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Variant Base Excision Repair Proteins: Contributors to Genomic Instability

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Abstract

Cells sustain endogenous DNA damage at rates greater than 20,000 DNA lesions per cell per day. These damages occur largely as a result of the inherently unstable nature of DNA and the presence of reactive oxygen species within cells. The base excision repair system removes the majority of DNA lesions resulting from endogenous DNA damage. There are several enzymes that function during base excision repair. Importantly, there are over 100 germline single nucleotide polymorphisms in genes that function in base excision repair and that result in non-synonymous amino acid substitutions in the proteins they encode. Somatic variants of these enzymes are also found in human tumors. Variant repair enzymes catalyze aberrant base excision repair. Aberrant base excision repair combined with continuous endogenous DNA damage over time has the potential to lead to a mutator phenotype. Mutations that arise in key growth control genes, imbalances in chromosome number, chromosomal translocations, and loss of heterozygosity can result in the initiation of human cancer or its progression.

Keywords

Base excision repair; mutator phenotype; cancer

1. DNA is Damaged Continuously in Cells Through a Variety of Processes

Endogenous DNA damage occurs in cells at a rate greater than 20,000 DNA adducts per cell per day (for an extensive review see [1]). The majority of these endogenous DNA adducts are non-bulky, and are repaired by the base excision repair (BER) pathway. Examples of endogenous DNA base damage include DNA hydrolysis, deamination, oxidation, and alkylation.

As Lindahl pointed out in 1993, [2] the N-glycosyl bond in DNA is very labile. Together with its presence in largely B-form DNA, which is fully hydrated, it is subject to hydrolysis, leading to the formation of abasic sites in DNA [2]. Base residues of DNA also undergo hydrolytic deamination, an example of which is the deamination of cytosine to uracil [1, 2]. Normal aerobic metabolism gives rise to reactive oxygen species (ROS), which damage DNA base residues (for an excellent review see [3]). As the human immune system mounts an inflammatory response to pathogens, leukocytes (including macrophages) release ROS, which has been shown to result in base damage to DNA (for a review see [4]). Lipid peroxidation also results in damage to DNA, generating a variety of damages including the

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etheno adducts. *S*-adenosylmethionine (SAM) functions in the methylation of DNA to regulate gene expression, but due to its reactive methyl group, it also participates in gratuitous methylation of DNA base residues [1, 5, 6]. Abasic sites and oxidized or methylated base residues all can give rise to mutations if not properly repaired. Given the extremely high rate of endogenous DNA damage, even slightly deficient DNA repair would be expected to result in the accumulation of mutations. This has been clearly demonstrated in bacterial cells where various mutant combinations of BER enzymes lead to very high spontaneous mutation frequencies (see for example [7]).

2. The BER Pathway Repairs Endogenous Base Damage

BER is an evolutionarily conserved DNA repair pathway that repairs non-bulky DNA base damage (for a review see [8]). There are five basic steps that occur during BER, as shown in Figure 1. An overview of BER proteins is given in Table 2. First, the damaged base is recognized and excised by a DNA glycosylase, leaving an abasic site. Second, the abasic site is removed, either by the lyase activity of a DNA glycosylase or by the enzymatic activity of apurimidinic endonuclease I (APE1). Third, the 3′ and/or 5′ ends of the DNA break are modified to generate 3′OH and 5′P. Fourth, insertion of one or more nucleotides by a DNA polymerase fills the gap, although it has recently been shown that deletions can arise by direct ligation of the 3′OH and 5′P without gap filling [9]. This suggests that initiation of the BER process can be mutagenic. The fifth general step of BER is ligation of the ends by a DNA ligase. Here, we will focus predominantly on short patch BER, in which Pol β usually inserts a single nucleotide during the gap-filling step. In long-patch BER greater than a single nucleotide is used to fill the gap. In this process, Pol β likely inserts the first base during gap filling and initiates strand displacement synthesis of the DNA 5′ to the gap. Replicative polymerases, along with PCNA fill the gap, and Flap endonuclease I (FEN1) processes the resulting 5′flap [10, 11].

3. Enzymes and Proteins of the BER Pathway

3.1. DNA Glycosylases

Several DNA glycosylases are expressed in mammalian cells (for reviews see [8, 12]). Here we will provide an overview of the DNA glycosylases and the adducts they act upon, as summarized in Table 1. In general, these enzymes recognize specific DNA adducts, flip the damaged base into their active sites, and cleave the N-glycosylic bond.

One of the most common mutagenic adducts that results from oxidative DNA damage is 7,8-dihydro-8-oxoguanine (8-oxoG) [8]. 8-oxoG can also be incorporated into DNA, but the 8-oxoGTP precursor is hydrolyzed by the MutT homologs (MTH) 1 or 2 [13]. Adenine pairs with 8-oxoG during DNA synthesis, ultimately resulting in transversions [14, 15]. The DNA glycosylase 8-oxoguanine glycosylase (OGG1) excises both 8-oxoG and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) from 8-oxoG:C base pairs, whereas the MutYH DNA glycosylase excises adenine from the 8-oxoG:A mispair [16–18]. OGG1 and MutYH are bifunctional DNA glycosylases that excise the damaged base and also use β-elimination to hydrolyze the DNA backbone.

