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Base excision repair: the long and short of it

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Abstract. Base excision repair (BER) is the primary DNA repair pathway that corrects base lesions that arise due to oxidative, alkylation, deamination, and depurinatiation/depyrimidination damage. BER facilitates the repair of damaged DNA via two general pathways – short-patch and long-patch. The short-patch BER pathway leads to a repair tract of a single nucleotide. Alternatively, the long-patch BER pathway produces a repair tract of at least two nucleotides. The BER pathway is initiated by one of many DNA glycosylases, which recognize and catalyze the removal of damaged bases. The completion of the BER pathway is accomplished by the coordinated action of at least three additional enzymes. These downstream enzymes carry out strand incision, gap-filling and ligation. The high degree of BER conservation between *E. coli* and mammals has lead to advances in our understanding of mammalian BER. This review will provide a general overview of the mammalian BER pathway. (Part of a Multi-author Review)

Keywords. Base excision repair, glycosylase, DNA damage, alkylation, oxidation, deamination.

Overview

DNA damage arises from a variety of sources, including oxidative damage [1], alkylation damage [2], and deamination events [3]. Many DNA lesions resulting from these types of damage can cause non-canonical base pairing which may eventually result in the incorporation of an incorrect base by a DNA polymerase, causing a mutation. In order to maintain genomic integrity the cell must repair these damaged bases. Evolution has developed a highly conserved pathway to repair DNA damage arising from alkylation, deamination, and oxidative damage; this pathway is known as the base excision repair pathway (BER). This repair pathway has the ability to fix many types of damaged bases in a cell, thus ensuring the integrity of the genome. This review will address the current understanding of the BER pathway. We begin this review discussing the necessity for the BER pathway followed by a brief overview of a currently accepted model for mammalian BER. A more detailed discussion of the enzymes involved in the BER pathway, including structural analysis, will follow, and we address the high degree of BER conservation throughout evolution. We will touch on some mouse deletions which provide insight into the necessity for BER in humans and, in conclusion, describe where we believe BER research is headed in the future.

The core base excision repair pathway

The core base excision repair pathway (Fig. 1) requires the function of only four proteins [4]: these proteins include a DNA glycosylase, an AP endonuclease or AP DNA lyase, a DNA polymerase, and a DNA ligase. The proteins involved in this core reaction function in concert to remove a damaged DNA base and replace it with the correct base. A currently accepted model for the core BER pathway reveals five distinct enzymatic steps for the repair of

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damaged bases. The first step in BER is the recognition of a damaged base by a DNA glycosylase. After recognition of the damaged base by the appropriate DNA glycosylase, this glycosylase catalyzes the cleavage of an N-glycosidic bond, effectively removing the damaged base and creating an apurinic or apyrmidinic site (AP site). The DNA backbone is cleaved by either a DNA AP endonuclease or a DNA AP lyase – an activity present in some glycosylases. AP endonuclease activity creates a single-stranded DNA nick 5′ to the AP site, contrasting with the nick being created 3′ to the AP site as resulting from DNA AP lyase activity. The newly created nick is processed by the AP endonuclease, creating a single-nucleotide gap in the DNA. Importantly, the gap created contains a 3′-hydroxyl and a 5′-phosphate, substrates compatible with the downstream enzymatic reactions in BER. A DNA polymerase fills in the gap with the correct nucleotide. Finally, a DNA ligase completes the repair process and restores the integrity of the helix by sealing the nick.

The BER pathway was discovered nearly 35 years ago by Lindahl. He was searching for an enzymatic activity that could catalyze the removal of the mutagenic uracil DNA base. In *Escherichia coli*, he identified an enzymatic activity that catalyzed the release of uracil as a free base [5, 6]. Other groups subsequently demonstrated that this enzyme, the uracil-DNA glycosylase, represented a highly conserved DNA repair enzyme that is present in most organisms [7, 8]. A few years later Laval successfully reconstituted the first two steps of the BER pathway with a DNA glycosylase which excised 3-methyladenine from DNA and an AP endonuclease [9]. Today it is widely accepted that two different enzymatic activities are capable of cleaving the DNA at the AP site. The first activity described is carried out by an AP endonuclease which incises the DNA 5′ to the AP site, creating a 5′ sugar moiety which must be processed by a DNA polymerase to allow for DNA ligation [10, 11]. Alternatively, the AP site is incised by an AP lyase, often associated with a bifunctional DNA glycosylase [12]. This lyase activity creates a DNA nick containing a 3′ sugar moiety which requires further processing by a DNA polymerase in order to provide a suitable substrate for a DNA ligase.

Matsumoto and Kim subsequently demonstrated that DNA polymerase beta (POLB) catalyzed the release of the 5′-terminal deoxyribophosphate (dRP) from the incised AP site [13]. The catalytic domain for this activity is located within an 8-kDa amino-terminal fragment of the polymerase. Thus, POLB carries out two distinct and essential enzymatic reactions in BER: it uses its DNA polymerase activity to fill in the one-nucleotide gap, and it also uses its 5′-deoxyribophosphatase activity to cleave the 5′ phosphate to allow for efficient ligation [13–15]. The one-nucleotide short-patch BER pathway is completed by the action of DNA ligase III (LIG3) [4, 16].

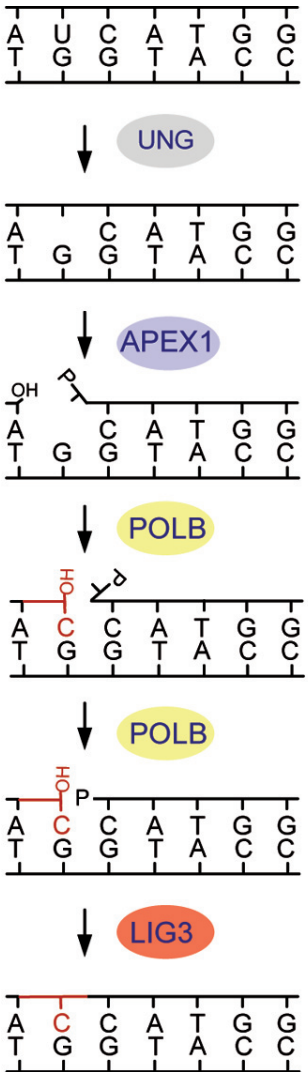

Figure 1. The core BER pathway. In this pathway, the UNG glycosylase catalyzes the excision of the damaged uracil base, creating an abasic (AP) site. The APEX1 endonuclease catalyzes the incision of the DNA backbone 5' to the AP site. POLB displaces the AP site and polymerizes DNA to fill in the gap. POLB then catalyzes the removal of the displaced AP site. Finally, LIG3 catalyzes the formation of a phosphodiester bond, completing the repair pathway. Abbreviations as in the legend to Figure 2.

