NHEJ pathway appears to have evolved as a back-up mechanism for classic NHEJ, and does not seem to be active unless core end-joining proteins, such as the Ku factors, are deficient [242-247]. As alternative end-joining is more error-prone than NHEJ, it is believed to play a significant role in driving genomic instability, namely translocations, and thus the tumorigenic process. At present, the degree to which alternative end-joining is influenced by a non-dividing status, particularly in comparison to the canonical NHEJ pathway, and thus contributes to DSBR in non-dividing cells is unknown.

Patients with a deficiency in certain NHEJ factors, such as Artemis [248], LIG4 [249] and XLF [250], show a severe combined immunodeficient (SCID) phenotype, stemming from a defect in V(D)J recombination, as well as radiosensitivity and microcephaly, presumably due to defective DSBR. Similarly, mutant mouse models in Ku70, Ku80, DNA-PKcs and Artemis show a dual radiosensitive/SCID phenotype (reviewed in [251]), which interestingly, is not seen in mice lacking XLF [252]. In addition, deficiency in XRCC4 [253] or LIG4 [253-257] in mice leads to embryonic lethality, in part, because of defective neurogenesis and excessive apoptosis of neurons. In particular, it was found that most of the apoptotic cell death in mice harboring a homozygous hypomorphic mutation in LIG4 (LIG4

Y288C) occurs in the intermediate zone, which contains nonreplicating differentiated neurons [258]. In a study using a mouse model for neuroretina, small molecule inhibition of DNA-PK results in the death of neurons isolated from embryos at day 14.5, suggesting a role for NHEJ in early retinal neurogenesis [259]. In the case of DNA-PKcs-deficient mice, however, they exhibit no obvious neuropathology, suggesting normal neurogenesis in the brain [260]. Moreover, cultured primary hippocampal neurons from DNA-PKcs-deficient mice show normal neurite growth and survival, although the neurons are hypersensitive to DNA-damaging and oxidative stress-inducing agents, which promote apoptosis [261,262]. These findings suggested that NHEJ factors play variable, albeit critical, roles in maintaining viability of non-proliferating cells like neurons.

Recently, it was reported that NHEJ genes are expressed in terminally-differentiated adipocytes [263], as well as in terminally-differentiated astrocytes established from murine embryonic stem (ES) cell-derived neural stem cells (NSCs) [214]. The adipocytes, which were created by differentiation of murine 3T3F442A pre-adipocyte cells, showed increased expression of DNA-PKcs and enhanced DSBR capacity, as inferred by the disappearance of γH2AX and DSBs (by pulse-field gel electrophoresis) after IR or treatment with the radiomimetic drug calicheamicin γ1. Interestingly, while the HR related-gene *MRE11* was strongly downregulated in terminally-differentiated astrocytes compared with NSCs, the NHEJ genes, *DNA-PKcs, Ku70, Ku80, LIG4*, and *XRCC4*, did not show any obvious differences in expression between the two cell types [214]. In terminally-differentiated astrocytes, DNA-PK inhibition resulted in a limited reduction in γH2AX foci after IR treatment, further supporting the idea that the NHEJ pathway plays a more critical role in non-dividing cells.

4. Concluding remarks

We have provided a general overview herein of the different DNA repair mechanisms, and how they appear to function in dividing and non-dividing cells (summarized in Table 1). Given the frequency of spontaneous hydrolysis, alkylation and oxidation of DNA, the MGMT protein and the BER pathway expectantly play important roles in both cell types. However, it is interesting that to date, only genetic mutations in proteins that operate in SSBR (*i.e.*, TDP1, APTX and PNKP), a sub-pathway of BER, have been directly linked to neurological disorders. Such an observation indicates a critical and non-redundant role for efficient SSB processing in non-dividing neuronal cells. Whether more subtle deficiencies in the core components of BER associate with brain disease needs to be more thoroughly clarified. Additionally, how unrepaired O^6-meG lesions induce neuronal cell death needs to be defined.

In the case of NER, GG-repair appears to be most critical to the viability and function of replicating cells, as damage accumulation and replicative bypass is likely behind the mutagenesis associated with the cancer development in XP patients. Defects in TC-NER appear to underlie the cell loss and severe brain atrophy seen in at least a subset of XP, CS and TTD patients. In this scenario, it is presumed that unrepaired endogenous DNA damage in transcriptionally active genomic regions results in transcription arrest and subsequent apoptotic cell death. It is noteworthy that for many of the disorders described herein, including those stemming from a defect in either GG-NER or TC-NER, the precise DNA lesion that drives the clinical pathologies is unclear. That said, it seems likely that some form of oxidative DNA damage, such as either a simple base or sugar modification or a more complex lipid peroxidation adduct, is the culprit.