Deamination of 5-methylcytosine (5-meC) to thymine is a common event that gives rise to the T:G mispair. In fact, the mutation rate of 5-meC to thymine is 10- to 50-fold higher than other transition mutations in humans [19–21]. The Thymine DNA glycosylase (TDG) and Methyl-CpG-binding domain protein 4 (MBD4) DNA glycosylases excise thymine from T:G mispairs. TDG is also able to excise adducts such as ethenocytosine that arise as a byproduct of lipid peroxidation [22]. The MBD4 enzyme, also known as MED1, has a domain that recognizes methylated and hemi-methylated CpG sites in DNA, as well as a glycosylase

domain [19, 23]. MBD4 prefers to bind to m5CpG:TpG mismatches, which arise as a result of deamination of 5-meC, and it catalyzes the excision of thymine from T:G or uracil from U:G mismatches [19]. MBD4 also catalyzes the removal of T opposite O^6-methylG, which can lead to futile cycling of excision of T and its incorporation opposite the G lesion by DNA polymerases, similar to what has been described for mismatch repair [24]. Phosphorylated MBD4 promotes excision of methylated DNA bases [25]. Interestingly, MBD4 interacts with MutL homolog 1 (MLH1) [26], and deletion of MBD4 leads to significant down-regulation of mismatch repair proteins [24], suggesting that the MBD4-initiated BER pathway and mismatch repair are associated in some way. Both of these glycosylases are monofunctional, excising only the damaged base.

In humans, there are two nuclear uracil DNA glycosylases, uracil DNA glycosylase 1 (UNG) and single-strand selective monofunctional uracil DNA glycosylase (SMUG1). UNG1 removes uracil during DNA replication [27], whereas SMUG1 likely excises uracil that arises from deamination of cytosine [28]. SMUG1 also excises 5-hydroxymethyluracil and 5-formyluracil and other oxidized bases [28, 29]. Both of these glycosylases are monofunctional.

Human NTH1, like all the EcoNth orthologs, possesses DNA-glycosylase/lyase activity on oxidized pyrimidines, formamidopyrimidines, 5-formyluracil and also incises AP sites [30–40]. In *E. coli*, a backup activity to EcoNth for oxidized pyrimidines, EcoNei, was identified and characterized (see for example, [41]). Its first eukaryotic homologs were found in humans and designated NEIL1, NEIL2 and NEIL 3 (NEI-like). NEIL1 and NEIL2 have been extensively characterized [4243–45]. An active form of mouse NEIL3 recently has been expressed and characterized [46]. Like NTH1, NEIL1 recognizes oxidized pyrimidines, formadopyrimidines, and thymine residues oxidized in the methyl group [42–4547, 48]. Unlike hNTH1, NEIL1 recognizes both stereoisomers of thymine glycol [49–51]. Thus far, the best substrates for hNEIL1 appear to be the hydantoin lesions, guanidinohydantoin (Gh), and spiroiminodihydantoin (Sp) [52] that are further oxidation products of 8-oxoG[52]. This also is true for NEIL3 [46]. NEIL1 is also capable of removing lesions from single-stranded DNA as well as from bubble and forked DNA structures [53, 54]. NEIL2 prefers oxidized pyrimidines, but shows a greater preference than NEIL1 for lesions in single-stranded and bubble structures [53] as does mouse Neil3 [53]. Because the expression of NEIL1 is cell-cycle dependent [43], it acts on forked DNA structures [55] and it interacts with PCNA [53] and FEN-1 [55]; accordingly, it has been proposed that NEIL1 functions in replication associated repair. The expression of NEIL2 is not cell-cycle dependent [43]; and because of its propensity for lesions in bubble structures, it has been proposed to act during transcription. The NTH1 and NEIL3 enzymes are bifunctional glycosylases that hydrolyze the DNA backbone by β-elimination. However, both NEIL1 and 2 are bifunctional enzymes that hydrolyze the DNA backbone using β, δ-elimination, resulting in APE1-independent downstream BER events.

Human 3-methyladenine DNA glycosylase (AAG) recognizes and excises a variety of methylated bases including 7-methylguanine, 3-methyladenine, and 1-N^6-ethenoadenine from DNA [56, 57]. This enzyme recently was shown to act on a variety of novel DNA substrates including 1-N^2-ethenoguanine and uracil [58]. AAG is a monofunctional DNA glycosylase.

3.2. Cleavage of the DNA Backbone

Cleavage of the DNA backbone is conducted by APE1 when a monofunctional glycosylase excises the damaged base, or with the lyase activity of a bifunctional DNA glycosylase.