The discovery by Matsumoto and Kim allowed for the reconstitution of the core BER pathway with human proteins. The human BER pathway was initially reconstituted using only four purified human enzymes – uracil-DNA glycosylase (UNG), AP endonuclease (APEX nuclease 1, APEX1), POLB and LIG3 or LIG1 [4].

The following year Dogliotti and co-workers described an alternative form of BER, termed long-patch

BER [17]. In long-patch BER, APEX1 catalyzes the formation of a nick 5′ to the AP site. This action recruits POLB or DNA polymerase delta (POLD), PCNA, flap structure-specific endonuclease 1 (FEN1), and probably the replicative DNA ligase ligase (LIG1). In a PCNA-dependent manner POLB strand displaces and polymerizes tracts of DNA longer than one base. The strand displacement activity of POLB produces a flapped substrate that is refractory to ligation. FEN1 resolves the problem of an unligatable DNA junction by catalyzing the removal of the flap generated by POLB. Since these discoveries long-patch BER has been reconstituted *in vitro* using purified human enzymes. The reconstitution of long-patch BER has demonstrated an absolute requirement for the endonuclease activity of FEN1 in long-patch but not short-patch BER [18, 19].

The decision to proceed via the long-patch or short-patch BER mechanism is poorly understood. Currently several hypotheses exist for the switch between long-patch and short-patch BER. One hypothesis suggests that the switch from short-patch to long-patch BER depends on the relative ATP concentration near the AP site, which is apparently modulated by LIG3 and X-ray cross-complementing protein 1 (XRCC1) [20]. It was shown that long-patch BER occurred more frequently at low ATP concentrations, whereas short-patch BER appeared to be the preferred mechanism with elevated concentrations of ATP. A second study has shown that the decision to proceed via long-patch BER or short-patch BER can be traced to the 5′-dRP intermediate produced by AP endonuclease activity. It was shown that if this dRP can be efficiently removed by dRP lyase activity of POLB, BER proceeds by the short-patch mechanism. However, if the dRP cannot be effectively removed the BER pathway proceeds by the long-patch mechanism, apparently to avoid generating a nick that is refractory to the action of a DNA ligase [19].

Interactions at the base excision repair pathways

XRCC1 is one of the first proteins recruited to the nick generated by the action of a glycosylase and/or AP endonuclease in short-patch BER. XRCC1, having no known enzymatic activity, has been shown to function as a scaffold protein, coordinating short-patch BER [4]. XRCC1 was identified as a major player in short-patch BER when it was shown to interact with LIG3, a core BER enzyme [21]. XRCC1 was shown to interact with POLB, required for short-patch BER, lending more support to the notion that XRCC1 functions as scaffold for short-patch BER [4].

Long-patch BER (Fig. 2) appears to require the abundant nuclear protein PCNA. The notion that PCNA is required for vertebrate long-patch BER was first suggested by Matsumoto and colleagues in 1994 when they fractionated *Xenopus* cell extracts. Extracts missing the PCNA processivity factor were unable to support long-patch base excision repair. This group also suggested that POLD or DNA polymerase epsilon (POLE) may function in long patch BER [17, 22]; however, which DNA polymerase functions in long-patch BER has not yet been fully elucidated. Further evidence for the necessity of PCNA in long-patch BER came from Frosina and colleagues in 1996. This group showed that long-patch BER was not supported in mammalian cell extracts that removed PCNA using an antibody [17]. Further support for PCNA functioning to coordinate long-patch BER came when it was shown that PCNA, the processivity factor for eukaryotic polymerases, also interacts with and stimulates the activity of FEN1 [23]. More recently PCNA was shown to stimulate long-patch BER when POLB or POLD was used for repair synthesis [19]. Taken together these findings suggest that PCNA promotes long-patch BER via its ability to interact with and coordinate the enzymatic activities of a DNA polymerase and FEN1, which are both required for long-patch BER.

As would be expected for efficient short-patch and long-patch BER, there are numerous interactions between DNA glycosylases and downstream components of BER which ensure completion of the BER repair pathway; these interactions are described in more detail in several reviews [24, 25]. Figure 2 shows the numerous interactions that are made by enzymes that might coordinate BER – the core BER enzyme, FEN1, and one of the proteins which has been shown to interact with FEN1, PCNA.

Glycosylases at the initial step of base excision repair

DNA glycosylases make BER possible – glycosylases recognize specific damaged bases and excise them from the genome, effectively initiating both long-patch and short-patch BER. To date 11 different mammalian glycosylases have been characterized (Table 1). The primary function of most DNA glycosylases is to recognize their substrate (the damaged base) and catalyze the cleavage of an N-glycosidic bond, releasing a free base and creating an abasic site [5]. In addition to catalyzing the cleavage of N-glycosydic bonds, some glycosylases are bifunctional having an additional AP lyase activity [10].

The uracil-DNA glycosylase (UNG) was the first DNA glycosylase to be identified and cloned [5].