The story for MMR is less clear. While it is evident that MMR plays a critical role in suppressing genetic (microsatellite) instability and carcinogenesis in dividing cells, whether

it functions in some capacity in non-dividing cells, perhaps as a damage-specific signaling complex, requires further investigation. In terms of recombination, the two major pathways of HR and NHEJ appear to have divided their responsibilities based on (i) the stage of the cell cycle and (ii) the nature of the DNA DSB. In particular, HR mainly operates during S phase and on replication-derived one-ended DSBs to faithfully resolve the damage, whereas the more error-prone NHEJ process functions primarily during G1 and on frank, juxtaposed two-ended DSBs. Mutations in the genes associated with HR or NHEJ give rise to range of clinical phenotypes, including radiosensitivity, ataxia and other brain abnormalities, immunodeficiency, cancer predisposition and premature aging characteristics. The role of HR proteins (if any) and the relative contributions of the alternative and canonical end-joining pathways in non-dividing cell viability and functional integrity awaits further investigation.

It is important to note that non-dividing cells can exist in multiple forms in a human body. In particular, as mentioned earlier, stem cells can be induced to terminally differentiate into specialized, non-dividing cell types, such as neurons. In addition, cells can undergo an essentially irreversible growth arrest that is simulated by a myriad of factors (*e.g.*, telomere loss), termed senescence, which in effect involves the limited ability of cells to proliferate. It is thought that senescence represents an alternative response to apoptosis and has evolved to suppress tumorigenesis, and thus, may contribute to aging. Moreover, cells can exist in a state of quiescence, which entails G0 arrest, but unlike senescence, is a cellular process that can be disengaged. Interestingly, brain adult NSCs are largely thought to be quiescent. We have focused herein mainly on the roles of the different DNA repair processes in terminally-differentiated cells within the brain. As such, how quiescence or senescence, or the phenomenon of differentiation for that matter, affects DNA repair gene expression or pathway function needs to be better elucidated.

Another important consideration regarding the disorders described within is the specific involvement of developmental defects versus adulthood degeneration in the disease manifestation. Specifically, many of the inherited neurological diseases, such as MCSZ, appear to stem from faulty development of the nervous system, whereas others, such as XP, seem to entail impaired protection of a fully mature nervous system from the harmful effects of endogenous DNA damage. Distinguishing the roles of the various DNA damage responses in either facilitating proper nervous system development or protecting against adult cell loss (progressive degeneration) is an important research endeavor for the future. Furthermore, more thorough characterization of the roles of the different DNA repair mechanisms in the various brain cell types, such as neurons, astrocytes, oligodendrocytes, microglia and *Schwann* cells, is another key focus going forward. How this information can ultimately be used in designing strategic clinical interventions is a major challenge ahead.

Finally, in most eukaryotic cells, mitochondria are the major source of energy, generating ATP via the process of oxidative phosphorylation. While mitochondria maintain their own genome, most mitochondrial proteins are encoded by the nuclear genome. Nevertheless, maintaining mitochondrial DNA integrity is critical for preserving mitochondrial function. As such, it is becoming more and more evident that several DNA repair proteins that operate within the nucleus, also function within mitochondria, often being translocated to this organelle as a distinct protein isoform. Indeed, several of the human disorders described above are now recognized as having mitochondrial dysfunction as part of the clinical disease. For example, the CS proteins [71,72,264,265], TDP1 [266], Aprataxin [150] and PNKP [267,268], have all been recently shown to reside in the mitochondria and contribute to maintaining proper mitochondrial function. Determining the relative contribution of their role(s) in the nucleus versus the mitochondria in terms of the disease etiology, particularly the associated neuropathology, is a critical undertaking going forward. For further

discussion of mitochondrial DNA repair, the reader is directed to several comprehensive reviews [112,269,270].

Acknowledgments

We thank Drs. Peter Sykora and Magdalena Misiak for their critical reading of the manuscript. This effort was supported entirely by the Intramural Research Program at the NIH, National Institute on Aging.