Excision of a damaged base by a monofunctional DNA glycosylase leaves an abasic site. APE1 binds to this site and cleaves the phosphodiester backbone 5′ to the abasic site, resulting in a 3′OH and 5′deoxyribosephosphate (dRP) (for a review see [59]). APE1 also has

a 3′ diesterase or phosphatase activity that can remodel a 3′ end to generate a 3′-OH. The 8kDa amino terminus of Polβ has dRP lyase activity and functions to remove the 5-′ dRP group after cleavage of the phosphodiester backbone by APE1.

After a bifunctional DNA glycosylase excises the damaged base leaving an abasic site, hydrolysis of the phosphodiester backbone by β-elimination leaves a 3′ unsaturated aldehyde and a 5′ phosphate. The 3′ phosphodiesterase activity of APE1 acts to restore the 3′OH.

The bifunctional glycosylases NEIL1, NEIL2 and NEIL3 hydrolyze the DNA backbone via β,δ-elimination, which leaves a 5′ and a 3′ phosphate on the ends of the DNA. The 3′ phosphate is removed by polynucleotide kinase/phosphatase (PNKP) to generate a 3′ OH in an APE1-independent reaction [60].

3.3. The XRCC1 protein acts as a scaffold during BER

The X-ray Cross-Complementing Factor 1 (XRCC1) has no intrinsic enzymatic activity, but interacts with several proteins that function in single-strand break repair and in BER, including APE1, Polβ, and LIGIIIα (for a review see [61]). Alteration of the XRCC1 gene results in strong sensitivity to alkylating agents [62], most likely because XRCC1 acts as a scaffold to recruit and facilitate interactions between proteins that repair single-strand breaks that result from cleavage of the phosphodiester backbone by APE1. During short-patch BER, the XRCC1-LIGIIIα complex is recruited to seal the nick generated by the action of APE1 and Polβ. The absence of XRCC1 in cells leads to a reduction in cellular levels of LIGIIIα, through an unknown mechanism.

3.4. DNA Polymerase Beta Fills the Gap

During short patch BER and after excision of the damaged base, cleavage of the DNA backbone, and end remodeling to generate 3′ OH and 5′P ends, Polβ fills in the gap, usually adding a single nucleotide, (for a review see [12]). During long-patch BER, which appears to be a minor cellular repair pathway [63], Polβ initiates gap filling and completion of this process is performed by DNA polymerases δ and/or ε. In this case, the Fen1 flap endonuclease removes the 5′ dRP group after strand displacement synthesis has occurred [64]. In both cases, the XRCC1/LIGIIIα complex catalyzes ligation of the resulting ends. In addition to Polβ, the closely related polymerase Polλ also has been shown to participate in BER [65]. The substitution of Polλ for Polβ is dependent on the type of DNA damage; Polλ functions more readily in the repair of oxidative damage, but not alkylating damage [66]. Similarly to Polβ, Polλ possesses both dRP lyase and polymerase activities. Polλ also interacts with the DNA glycosylases SMUG1 [67], AAG, and OGG1 [68] further implicating its role in BER.

4. Genetic Variation in BER Genes and Mutagenesis

Importantly, over 100 different germline polymorphisms exist within the normal population that are predicted to result in nonsynonymous amino substitutions within various BER proteins, including DNA glycoslyases, APE1, XRCC1, and Polβ exist within the normal population (www.genome.utah.edu/genesnps/). In addition, variants of several BER proteins also are found in tumors (for a review see [69]). The majority of these variants are heterozygous. Thus, they may exert their effects via a dominant phenotype. Alternatively, as work from our lab indicates, a dominant phenotype is not necessary, as subtle variations in activity can lead to mutagenesis and cellular transformation [70, 71]. These variants may not function as well as their wild type counterparts with the end result being aberrant BER. Because BER is a highly coordinated DNA repair pathway that involves several protein-protein interactions, we speculate that even subtle alterations in specific proteins that function in BER could result in mutagenesis and/or genomic instability. Here we define

mutagenesis as small changes in the base sequence of DNA and genomic instability as large deletions, copy number variation, or chromosomal aberrations.