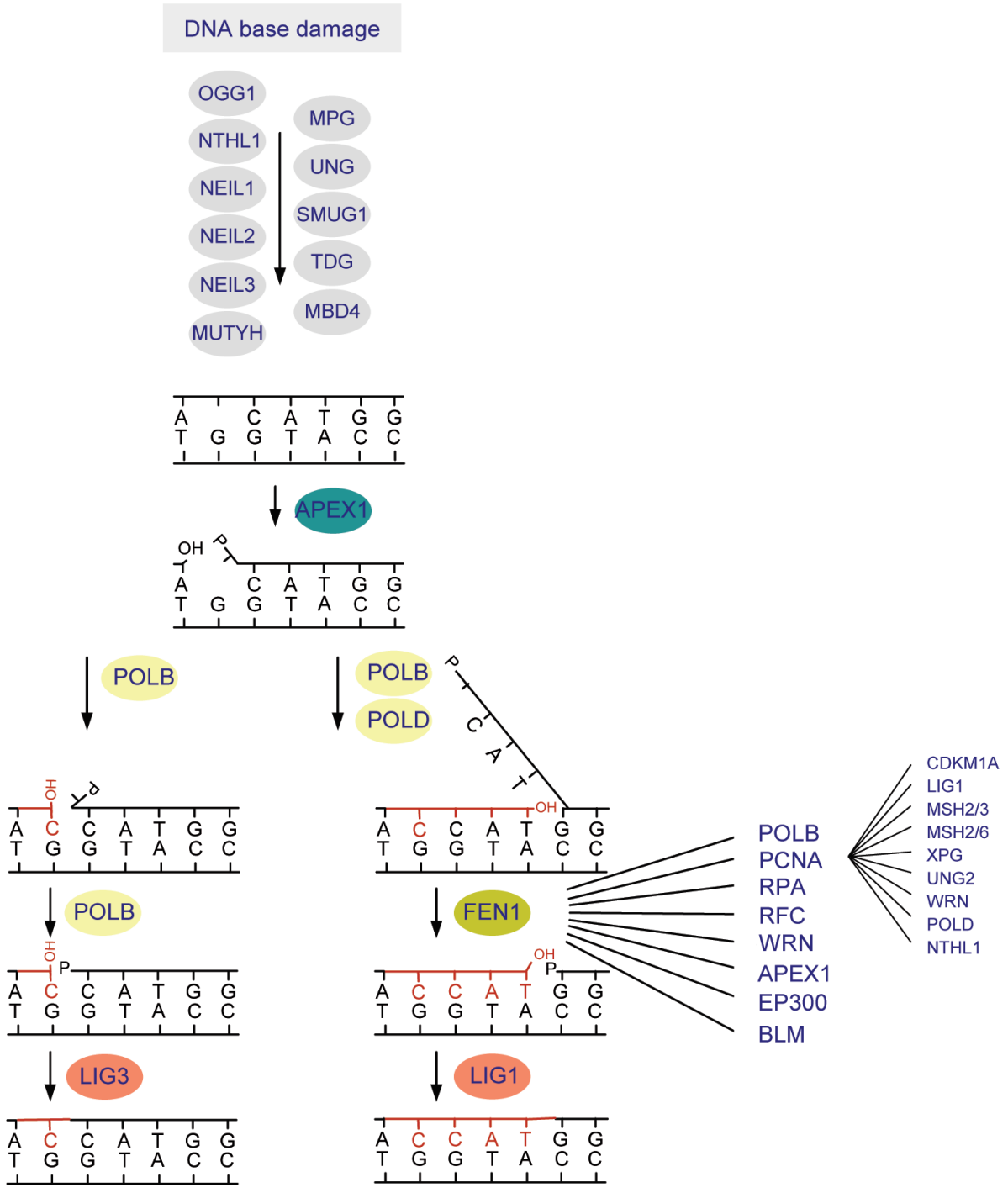

Figure 2. Selected protein interactions in the BER pathway. This figure shows the plethora of proteins and DNA interactions in both the short-patch (left branch) and long-patch (right branch) mammalian BER pathways. OGG1, 8-oxoguanine-DNA glycosylase; NTHL1, nth endonuclease III-like 1; NEIL, nei endonuclease VIII-like; MUTYH, muty homolog; MPG, N-methylpurine-DNA glycosylase; UNG, uracil-DNA glycosylase; SMUG1, single-strand-selective monofunctional uracil-DNA glycosylase; TDG, thymine-DNA glycosylase; MBD4, methyl-CpG binding domain protein 4; APEX1, APEX nuclease 1; POL, DNA polymerase; FEN1, flap structure-specific endonuclease 1; LIG, DNA ligase; PCNA, proliferating cell nuclear antigen; RPA, replication protein A; RFC, replication factor C; WRN, Werner syndrome protein; EP300, E1A binding protein (alias p300); BLM, bloom syndrome protein; CDKM1A, cyclin dependent kinase inhibitor 1A (alias p21); MSH, mutS homolog, ERCC5, excision repair cross-complementing rodent repair deficiency protein (alias XPG). Gene names by www.genenames.org.

Table 1. Core base excision repair enzymes (human nomenclature) with a few biochemical properties and gene-targeted deletion phenotypes indicated.

Enzyme	Enzymatic activities	Mouse mutant phenotypes
UNG	uracil	B-cell lymphomas (after 18 months)
SMUG1	uracil, 5-OH-meU	*
TDG	T, U and ethenoC (CpG sites)	*
MBD4	T and U opposite G (CpG sites)	normal (increase in C-to-T transitions)
MUTYH	A opposite 8-oxoG	normal
OGG1	8-oxoG, fapyG	normal (increase in G-to-T transversions
NTHL1	Tg, fapyG, DHU, 5-OHU, 5-OHC	normal
NEIL1	as NTH1 and fapyA, 8-oxoG	normal/metabolic syndrome
NEIL2	overlap with NTH1/NEIL1	*
NEIL3	unknown	normal
MPG	3-meA, hypoxanthine, ethenoA	normal
* Not yet available.		
APEX1	AP-endonuclease activity	lethal (E5.5/E8.5)
POLB	DNA synthesis, 5′ dRPase activity	lethal (E18.5)
FEN1	5′ single-stranded flap endonuclease	lethal (E4.5)
LIG3	ligates 3′-OH/5′-P nicks	lethal (E9.5)

DNA glycosylases in the upper table and enzymes of the intermediate and later stages of BER in the lower table. Data from [79] and [65].

MPG, N-methylpurine-DNA glycosylase, and OGG1, 8-oxoguanine-DNA glycosylase, represent some extensively studied DNA glycosylases [2, 3, 26]. Here we give a brief overview of these three well-characterized DNA glycosylases.

Uracil in DNA arises as a result of the hydrolytic deamination of cytosine. Here one can easily see the need for the removal of uracil from the DNA – this newly generated uracil base will base-pair with adenine, causing a C-to-T transition mutation [27]. Enzymes that catalyze the excision of uracil from the genome are present in many species. In mammalian species the UNG protein is coded for by the *UNG* gene which has both a nuclear (UNG2) and a mitochondrial (UNG1) form, depending upon splice variants of the RNA [28]. Both forms of UNG have the most efficient DNA glycolase activity when its substrate is uracil in DNA. UNG can catalyze the excision of uracil from both single-stranded and double-stranded DNA [29]. Compared with all other known DNA glycosylases, UNG has an incredibly high turnover number; it is capable of catalyzing the removal of 1000 uracil residues from DNA every minute [3]. UNG is also capable of catalyzing the excision of cytosine-derived products of oxidative DNA damage, albeit at lower efficiencies. Isodialuric acid, 5-hydroxyuracil, and alloxan have all been described as substrates for UNG [30].

SMUG1, single-strand-specific monofunctional uracil-DNA glycosylase, is another DNA glycosylase that has been shown to efficiently catalyze the excision of uracil residues from DNA [31]. However, combined disruption of SMUG1 and UNG demonstrates that these enzymes have similar, as well as distinct and non-redundant roles [32].