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Abbreviations

AOA1	ataxia with ocular motor apraxia 1
AP	apurinic/apyrimidinic
APE1	AP endonuclease 1
APTX	aprataxin
ATM	ataxia telangiectasia mutated
CPDs	cyclobutane pyrimidine dimers
CS	Cockayne syndrome

CSR	class switch recombination
DAR	transcription domains-associated repair
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
dRP	deoxyribose-5-phosphate
DSBR	DNA double strand break repair
ERCC1	excision repair cross complementing 1
FEN1	flap endonuclease 1
GG-NER	global genome-NER
HNPCC	hereditary nonpolyposis colorectal cancer
HR	homologous recombination
IR	ionizing radiation
MAP	MUTYH-associated polyposis
MCSZ	microcephaly with early-onset, intractable seizures and developmental delay
MGMT	O^6-methylguanine-DNA methyltransferase
MMR	mismatch repair
MPG	N-methylpurine-DNA glycosylase
MUTYH	human mutY homolog
NEIL1	endonuclease VIII-like 1
NER	nucleotide excision repair
NHEJ	nonhomologous end joining
NTH1	endonuclease III-like 1
NSC	neural stem cells
OGG1	8-oxoguanine DNA glycosylase
PARP1	poly(ADP-ribose) polymerase-1
PCNA	proliferating cellular nuclear antigen
PG	phosphoglycolate
PNKP	polynucleotide kinase 3’-phosphatase
Pol β	DNA polymerase β
RFC	replication factor C
RNAP	RNA polymerase
RPA	replication protein A
SCAN1	spinocerebellar ataxia with axonal neuropathy-1
SCID	severe combined immunodeficient
SDSA	synthesis-dependent strand annealing
SSA	single-strand annealing
SSBR	DNA *single strand* break repair

SSBs	DNA single strand breaks
TC-NER	transcription-coupled NER
TDP1	tyrosyl-DNA phosphodiesterase 1
TFIIH	transcription factor II H
TOP1	topoisomerase 1
Top1cc	Top1 cleavage complex
TTD	trichothiodystrophy
UNG	uracil-DNA glycosylase
XP	xeroderma pigmentosum
XRCC1	X-ray repair cross-complementing protein 1
PUA	phospho-α,β-unsaturated aldehyde
6-4PPs	pyrimidine-(6,4)-pyrimidone photoproducts

Highlights

A review of the contribution of the different DNA repair mechanisms in dividing and non-dividing cells is provided.

The most recent findings on the molecular mechanisms of DNA repair in proliferating and terminally-differentiated brain cells are described.

The relationship between a deficiency in a particular DNA repair pathway and human disease, with an emphasis on neuropathologies, is reviewed.