4.1. DNA Glycosylase Variants

Variant BER enzymes could lead to increased genomic instability and here, we outline our view of how genomic instability could occur as a result of the presence of an altered DNA glycosylase. Some glycosylase variants have been characterized and were shown to contain altered or low enzymatic activity (see for example [72]), but a complete study of all known glycosylase variants has not been undertaken. Fewer damaged bases are likely to be removed in cells expressing variants with low substrate binding affinity or low enzymatic activity. Error-prone lesion bypass could result in incorrect base incorporation, leading to the accumulation of mutations. Should these mutations occur in key growth control genes, leading to an alteration in either the expression or function of a protein, cancer could result. Replication fork arrest by lesions per se -- or that result from a large number of lesions -- could be processed via homology dependent repair (HDR) or non-homologous end joining (NHEJ), which could lead to the accumulation of chromosomal aberrations. Although many chromosomal aberrations are incompatible with life, several of them are associated with human cancer. These include imbalances in chromosome number, translocations, and loss of heterozygosity. Slowing of fork elongation in repetitive sequences including fragile sites also has been shown to result in replication stress, likely resulting in the firing of additional origins of replication. This leads to higher than normal levels of single-stranded DNA, ultimately resulting in chromosomal translocations which are associated with cancer [73]. The slowing of replication fork movement due to the presence of an unrepaired lesion could also lead to massive genomic instability. Low turnover by an enzyme with normal or increased substrate binding affinity would result in the removal of fewer damaged bases and perhaps an increase in protein-DNA complexes at the lesion site. Transcriptional mutagenesis has also been shown to result from mutagenic bypass of DNA lesions by RNA polymerases (see for example [74]). Translation of mutated RNA transcripts has been shown to lead to phenotypic changes in cells.

DNA glycosylase variants could also possess altered substrate specificity, resulting in the excision of normal bases, or excision of damaged bases outside the normal sequence context. One example of such is the S326C OGG1 variant, ranging in allele frequency between 0.13 and 0.62 in the normal population. S326C has 2–6-fold decreased catalytic efficiency for the removal of 8-OxoG and abasic site cleavage, and is highly dependent upon the base opposite the lesion [75, 76]. The decreased catalytic efficiency of this variant is most likely due to its decreased binding affinity for lesions in the DNA [75]. Importantly, enzymatic turnover of the S326C variant is not stimulated by APE 1, as is the WT; in addition, S326C has a dimeric conformation, unlike WT, which is monomeric. The dimeric conformation is thought to interfere with the stimulation of S326C by APE1 [75]. A more subtle possibility is that the variants might exhibit altered sequence context specificity. It is known that the WT versions of these enzymes exhibit sequence context specificity (for examples see [52, 75]). Variants could excise damaged bases with a higher rate in certain sequence contexts, for example, either nearest neighbors or dependence upon the base opposite the damage. Cells with variants exhibiting altered sequence context specificity might have increased mutagenesis within certain sequence contexts or non-B-DNA structures. Should this increased mutagenesis occur in key growth control genes, cancer could result.

Mutations in the MYH gene are associated with colon cancer that arises from genomic instability [77, 78]. The MYH DNA glycosylase excises A opposite 8-oxoG, so a deficiency in the enzymatic activity of this protein leads to an accumulation of G to T transversions that appear to accumulate in genes, including APC and KRAS2. Importantly, loss of glycosylase activity has been shown in variants of MYH associated with colon cancer [79, 80].

DNA glycosylase-associated AP lyase activity could be altered in germline variants. A decrease in lyase activity would likely result in an increase in AP sites in the DNA. If not removed by APE1 due to structural or other constraints, AP site bypass by error prone polymerases could lead to mutation accumulation. Increased AP lyase activity could result in an accumulation of single-strand breaks (SSBs), which are converted to DSBs upon replication, or by clustering of the SSBs themselves. DSBs are repaired via HDR or NHEJ, the latter process of which may lead to chromosomal aberrations. Both mutagenesis and chromosomal aberrations can lead to cancer, as described above.

The variants might also have altered affinity for their protein partners, many of which are likely unknown; or they may interact aberrantly with other proteins with which they usually do not interact. This could result in sequestration of repair proteins that are needed for BER or other repair processes, ultimately leading to defective repair and mutagenesis. Aberrant protein interactions also could result in imprecise handoff to the next enzyme in the pathway (i.e. glycosylase to AP endonucleaset to polymerase) leading to unprotected accumulation of BER intermediates such as AP sites and breaks. This could also result in genomic instability. Most of the scenarios listed here predict that the cell will sustain increased DNA damage and genomic instability, which could lead to cancer should alterations in cellular growth control result.

4.2. APE1 Variants

APE1 germline variants that have amino acid alterations within the active site of the protein have been characterized. Some of these proteins exhibit reduced DNA cleavage activity [81]. The activities of variants with alterations outside the active site are not known. Because APE1 interacts with a variety of other BER proteins, complete characterization is important. APE1 variants with low enzymatic activity would likely result in an increase in abasic sites in cells, which could lead to the accumulation of mutations via error-prone translesion bypass. Mutations in key growth control genes could lead to a malignant phenotype. Variants of APE1 with reduced specificity could cleave the phosphodiester backbone in the absence of abasic sites, resulting in gratuitous single-strand breaks. Compromised ability of an APE1 variant to remove a 3′-dRP would result in ends that are not ligated, which could lead to formation of a DSB or fork collapse during DNA replication, and ultimately, genomic instability (see for example [82]). Should the genomic instability lead to translocations, loss of heterozygosity, or an imbalance in chromosome numbers, cancer could result.