The *OGG1* gene was discovered in the mid-1990s in a random genetic screen using a mutator strain of *E. coli* (*fpg mutY*). This screen was attempting to identify *Saccharomyces cerevisiae* genes that would complement the deletion of the *fpg mutY* genes in *E. coli.* It was demonstrated that the *S. cerevisiae OGG1* gene could complement the deletion of the *E. coli fpg* and *mutY* genes, leading to a reduction in the bacterium's spontaneous mutation frequency [33]. The *OGG1* gene was shown to encode an enzyme that could effectively catalyze the cleavage of an N-glycosidic bond between a deoxyribose sugar and the damaged base, 8-oxoguanine (8-oxoG) (see Fig. 3 for structures of damaged bases). 8-oxoG results from the oxidative damage of a guanosine base, causing this base to pair with adenine as well as with cytosine, resulting in an G-to-T transversion mutation [34]. The human OGG1 enzyme was identified because of its high degree of homology to the *S. cerevisiae OGG1* gene [26].

The OGG1 enzymes are structurally unrelated to the *E. coli Fpg* enzyme, and there is no indication of any sequence homology between the two proteins. How-

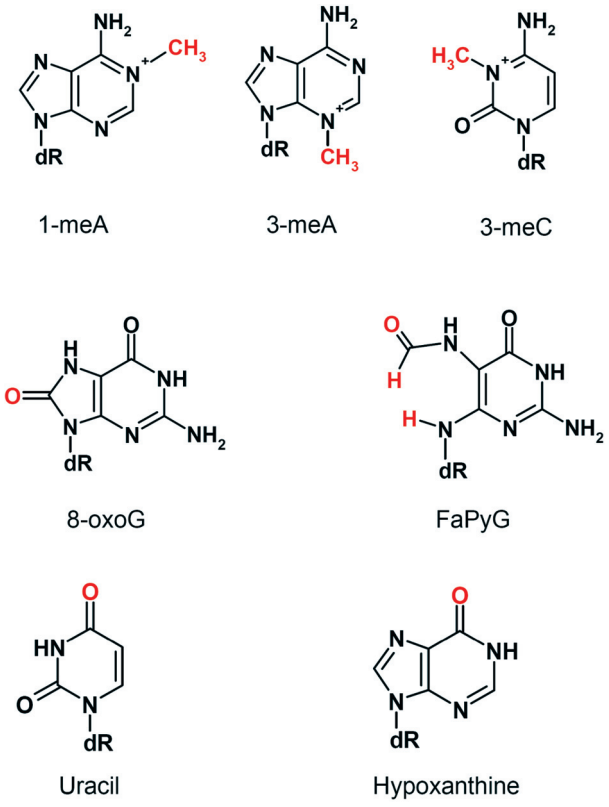

Figure 3. Several damaged DNA bases are processed by the BER pathway. This figure shows the structures of many damaged bases that can be repaired by BER. 1-meA, 1-methyl adenine; 3-meA, 3-methyl adenine; 3-meC, 3-methyl cytosine; 8-oxoG, 8-oxoguanine; FaPyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine.

ever, the activity of these two enzymes on 8-oxoG bases in DNA is comparable. In fact, the activities are so similar that the eukaryotic enzymes can complement a deletion of the *fpg* gene in *E. coli* [35, 36]. The OGG1 glycosylase, like many glycosylases that catalyze the excision of oxidized bases, is a bifunctional DNA glycosylase [37]. The initial characterization of purified OGG1 from *S. cerevisiae* revealed that this glycosylase could also catalyze the removal of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) lesions in addition to 8-oxoG [12]. FapyG and 8-oxoG bases in DNA are both substrates for the mouse Ogg1 and human OGG1 enzyme [38]. Further characterization revealed that OGG1 could catalyze the removal of a 7,8-dihydro-8-oxoadenine (8-oxoA) lesion [39, 40]. Although OGG1 repairs all these lesions with a similar efficiency, the vast majority of research has focused on 8-oxoG repair. Because of the abundant research on 8-oxoG, it is worthy to note that FapyG and 8-oxoA are induced at significant rates by oxidative stress, and therefore must be repaired by BER initiated with the OGG1 glycosylase.

E. coli contains two DNA glycosylases that are capable of catalyzing the removal of alkylated DNA bases; however, only one mammalian DNA glycosylase that can catalyze the removal of an alkylated DNA base has been identified. The mammalian enzyme has been termed N-methylpurine-DNA glycosylase (MPG) [41–43]. MPG can catalyze the removal of 3-methyladenine from DNA; however, MPG has a broad substrate specificity including 7-methylguanine (7-meG), hypoxanthine, and 1,N^6-ethenoadenine. Additionally, MPG has been shown to catalyze the removal of unmodified adenine and guanine bases from DNA at very slow rates [44]. This glycosylase activity on normal bases could seemingly represent a major threat to the integrity of the genome. However, the slow velocity of the excision of these bases probably has little relevance *in vivo,* especially given that both adenine and guanine are continuously lost at much higher rates due to spontaneous hydrolytic depurination [2].

Interestingly, some organisms remove identical DNA damage using remarkably different pathways. 1-methyl adenine (1-meA) and 3-methyl cytosine (3-meC) lesions are substrates for the AfAlkA glycosylase in *Archaeoglobus fulgidus* (AfAlkA) [45]. In *E. coli*, and in humans, these lesions are processed by the AlkB dioxygenase, which directly catalyzes the removal of the methyl group and not the entire base [2].

Structures in base excision repair

Crystal structures have been an invaluable resource for understanding and elucidating the enzymatic mechanisms of the enzymes involved in BER. For instance, a DNA glycosylase has an incredibly complex enzymatic mechanism that could not realistically be understood without the use of crystal structures. Using crystallography, DNA glycosylases have been shown to (i) recognize damaged DNA bases, (ii) cause helical distortions in the DNA resulting in the flipping out of the damaged base from the DNA helix, (iii) catalyze the cleavage of the N-glycosidic bond between the damaged base and the deoxyribose sugar, and (iv) release the damaged base into solution.

The crystal structures of many bacterial BER-initiating proteins have been solved. For a review of these structures see Huffman et al. [46]. In sharp contrast to the bacterial enzymes, relatively few structures of mammalian BER enzymes have been solved – leaving researchers in the dark as to the enzymatic mechanism of these enzymes. In this section, we will summarize the structural information from a few mammalian BER enzymes (Fig. 4).