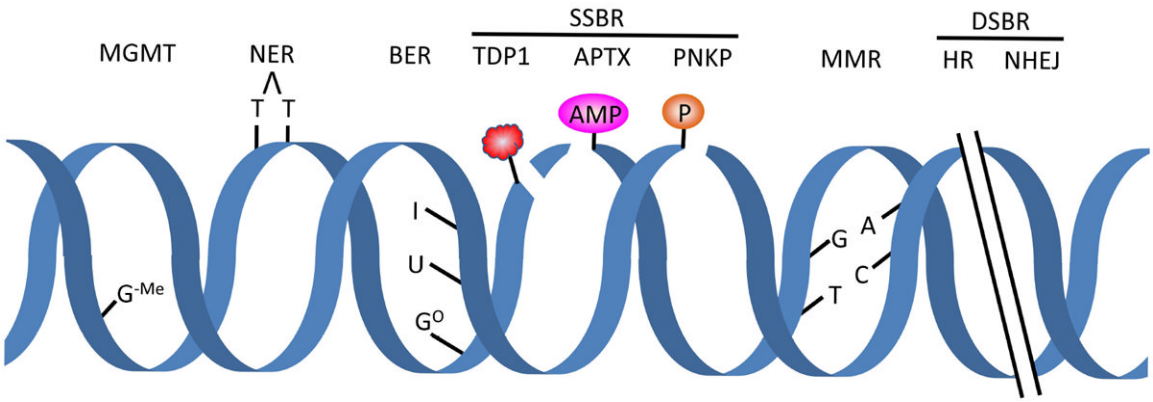

Figure 1. DNA damage and repair responses

DNA repair pathways (top) and examples of corresponding DNA damage (bottom). The detailed molecular mechanisms for the repair responses are provided in text. APTX, aprataxin; BER, base excision repair; DSBR, DNA double strand break repair; HR, homologous recombination; *MGMT,* O^6-methylguanine-DNA methyltransferase; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; PNKP, polynucleotide kinase 3'-phosphatase; SSBR, DNA *single strand* break repair; SSBs, DNA single strand breaks; TC-NER, transcription-coupled NER; TDP1, tyrosyl-DNA phosphodiesterase 1; G-Me, O^6-Methylguanine; T^T, thymine dimer; I, inosine; U, uracil; G^o, 8-oxoguanine.

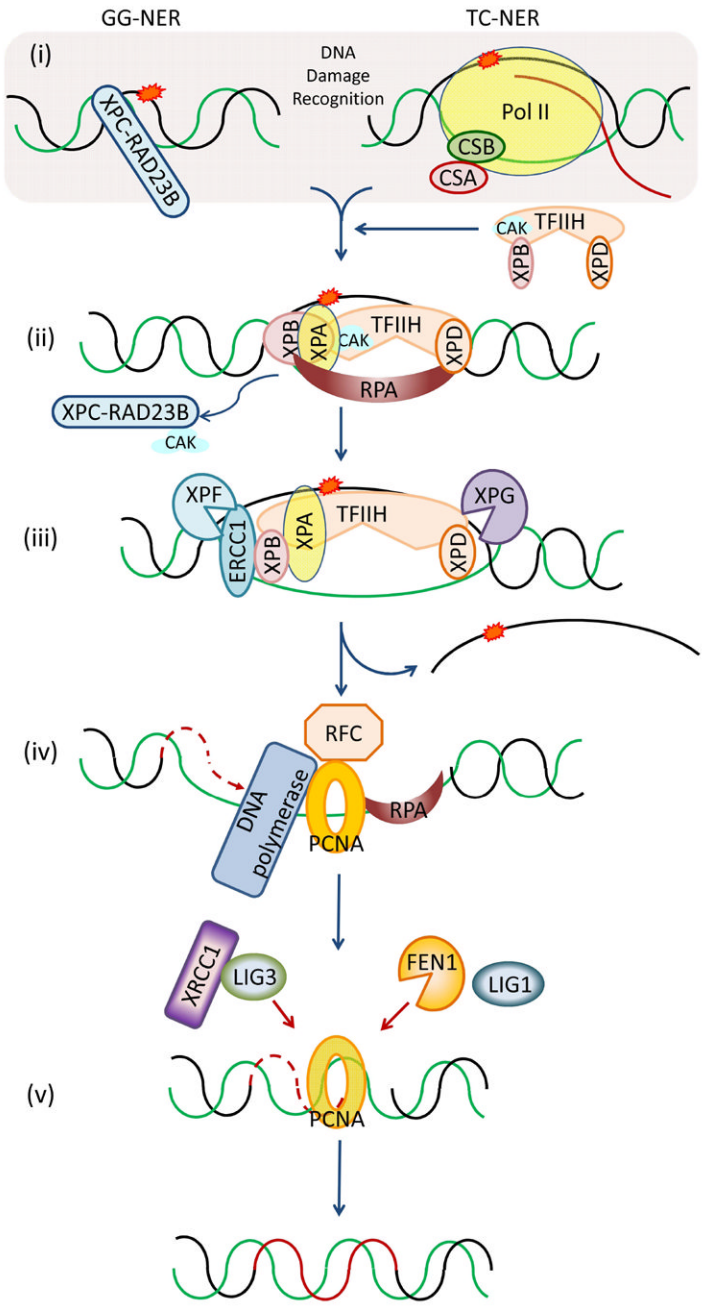

Figure 2. Nucleotide excision repair pathways

Two subpathways of mammalian NER: GG-NER and TC-NER. (i) XPC-RAD23B recognizes DNA damage-induced structural change as the initiation step of GG-NER. TC-NER is initiated by stalling of an elongating RNAP at a blocking lesion on the transcribed strand within an active gene. After these initial recognition steps, GG-NER and TC-NER pathways involve many of the same protein components. (ii) Following recognition, the TFIIH complex is recruited. Through the activity of the helicase subunits, XPB and XPD, TFIIH promotes opening of the DNA duplex around the lesion, facilitating recruitment of XPA and RPA. (iii) The XPF–ERCC1 complex is recruited to the lesion via a direct interaction with XPA, while XPG is specifically engaged through an interaction with TFIIH. The two endonucleases, XPF–ERCC1 and XPG, are responsible for carrying out incision 5’