APE1 interacts with Werner protein (WRN) and has been found to impede its helicase activity [83]. APE1 physically associates with XRCC1, which results in an increased efficiency of its AP endonuclease and 3′phosphodiesterase activities [84] (and for a review see [85]). APE1 physically associates with FEN1 and PCNA, suggesting that it may function in long patch BER [86]. APE1 stimulates the glycosylase activity of OGG1 by facilitating product release in such a way as to perhaps inhibit the lyase activity of OGG1 [87, 88]. Demple and colleagues have shown that APE1 interacts with, and stimulates Polβ [89–91]. Variant forms of APE1 might exhibit aberrant interactions with some of its protein partners; this could lead to genomic instability. Higher affinity for a protein partner could lead to its sequestration and inability to function in DNA repair. Lower affinity for a protein partner could lead to less modulation of its activity. For example, a low affinity APE1 protein that does not bind tightly to OGG1 would result in lower stimulation of its AP lyase activity. Ends unable to be ligated would accumulate and lead to genomic instability as described above. Finally, APE 1 undergoes posttranslational modification [85] and disruption of this could result in an enzyme that participates aberrantly in BER, leading to genomic instability.

4.3. XRCC1 Scaffold Variants

Chinese hamster ovary (CHO) cell lines that are XRCC1-deficient exhibit strong sensitivity to alkylating agents, increased sister chromatid exchanges and micronuclei, a decrease in the repair of single-strand breaks, and an increase in deletions in response to treatment with an alkylating agent (see for example [62, 92]). Recent work also shows that the R280H human germline variant of XRCC1, previously suggested to be associated with deficient DNA repair [93, 94], exhibits decreased DNA binding and retention time at DNA breaks *in vivo* [95]. These studies provide strong evidence that alterations of XRCC1 induce genomic instability.

It is important to point out that a potentially cytotoxic and mutagenic single-strand break is a central intermediate DNA substrate of each of the BER sub-pathways. XRCC1 is recruited by PolyADPribose polymerase 1 (PARP1) to the break, where it mediates interactions between key BER proteins, including Polβ and XRCC1-LIGIIIα. It follows that a decrease in retention at breaks by the R280H XRCC1 variant would not provide the most efficient scaffolding of these proteins at the site of breaks, leading to a decrease in their repair. Even a subtle decrease in break repair is likely to result in genomic instability, leading to cancer, as described above. For example, we have shown that an inability to fill in single nucleotide gaps by the E295K Polβ variant (see below) leads to massive genomic instability [82]. The presence of unrepaired single-strand breaks can lead to replication fork collapse, the formation of double-strand breaks, and, if not cytotoxic, error-free or error-prone repair of these breaks. Error-prone repair by end joining pathways can lead to deletions, translocations, and loss of heterozygosity. The encounter of a single-strand break by the replication fork can also lead to slowing of elongation which has been shown to result in replication stress and additional firing of origins [96]. Breaks present within fragile sites and repetitive DNA sequences that induce replication stress increase the levels of single-stranded DNA in cells, likely from aberrant lagging-strand replication. Processing of these regions of single-strandedness leads to chromosomal aberrations, including translocations and breaks (for a review see[73]), and can result in cancer.

4.4. PARP1 Is Important for Efficient Damage Processing

PolyADPribose polymerase 1 (PARP1) binds to DNA breaks, becomes activated by ADP ribosylation, and then recruits repair proteins to the site of DNA damage (for an excellent review see [97]). Interestingly, cells deleted of PARP1 are moderately sensitive to alkylating agents, whereas treatment of $PARP1^{+/+}$ cells with PARP inhibitors results in hypersensitivity to alkylating agents. This indicates that an enzymatically-inhibited PARP1 protein must be present in the cell to for it to be defective in the repair of breaks. There are several germline SNPS in the gene encoding PARP1, many of which result in nonsynonymous amino acid substitutions. Some of these variants may exhibit lower DNA binding, activation, or recruitment activity, similar to what occurs in the presence of PARP inhibitors. Hypersensitivity to alkylating agents is indicative of a defect in break repair, meaning that single-strand breaks remain in the DNA. As discussed above for XRCC1, this could lead to genomic instability via a double-strand break intermediate [98].

4.5. Polβ Variants

We have previously reported that mutations in Polβ are found in 30% of tumors examined [99], but are not found in matched normal tissues. Many of these are nonsynonymous point mutations. Thorough structural and biochemical analyses of Polβ have provided us with the ability to predict the outcomes of certain mutations based upon where they are found within the protein. Mutations that even slightly alter the positioning or folding of the protein may alter its catalytic activity, resulting in an aberrant phenotype. For example, the hydrophobic hinge located in the 31 kDa polymerase domain is required for the fidelity of polymerase due to its involvement in the conformational change that occurs

during dNTP binding [100–103]. Mutations in these hinge residues, such as I260, I174, or Y265, affect the enzyme's ability to discriminate between correct and incorrect dNTPs and result in increased mutagenesis. When a mutation causes an increase in the mutation frequency, we hypothesize that this variant would make more errors during the repair of endogenous DNA damage compared to the WT enzyme. If these errors occur in genes that regulate important cellular processes such as cell cycle, proliferation, or apoptosis, this could lead to cancer progression.