The structure of the human MPG enzyme is well characterized. Structural information about MPG is available for the protein in its native state, and

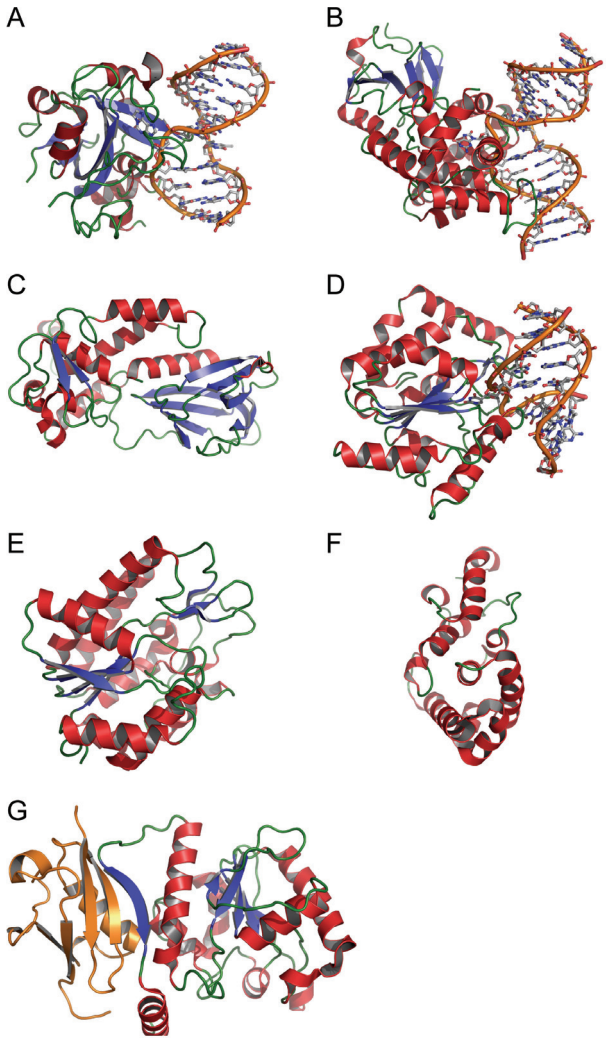

Figure 4. Crystal structures of eukaryotic DNA glycosylases with PDB accession numbers. human N-methylpurine-DNA glycosylase (MPG; 1EWN) in complex with DNA containing an 1,N^6-ethenoadenine (εA) moiety (*A*). 8-oxoguanine-DNA glycosylase (OGG1; 1EBM) complexed with DNA containing 8-oxoguanine (*B*). Human nei endonuclease VIII-like 1 protein (hNEIL1; 1TDH) (*C*). Human uracil-DNA glycosylase (UNG; 1EMH) in complex with DNA containing the substrate analog pseudouracil (*D*). *Xenopus laevis* single-strand specific monofunctional uracil-DNA glycosylase 1 (xSMUG1; 1OE6) (*E*). The C-terminal mismatch-specific thymine glycosylase domain of the methyl-CpG binding domain protein 4 (MBD4; 1NGN) (*F*). Human thymine-DNA glycosylase (TDG; 1WYW) conjugated to SUMO-1, here indicated in orange (*G*). The proteins are shown as cartoons and colored according to secondary structure, with α-helices shown in red, β-strands in blue, and loop regions in dark green. For DNA complexes, the DNA backbone is shown as orange tubes, with bases included as atom-colored sticks. The figure was prepared using PYMOL (http://www.pymol.org).

available for an inactive mutant of the protein complexed with DNA containing a 1,N^6-ethenoadenine lesion [47, 48]. Most BER DNA glycosylases have a relatively conserved structure; however, MPG has an atypical structure consisting of a single α/β domain with a β-hairpin protruding into the minor groove of the DNA when MPG recognizes its substrate (Fig. 4a). The damaged and often positively charged base is flipped into the active site of the enzyme where it is stabilized by planar stacking and cation-π interactions. These planar stacking and cation-π interactions are important in the substrate selectivity of MPG.

MPG is the only known mammalian alkylbase-DNA glycosylase that functions to repair alkylated DNA damage via the BER pathway. However, it is worthwhile to note that other mammalian enzymes can repair alkylated DNA damage using other repair activities – including suicide mechanisms (O^6-alkylguanine-DNA alkyltransferase (AGT)) or direct repair mechanisms in the case of 1-methyladenine (1-meA) or 3-methylcytosine (3-meC) catalyzed by AlkB-homolog 3 (ALKBH3). Crystal structures are available for both AGT and ALKBH3 [49, 50].

In contrast to MPG there are several mammalian BER glycosylases that can initiate the BER pathway in response to oxidative DNA damage (Table 1). These glycosylases include human 8-oxoG-DNA glycosylase (OGG1) and human endonuclease VIII-like 1 (NEIL1). The crystal structure of a catalytically inactive mutant of OGG1 (Fig. 4b) in complex with 8-oxoG has been determined [51] and shows that the overall structure of OGG1 is similar to bacterial endonuclease III and AlkA. A helix-hairpin-helix (HhH) motif, which generally implies DNA binding, in OGG1 is instrumental in making contact with the DNA and flipping the damaged base out of the double helix and into the recognition pocket.

The crystal structure of human NEIL1 (Fig. 4c) protein has been solved. Furthermore, NEIL1 has been shown to have a biochemical activity that catalyzes the removal of oxidized pyrimidine bases (thymine glycol and 5-hydroxyuracil) [52, 53]. The overall structure of NEIL1 consists of two domains, where the N- and C-terminal domains mainly contain β-strands and α-helices, respectively. Two helices in the C-terminal domain that contribute to a helix-2turn-helix (H2TH) motif are most likely involved in DNA binding.

There are a large number of BER glycosylases capable of initiating BER when presented with a uracil or misincorporated DNA base. BER glycosylases that catalyze the excision of uracil show up frequently in most organisms. The human uracil-DNA glycosylase (UNG; Fig. 4d) has been shown to catalyze the excision of uracil from both single-stranded and double-stranded DNA. UNG catalyzes the excision of the uracil base in DNA independent of the base opposite to the uracil base [54]. The catalytic domain of UNG consists of a mixed α/β structure with a central parallel β-sheet containing four β-strands [55].

The substrate binding pocket is located at the C-terminal end of this β-sheet, and because of the pocket's shape and amino acid composition it has a preference for uracil over other bases. A co-crystal structure of UNG in complex with a pseudouracil base has been solved [56]. The solution of this structure has contributed to the elucidation of the reaction mechanism catalyzed by UNG.

Although the SMUG1 glycosylase (Fig. 4e) was initially characterized as a single-strand-selective and monofunctional uracil-DNA glycosylase, this enzyme has later been demonstrated to efficiently catalyze the excision of uracil from both U:G mismatches and U:A base pairs [54]. To date, the only crystal structure of a SMUG1 enzyme comes from *Xenopus laevis* (xSMUG1). The structure of xSMUG1 has the same overall fold as UNG; however, upon base-flipping, the disruption of the DNA base stacking appears to be more extensive in xSMUG1 compared to UNG [57].