and 3', respectively, to the DNA damage. (iv) After dual incision and removal of the damage-containing oligonucleotide fragment, a DNA polymerase carries out gap-filling repair synthesis in cooperation with RFC and PCNA. (v) Finally, the nick is sealed by either XRCC1–LIG3α or a FEN1–LIG1 complex. CAK, the cyclin-dependent kinase (CDK)-activating kinase; GG-NER, global genome-NER; RFC, replication factor C; RPA, replication protein A; TC-NER, transcription-coupled NER; TFIIH, transcription factor II H.

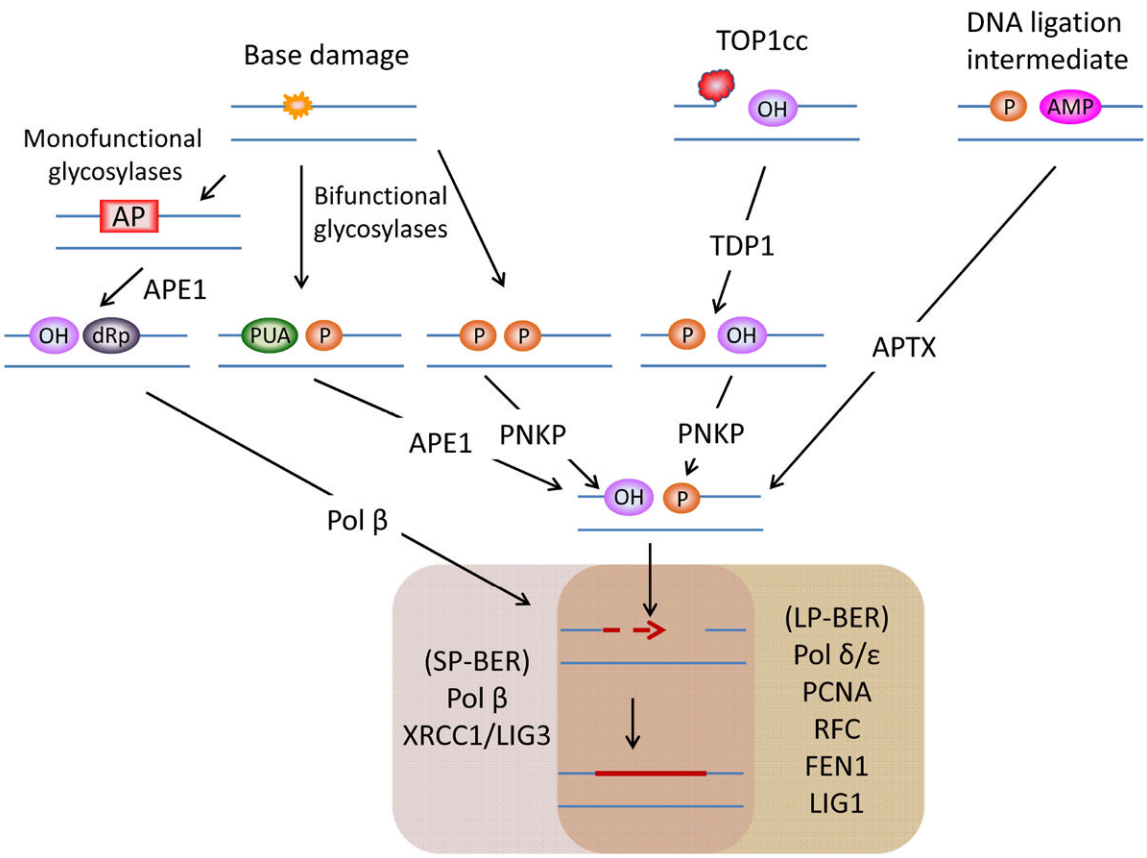

Figure 3. Base excision repair pathways

In BER, base damage is recognized and removed by a lesion-specific DNA glycosylase. Monofunctional DNA glycosylases include UNG and MPG, whereas bifunctional DNA glycosylases are OGG1, MUTYH, NTH1 and NEIL1. The monofunctional DNA glycosylases create an AP site by removing the substrate base. Such AP sites are incised by APE1, creating a 5'-dRP and 3'-OH strand break product. Pol β removes the 5'-dRP moiety via an intrinsic lyase activity. Bifunctional DNA glycosylases excise a damaged base, and can also incise the DNA backbone immediately 3' to the AP site product and produce a SSB with a 3'-PUA or 3'-P, respectively. APE1 removes the 3'-PUA residue, while PNKP excises the 3'-P moiety. TOP1cc can be removed by TDP1, leaving behind a 3′-P and 5′-OH terminus; both ends of this SSB are converted by PNKP to 3′-OH and 5′-P. APTX processes 5'-AMP groups, resulting from a failed DNA ligation event, to normal 5'-P ends at nicks or breaks. After creating the 3'-OH and 5'-P termini at a SSB, SP- or LP-BER performs repair synthesis and ligation. In SP-BER, Pol β replaces the missing nucleotide and the XRCC1 LIG3α complex seal the nick. In LP-BER, Pol δ/ε, RFC and PCNA incorporate 2–13 nucleotides and then FEN1–LIG1 completes the repair process. AMP, adenosine monophosphate; AP, apurinic/apyrimidinic; APE1, AP endonuclease 1; APTX, aprataxin; FEN1, flap endonuclease 1; LIG1, DNA ligase I; LIG3, DNA ligase III; MPG, N-methylpurine-DNA glycosylase; MUTYH, mutY homolog; NEIL1, endonuclease VIII-like 1; NTH1, endonuclease III-like 1; OGG1, 8-oxoguanine DNA glycosylase; PCNA, proliferating cellular nuclear antigen; PG, phosphoglycolate; PNKP, polynucleotide kinase 3'-phosphatase; Pol β, polymerase β; SSBR, DNA *single strand* break repair; SSBs, DNA single strand breaks; TDP1, tyrosyl-DNA phosphodiesterase 1; TOP1, topoisomerase 1; Top1cc, Top1 cleavage complex; UNG, uracil-DNA glycosylase; 3'-PUA, 3'-phospho-α,β-unsaturated aldehyde.