Importantly, a mutator phenotype may also occur when a variant does not affect the overall mutation frequency, but instead increases certain types of mutations compared to WT. This idea is supported by our findings with the cancer-associated variant I260M. I260M has a unique mutational spectrum compared to the WT enzyme and was found to induce more transversions and frameshift mutations than the WT enzyme [71]. Expression of I260M results in cellular transformation [70] that continued even after the expression was extinguished, suggesting that there is a mutational basis for this transformation, possibly due to the unique mutations induced by the variant.

Polβ has two catalytic activities: dRP lyase and polymerase activities. The dRP lyase activity lies within the N-terminal 8 kDa domain and also contains the DNA-binding domain which requires residues K41, K60, H34, R40, Y39, K68, K72, and R83 [104]. Sobol, et al., demonstrated that reconstitution of only the dRP lyase activity is able to rescue Polβ-deficient cells from DNA damage [105]. We have also shown that a Leu to Pro mutation at residue 22 found in gastric cancer has no dRP lyase activity, is deficient in base excision repair, and is unable to complement Polβ null cells in response to DNA damaging agents [100]. Although L22P is not directly involved in forming the DNA binding pocket, it has decreased DNA binding affinity. The mutation may alter the organization of the binding pocket, preventing Polβ from binding DNA efficiently and preventing polymerization from occurring. Hence, any mutations in the dRP lyase domain, despite whether or not they are in critical residues, can prevent the enzyme from participating in BER. These mutations could prevent the removal of the 5′-dRP group and the filling of the gap. They could also prevent Polβ from binding the DNA that would result in unrepaired lesions. These variants could result in an accumulation of BER intermediates leading to genomic instability. Unrepaired DNA can cause stalled and collapsed replication forks leading to the formation of double-strand breaks [106]. These breaks could be repaired by HDR or the error- prone NHEJ, and chromosomal aberrations and fusions could accumulate, resulting in genomic instability.

The other role of Polβ in BER is to fill in the gap. Mutations within the 31 kDa polymerase domain can result in a protein with aberrant polymerase function. No base will be incorporated if the mutation renders the enzyme inactive. For example, the gastric cancer variant E295K binds DNA with the same affinity as WT Polβ, but lacks polymerase activity and does not participate in BER. The expression of E295K in Polβ-deficient cells does not rescue the cells from MMS-induced cytotoxicity [82]. Since E295K is able to bind DNA with anaffinity similar to WT, it is likely that E295K is acting in a dominant negative fashion and it is able to compete with WT and prevent the repair of the damage. Our studies with E295K showed an increase in sister chromatid exchanges compared to wild type; this most likely is due to the lack of polymerase activity of E295K. If the variant is inactive and unable to bind DNA, it could still interact with BER proteins and sequester them from repairing other damage and indirectly cause an accumulation of damage. Even variants that have a lower rate of catalysis can be detrimental. If the variant binds the DNA and is slow to polymerize, this could cause the replication fork to slow or stall, resulting in double strand breaks that may lead to overall genomic instability.

It is important to note that a proper balance of Polβ expression must be maintained. As we discussed above, expression of non-functional or dysfunctional Polβ can cause deleterious effects. Likewise, over-expression of Polβ can be detrimental. Elevated levels of Polβ have been detected in tumors [107]; this was suggested to be due to the larger need for Polβ as a result of the higher rate of DNA damage in the tumor. Alternatively, the up-regulation of Polβ could disrupt other cellular processes and cause more damage to the cell. One possibility is that Polβ could substitute for replicative polymerases. Polβ has a lower fidelity than replicative polymerases and has no known robust proofreading abilities [108] which ultimately could result in increased mutagenesis. Over-expression of Polβ interferes with normal replication and impairs replication fork progression without activating a checkpoint response [96]. Genomic instability could occur by increasing chromosomal rearrangements and common fragile sites [96].

Polβ also interacts with proteins involved in BER (i.e. XRCC1, APE1, gycosylases, LigaseIIIα). If additional Polβ is available, it may not participate in BER, and instead sequester the other proteins leading to inefficient BER and thus increase BER intermediates that could result in genomic instability.

4.6. DNA LigaseIIIα and Lack of Variants

There are several germline polymorphisms within the DNA ligase III gene, according to the Environmental Genome Project (http://egp.gs.washington.edu/). However, only one of these is predicted to result in a non-synonymous substitution within the protein. This alteration is predicted to be well-tolerated, so it is unlikely to result in a phenotype associated with genomic instability. Because DNA ligases are critical to a number of DNA repair processes and as well as to replication, perhaps even the presence of subtle variations in a ligase would not be compatible with life.