MBD4 is a member of the methyl-CpG-binding protein family, and contains two domains – an N-terminal methyl-CpG binding domain (MBD) and a C-terminal mismatch-specific glycosylase domain. As the name suggests, the MBD domain binds to methylated CpG regions of DNA while the C-terminal domain is able to catalyze the excision of thymine from G:T mismatches in DNA [58]. Only the C-terminal domain of MBD4 has been crystallized (Fig. 4f). This structure shows that the glycosylase domain has an all α-helical structure and contains an HhH structural motif [59].

Human thymine-DNA glycosylase (TDG; Fig. 4 g) has been shown to to catalyze the removal of both thymine and uracil bases that are incorrectly paired with guanosine [60]. The crystal structure of TDG has been determined while conjugated to the small ubiquitin-like modifier-1 (SUMO-1). The SUMO-1 modification has been reported to be important in order to reduce the AP site binding of TDG [61]. The glycosylase domain of TDG has an overall structure that is similar to UNG.

Conservation of enzymes in base excision repair

Many of the genes involved in BER are highly conserved from bacteria to humans. This high degree of conservation indicates that BER is a fundamental repair pathway in many living organisms. Major BER genes and their symbols in selected organisms are shown in Table 2.

The BER gene that is most highly conserved throughout evolution is probably the uracil-DNA glycosylase gene (*UNG*). UNG is conserved both in terms of the number of different organisms that have a functional homolog and the degree of sequence conservation across species [62]. Homologs of the *UNG* gene have been discovered in nearly every organism examined, including several viral genomes (including cytomegalovirus and the Epstein-Barr virus). A multiple sequence alignment of the uracil-DNA glycosylase proteins from humans, yeast, bacteria, and human cytomegalovirus is shown in Figure 5. A pairwise alignment of *E. coli* Ung and human UNG proteins demonstrates that the two proteins are 58 % identical, indicating an exceptionally high level of sequence conservation between the two species. UNG is in a structural superfamily of enzymes that include the UNG homolog found in thermophilic archea and several bacteria (AUDG), the single-strand-selective monofunctional uracil-DNA glycosylase (SMUG1), the mismatch-specific uracil-DNA glycosylase (MUG) and the T:G mismatch-specific thymine-DNA glycosylase (TDG), as well as other proteins [63].

Another highly conserved BER gene is the *nth* gene that codes for endonuclease III [64]. Endonuclease III catalyzes the removal of many oxidatively damaged bases. Homologs of the *nth* gene are also found in nearly every organism studied, and the sequences of *nth* homologs are well conserved, although not as highly conserved as the *ung* gene. Endonuclease III belongs to a large and versatile structural superfamily of enzymes characterized by an HhH motif [62]. This superfamily includes the A:G-mismatch repair DNA glycosylase (mutY) found in a wide range of organisms. Other glycosylases belonging to this superfamily include MBD4, AlkA, MAG1, Tag and OGG1. These enzymes are capable of binding or removing a wide range of both alkylated bases and oxidized bases.

The major nucleases that make incisions 5′ or 3′ to the AP site are primarily carried out by a glycosylase, exonuclease III (*xthA*, APEX1, APEX2), or endonuclease IV (*nfo*) families of genes. The *xthA* family of genes seems to be slightly more prevalent in known organisms than the *nfo* genes; however, these genes are far from being ubiquitously present throughout evolution.

Base excision repair mutants

In order to understand the effects of the loss of BER proteins have on mammals, many of the genes that code for BER enzymes have been knocked out in mice, and the effects of these mutations have been described (Table 1) [65, 66]. These knockout models have revealed that the BER enzymes have unique effects on the mice even if there is some redundancy in

Table 2. Major BER genes.

Gene name	*E. coli*	*S. cerevisiae*	*M. musculus*	*H. sapiens*
DNA glycosylases and related genes				
3-Methyl-adenine DNA glycosylase I	tag			
3-Methyl-adenine DNA glycosylase II	alkA	MAG1		
8-Oxoguanine DNA glycosylase		OGG1	Ogg1	OGG1
Endonuclease III/endonuclease III-like 1	nth	NTG1/NTG2	Nthl1	NTHL1
Adenine-DNA glycosylase	mutY		Mutyh	MUTYH
Methyl-CpG binding domain protein 4			Mbd4	MBD4
Uracil-DNA glycosylase	ung	UNG1	Ung	UNG
Single-strand-selective monofunctional uracil-DNA glycosylase 1			Smug1	SMUG1
G:U-mismatch-specific DNA glycosylase/thymine-DNA glycosylase	mug		Tdg	TDG
Formamidopyrimidine DNA glycosylase	mutM			
Endonuclease VIII	nei			
Endonuclease VIII-like 1			Neil1	NEIL1
endonuclease VIII-like 2			Neil2	NEIL2
N-Methylpurine-DNA glycosylase			Mpg	MPG
Endonucleases and related genes				
Exonuclease III/APEX nuclease (apurinic/apyrimidinic endonuclease) 1	xthA		Apex1	APEX1
APEX nuclease (apurinic/apyrimidinic endonuclease) 2		APN2	Apex2	APEX2
Endonuclease IV	nfo	APN1		
Flap structure-specific endonuclease 1		RAD27	Fen1	FEN1
Polymerases and related genes				
DNA polymerase I	polA			
DNA polymerase beta			Polb	POLB
Proliferating cell nuclear antigen/DNA polymerase sliding clamp		POL30	Pcna	PCNA
Poly(ADP-ribose) polymerase family, member 1			Parp1	PARP1
Poly(ADP-ribose) polymerase family, member 2			Parp2	PARP2
Ligases and related genes				
Ligase III, ATP-dependent			Lig3	LIG3
DNA ligase, NAD(+)-dependent	lig1			
X-ray repair complementing defective repair in Chinese hamster cells 1			Xrcc1	XRCC1

the specificity of an enzyme. Mouse models have led to the identification of residual activities for the repair of specific DNA base lesions. Often no overt phenotype is associated with mice lacking a single DNA glycosylase. The lack of a phenotype is somewhat surprising given the known mutagenic effects of unrepaired base lesions. On the other hand, the targeted deletion of BER enzymes other than glycosylases usually leads to a very severe and often lethal phenotype. We will describe in more detail the effects of removing one or more of the BER genes from mice.