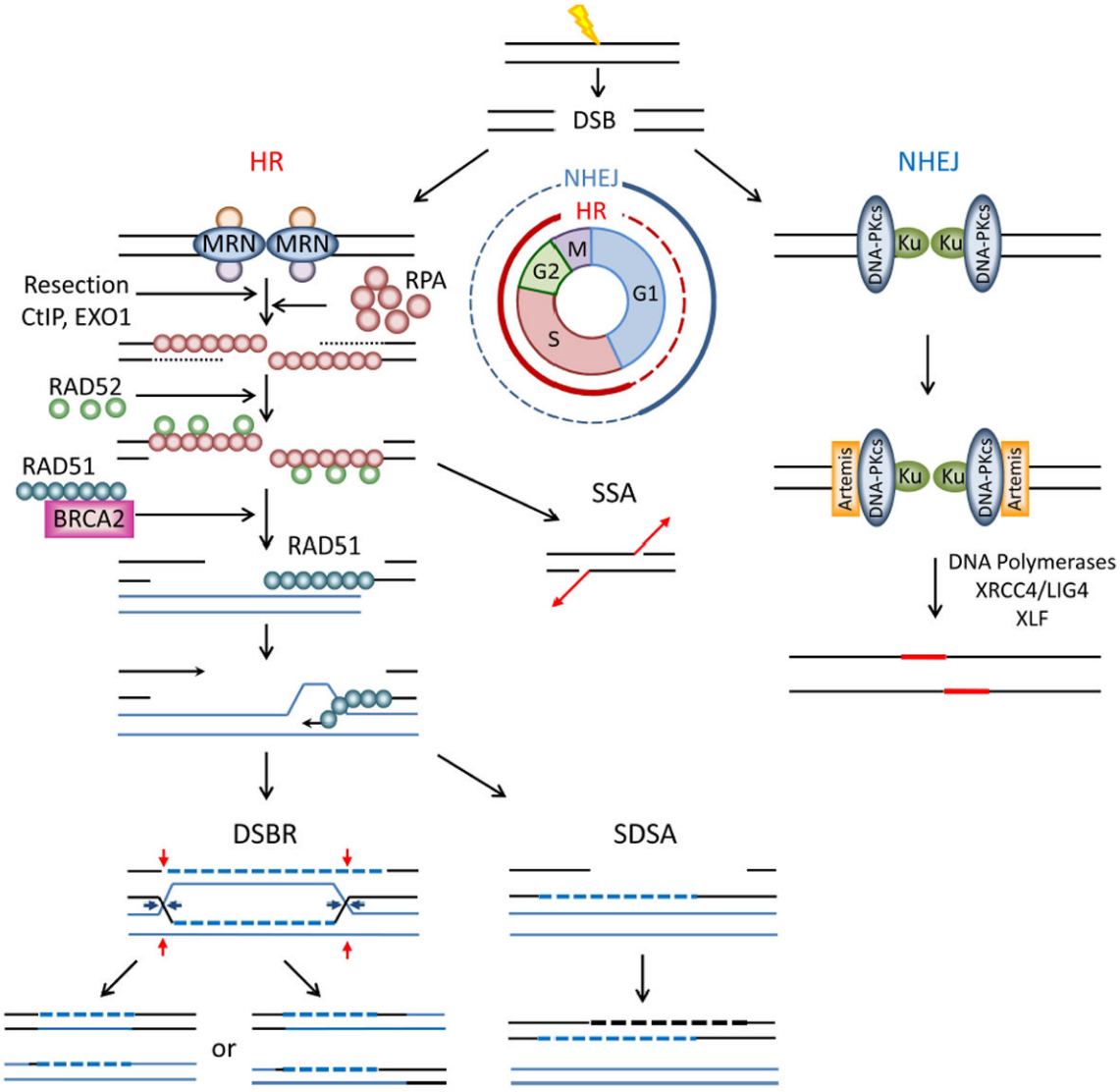

Figure 4. Recombination pathways

DSBR is divided into two major pathways: HR and NHEJ. HR operates in dividing cells and in S phase, whereas NHEJ can function in both dividing and non-dividing cells and independent of cell cycle. HR has been proposed to be initiated by recognition of the DSB by the MRN complex (MRE11-RAD50-NBS1). The MRN complex associates with CtIP, which initiates 5′–3′ end resection to create the 3′ ssDNA overhang. Further resection is carried out by exonucleases (possibly EXO1), and the resulting ssDNA is stabilized by binding of RPA. RAD52 is recruited to RPA. The RAD51-BRCA2 complex then replaces the RAD52-RPA complex to form RAD51 nucleoprotein filaments, whereas, in SSA, RPA and RAD52 carry out the recombination process in a RAD51-independent manner. RAD51-coated ssDNA enables strand invasion of the intact homologous DNA region. In classic DSBR, the second DSB end can be captured by the D-loop to form an intermediate with double Holliday junctions, which can result in a non-crossover (cleavage at blue arrows) or a crossover (cleavage at blue arrows on one side and red arrows on other side) products. In SDSA, the newly synthesized strand is displaced to permit annealing to the other DSB end, resulting in a non-crossover product. NHEJ is initiated by recognition of the DSB ends by