5. Imbalances of BER Proteins Might Be Mutagenic

Spontaneous mutation rates have been known for some time to be significantly affected by imbalances in BER proteins, as shown in the classic study in yeast by Samson and colleagues [109]. Over-expression of AAG1 and APE1 is associated with chronic inflammation and microsatellite instability in ulcerative colitis [110]. These enzymes can be up-regulated at the transcriptional level, either as a result of induction by the presence of DNA damage that would be expected to be present during inflammation, or by posttranslational modification. Over-expression of the BER proteins could lead to the removal of normal, non-damaged bases, initiating gratuitous BER.

6. Mouse Models of Aberrant BER and Genomic Instability

Investigations into the links between BER genes, a mutator phenotype, and cancer in an organism have largely been initiated by knocking out, or deleting, genes that function in BER; and much of this field has been reviewed by Klungland [111]. In general, deletion of DNA glycosylase genes results in accumulation of the lesions excised by the specific glyosylase studied; but genome instability and cancer rarely are observed. However, cancer and genome instability have been observed in mouse models in which at least two DNA glycosylases have been deleted (see for example [112, 113]). Deletion of genes that encode proteins that act downstream of the DNA glycosylases usually results in embryonic or early onset lethality inmice. Some of these mice have aberrant neuronal phenotypes, suggesting the importance of BER in neuronal DNA maintenance. It is suggested that BER is critical for development and maintenance of certain types of cells and that deletion of BER genes is not compatible with life. Indeed, deletions of BER genes are rarely, if ever, observed to occur in the germline of humans. Thus, it is suggested that knock-in mouse models,

harboring specific BER polymorphisms, might act as better models for the study of genomic instability, cancer, and possibly other human diseases that might arise from aberrant BER.

7. Genetics and Environment Contribute to Genomic Instability and Cancer

Although children suffer from cancer, the highest percentage of people diagnosed with cancer are adults, many of whom are older adults. This, along with the existence of many low frequency germline SNPs in BER genes, indicates that a genetic predisposition, combined with long-term environmental exposure, lead to a mutator phenotype and cancer. In the case of BER, the exposures likely result from endogenous damage due to the inherently unstable nature of DNA, metabolism of oxygen, and aberrant DNA methylation. Subtle deficiencies in BER of these endogenous lesions can lead to genomic instability and cancer after several years of exposure combined with subtly aberrant BER. The mutations arising from aberrant BER need to accumulate in genes and regulatory regions that are critical for growth control, genome stability, and damage signaling in order for mutagenesis, genomic instability, and tumor initiation to occur. For example, many of the polymorphic variants of Polβ we have studied possess subtle mutator phenotypes that lead to cellular transformation after many passages in tissue culture (for example see [70]).

8. Conclusions

Endogenous DNA damage occurs at very high rates in human cells. These lesions normally are repaired and the DNA sequence is restored. However, in the presence of environmental stress and BER polymorphic variants, BER is aberrant and mutations accumulate in the genome which have the potential to lead to genomic instability and cancer.

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Abbreviations

BER	base excision repair
ROS	reactive oxygen species
SAM	*S*-adenosylmethionine
APE1	apurimidinic endonuclease I
MTH	MutT homolog
OGG1	DNA glycosylase 8-oxoguanine glycosylase
FapyG	2,6-diamino-4-hydroxy-5-formamidopyrimidine
5-meC	5-methylcytosine
MBD4	methyl-CpG-binding domain protein 4
TDG	thymine DNA glycosylase
MLH1	MutL homolog 1
UNG	uracil DNA glycosylase 1
SMUG1	single-strand selective monofunctional uracil DNA glycosylase

AAG	3-methyladenine DNA glycosylase
DRP	5′deoxyribosephosphate
XRCC1	X-Ray Cross-Complementing Factor 1
LIGIIIα	DNA ligaseIIIα
Polβ	DNA polymerase beta
Polλ	DNA polymerase lambda
HDR	homology dependent repair
NHEJ	non-homologous end joining
SSBs	single-strand breaks
DSBs	double-strand breaks
PARP1	polyADPribose polymerase 1