Mice deficient for the *Ung* gene showed only a modest increase in spontaneous mutation frequency [67]. Using mice deficient for the *Ung* gene, it was shown that Ung corrected U:A mispairs that arise from the misincorporation of dUTP by a DNA polymerase, in addition to its role in correcting U:G mispairs that result from hydrolytic deamination of cytosine. Nilsen and colleagues subsequently demonstrated that *Ung*-deficient mice display an increased incidence of B-cell lymphomas as they get older [68]. Class-switch recombination of immunoglobulins requires the Ung processing of induced U:G mispairs resulting from enzymatic deamination of cytosine [69]. The production of these mispairs in B-cells allows for the formation of double-stranded DNA breaks necessary for class-switch recombination. The observed B-cell lymphomas are believed to arise because a deficiency in Ung interferes with B-cell differentiation [68]. The finding that Ung mice developed B-cell lymphomas more rapidly than their wild-type cohorts demonstrated, for the first time in mice, that a DNA glycosylase could have a role in mouse oncogenesis. Consistent with the *ung* mouse model, humans deficient for the *Ung* gene show significant problems with immunoglobulin class-switch recombination [70].

Mice deficient in the repair of 8-oxoG have been generated by disruption of the gene that codes for Ogg1 [38, 71]. The *Ogg1*-deficient mice produced are viable, fertile, and lack a visible phenotype. This finding suggests overlapping activities for the repair of 8-oxoG lesions [38, 71]. Further inspection of liver tissue from *Ogg1* null mice show a two- to threefold increase in the spontaneous mutation rate resulting from an increase in 8-oxoG lesions [38, 71]. Most of

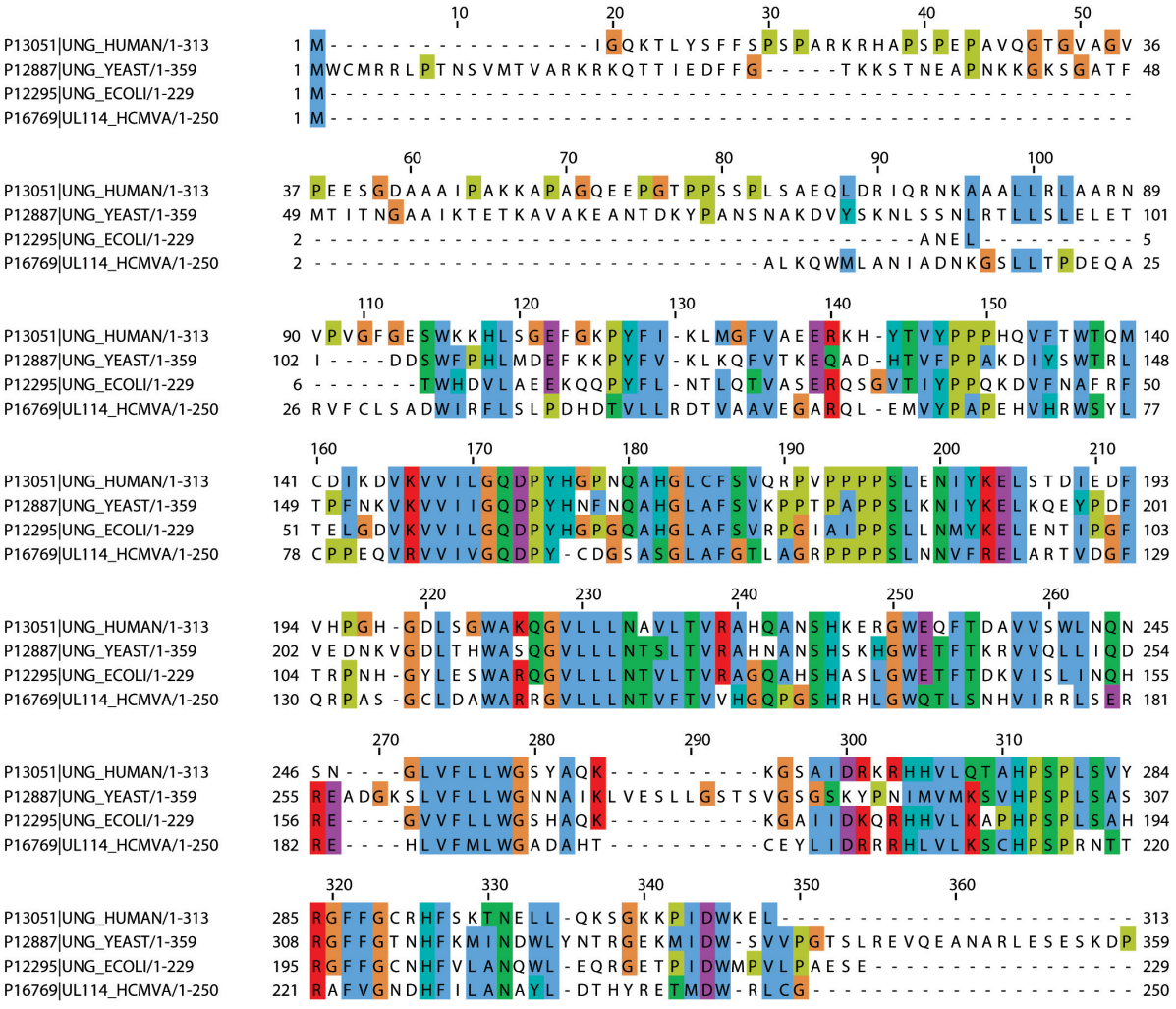

Figure 5. Multiple sequence alignment of uracil DNA glycosylases. Top line to bottom line: *Homo sapiens*, *Saccharomyces cerevisiae*, *Escherichia coli* and human cytomegalovirus (HHV5). Aligned by MAFFT [63a] and drawn by Jalview [63b].

these spontaneous mutations were in the form of GC-to-TA transversion mutations [38, 71]. The *Mutyh*, mutY homolog, knockout mouse also has no obvious tumor phenotype [72]. Interestingly a deletion of both *Mutyh* and *Ogg1* genes in mice results in a 65.7% higher incidence of tumors and suggests that Mutyh and Ogg1 have redundant activities [72]. This finding, in addition to the observations of the *Ung* deletion, established an obvious link between BER deficiency and tumor formation.

Biochemically the Mutyh DNA glycosylase catalyzes the removal of an adenine that has been misincorporated across from 8-oxoG. The action of Mutyh gives Ogg1 another opportunity to correctly repair this lesion prior to the mutation becoming fixed. Because of this finding it becomes clear that the *Mutyh Ogg1* double mutant is required for the accumulation of unrepaired 8-oxoG to become mutagenic and contribute to tumorigenesis. The synergy of the *Ogg1* and *Mutyh* deficiency parallels the situation observed in *E. coli*, where a hundredfold increase in spontaneous G-to-T transversions are seen in a *mutM mutY* double mutant [72, 73].