the Ku (Ku70/Ku80) complex, followed by recruitment of DNA-PKcs. DNA-PKcs activates Artemis, which generates terminal overhangs prior to ligation. To complete the process, DNA synthesis is performed to fill-in the gaps and end joining is carried out by XRCC4–LIG4 in collaboration with XLF. CtIP, C-terminal binding protein-interacting protein; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSBR, DNA double strand break repair; HR, homologous recombination; NHEJ, nonhomologous end joining; SDSA, synthesis-dependent strand annealing; SSA, single-strand annealing; XRCC4, X-ray repair cross-complementing protein 4.

Table 1

Functional capacity of the different DNA repair pathways in dividing/non-dividing cells and their connection to inherited cancer/neurological disorders.

Repair Pathway		Dividing cells	Non-dividing cells	Disease relationship	
		Capacity of DNA repair		Cancer	Neurological
MGMT		++	+	O	X
NER	TC-NER	++	+	X	O
	GG-NER	+	+	O	O
BER		+	+	O	X
SSBR	TDP1	++	+	X	O
	APTX	++	+	X	O
	PNKP	++	+	X	O
MMR		++	+/-	O	X
DSBR	HR	++	+/-	O	O
	NHEJ	+	++	O	O

Repair capacity: ++, high; +, low; +/-, very low or no detectable activity. Disease relationship: O, associated; X, not associated.