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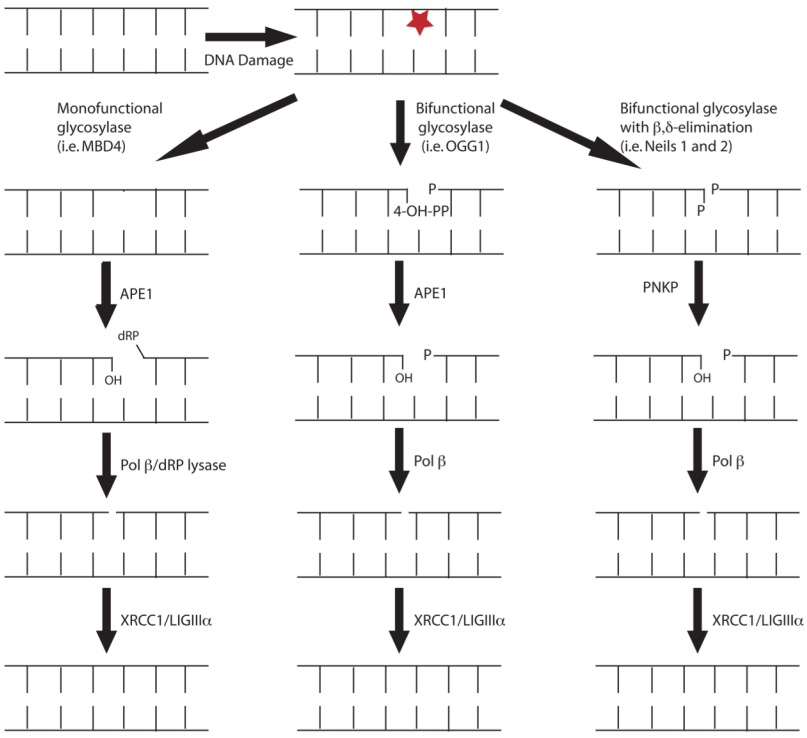

Figure 1. The BER pathway

There are five major steps in the BER pathway: 1) excision of the damaged base; 2) AP site incision; 3) modification of the ends of the DNA break; 4) gap filling; and 5) ligation of the nick. For a monofunctional glycosylase (i.e. MBD4), the glycosylase removes the damaged base leaving an abasic site. Ape1 incises the abasic site leaving a 3′ OH and 5′ dRP. Polβ removes the dRP group with its lyase activity and fills in the missing nucleotide. In the case of bifunctional glycosylases (i.e. OGG1), the damaged base is removed and the glycosylase incises the abasic site leaving a 3′ 4-OH pentenal phosphate (4-OH-PP) and 5′ phosphate. Ape1 modifies the end to 3′ OH and Polβ fills in the gap. In an Ape1-independent pathway, bifunctional glycosylases (i.e. Neils 1 and 2) remove the damaged base and incise the abasic site via with β,δ elimination, leaving 3′ and 5′ phosphates. PNKP modifies the 3′ end to an OH and Pol β fills in the gap. In the final step, the DNA is ligated by the XRCC1/LIGIIIα complex.

Table 1

Summary of DNA Glycosylases

Gene Symbol	Gene	Type of Glycosylase	Substrates
OGG1	8-oxoguanine DNA glycosylase	bifunctional	8-oxoG:C/G/T; 8-oxoG-FapyG;
MUTYH	mutY homolog (E. coli)	monofunctional	8-oxoG:A
TDG	thymine-DNA glycosylase	monofunctional	T:G; ethenoC:G
MBD4	methyl-CpG binding domain protein 4	monofunctional	T:G; 5-meCpG:TpG; O^6-meG:T
UNG	uracil-DNA glycosylase	monofunctional	uracil
SMUG1	single-strand-selective monofunctional uracil-DNA glycosylase 1	monofunctional	U:G; 5-hydroxymethyluracil; 5-formyluracil
NTHL1 (NTH1)	nth endonuclease III-like 1 (E. coli)	bifunctional	oxidized pyrimidines, formamidopyrimidines, 5-formyluracil; incises AP sites
NEIL1	nei endonuclease VIII-like 1 (E. coli)	bifunctional; APE-independent	oxidized pyrimidines, formamidopyrimidines, thymine glycol, hydantoin, guanidinohydantoin, spiroiminodihydantoin; removes lesions in single-stranded, bubble, and forked DNA structures
NEIL2	nei endonuclease VIII-like 2 (E. coli)	bifunctional; APE-independent	oxidized pyrimidiines; removes lesions in single-stranded and bubble DNA structures
NEIL3	nei endonuclease VIII-like 3 (E. coli)	bifunctional	hydantoin, guanidinohydantoin, spiroiminodihydantoin; removes lesions in single-stranded and bubble DNA structures
AAG (MPG)	N-methylpurine-DNA glycosylase	monofunctional	methylated bases (i.e. 7-meG, 3-meA); 1-N^6-etheno-A, 1-N^2-ethenoG, uracil

Table 2

Summary of BER proteins.

Gene Symbol	Gene	Function
APEX1 (APE1)	APEX nuclease (multifunctional DNA repair enzyme) 1	binds to abasic site and cleaves backbone; remodels 3′ end in the case of bifunctional glycosylases
XRCC1	X-ray complementing defective repair in Chinese hamster cells 1	scaffold protein that helps recruit BER proteins
POLB	polymerase (DNA directed), beta	main polymerase in BER
POLL	polymerase (DNA directed), lambda	substitutes for Pol β in BER
PARP1	poly (ADP-ribose) polymerase 1	binds to breaks in DNA and recruits other BER proteins
LIG3	ligase III, DNA, ATP-dependent	ligates the ends of the repaired DNA