The Mpg glycosylase was the first glycosylase to be knocked out in mice. Two different research groups have produced these null mice. In both cases the animals were viable, fertile, and had a normal life span; interestingly, the fibroblasts derived from these mice were also only moderately sensitive to alkylating agents [74, 75]. This moderate sensitivity may suggest the existence of a redundant DNA glycosylase that can compensate for this activity, or the existence of another repair pathway that would repair these alkyl lesions.

In contrast to the deletion of glycosylases in mice described above, the deletion of murine genes that function downstream of these glycosylases in the BER pathway is not viable (summarized in Table 1). It is

reasonable to speculate that the reason the deletion of these genes is not viable is because BER, which can be initiated by many different DNA glycosylases, proceeds through identical downstream enzymes. The numbers of lesions handled by a single downstream enzyme are most likely severalfold higher than those handled by a single DNA glycosylase. Thus, it would appear that the removal of one of these downstream enzymes effectively abolishes BER altogether. Alternatively, it seems reasonable to believe that the activity of a DNA glycosylase creates a lesion in the double helix which must be processed by downstream BER enzymes. These lesions generated by glycosylases left unrepaired may be highly mutagenic and potentially lethal. In either scenario, it is known that the inactivation of the genes involved in the late stages of the BER pathway leads to embryonic lethality in mice [66], it seems likely that repair of spontaneous base damage in DNA is essential for embryogenesis.

Current challenges

Although the BER pathway has been extensively studied for more than 30 years, there are still challenges which remain. For instance, most biochemical studies that look at the mechanisms of the BER pathway have been carried out on naked DNA. DNA in mammalian cells is likely to be tightly wrapped into nucleosomes. The presence of nucleosomes may hinder the ability of BER enzymes to access damaged DNA. However, it has been demonstrated that at least one glycosylase, NTHL1, can access damaged bases in the presence of nucleosomal particles [76]. It remains to be shown that the BER pathway can be completed when damaged DNA is wrapped around histones. One study indicates that the two first steps of BER, DNA glycosylase and AP endonuclease, can carry out their activities on DNA substrates bound by nucleosomes [77]. However, the efficiency is only about 10% as compared with repair on naked DNA. The reaction is dependent on the rotational settings of the lesion, and the synthesis of new DNA by POLB was shown to be completely inhibited by nucleosomes. It is also possible that BER enzymes have additional activities that would loosen chromatin, allowing the enzymes to repair DNA damage in tightly wound chromatin. Another intriguing possibility is that BER enzymes can interact with chromatin modification enzymes which would loosen chromatin and allow for repair. One such example is the SIR2 histone deacetylase, which functions as a chromatin silencer regulating genomic stability and importantly has a role in BER [78]. Further analysis on this topic should include, like the study cited above, the study of mouse models deficient for chromatin modification proteins and the effects of these deficiencies in mice.

1 Lindahl, T. (1993) Instability and decay of the primary structure of DNA. Nature 362, 709–715.
2 Sedgwick, B., Bates, P. A., Paik, J., Jacobs, S. C. and Lindahl, T. (2007) Repair of alkylated DNA: recent advances. DNA Repair (Amst) 6, 429–442.
3 Kavli, B., Otterlei, M., Slupphaug, G. and Krokan, H. E. (2007) Uracil in DNA – general mutagen, but normal intermediate in acquired immunity. DNA Repair (Amst) 6, 505–516.
4 Kubota, Y., Nash, R. A., Klungland, A., Schar, P., Barnes, D. E. and Lindahl, T. (1996) Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase beta and the XRCC1 protein. EMBO J. 15, 6662–6670.
5 Lindahl, T. (1974) An N-glycosidase from *Escherichia coli* that releases free uracil from DNA containing deaminated cytosine residues. Proc. Natl. Acad. Sci. USA 71, 3649–3653.
6 Lindahl, T. (1980) Uracil-DNA glycosylase from *Escherichia coli.* Methods Enzymol. 65, 284–290.
7 Friedberg, E. C., Ganesan, A. K. and Minton, K. (1975) N-Glycosidase activity in extracts of *Bacillus subtilis* and its inhibition after infection with bacteriophage PBS2. J. Virol. 16, 315–321.
8 Olsen, L. C., Aasland, R., Wittwer, C. U., Krokan, H. E. and Helland, D. E. (1989) Molecular cloning of human uracil-DNA glycosylase, a highly conserved DNA repair enzyme. EMBO J. 8, 3121–3125.
9 Laval, J. (1977) Two enzymes are required from strand incision in repair of alkylated DNA. Nature 269, 829–832.
10 O'Connor, T. R. and Laval, J. (1989) Physical association of the 2,6-diamino-4-hydroxy-5N-formamidopyrimidine-DNA glycosylase of *Escherichia coli* and an activity nicking DNA at apurinic/apyrimidinic sites. Proc. Natl. Acad. Sci. USA 86, 5222–5226.
11 Robson, C. N. and Hickson, I. D. (1991) Isolation of cDNA clones encoding a human apurinic/apyrimidinic endonuclease that corrects DNA repair and mutagenesis defects in *E. coli* xth (exonuclease III) mutants. Nucleic Acids Res. 19, 5519–5523.
12 Boiteux, S., O'Connor, T. R. and Laval, J. (1987) Formamidopyrimidine-DNA glycosylase of *Escherichia coli*: cloning and sequencing of the fpg structural gene and overproduction of the protein. EMBO J. 6, 3177–3183.
13 Matsumoto, Y. and Kim, K. (1995) Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. Science 269, 699–702.
14 Singhal, R. K. and Wilson, S. H. (1993) Short gap-filling synthesis by DNA polymerase beta is processive. J. Biol. Chem. 268, 15906–15911.
15 Sobol, R. W., Horton, J. K., Kuhn, R., Gu, H., Singhal, R. K., Prasad, R., Rajewsky, K. and Wilson, S. H. (1996) Requirement of mammalian DNA polymerase-beta in base-excision repair. Nature 379, 183–186.
16 Wei, Y. F., Robins, P., Carter, K., Caldecott, K., Pappin, D. J., Yu, G. L., Wang, R. P., Shell, B. K., Nash, R. A., Schar, P. et al. (1995) Molecular cloning and expression of human cDNAs encoding a novel DNA ligase IV and DNA ligase III, an enzyme active in DNA repair and recombination. Mol. Cell Biol. 15, 3206–3216.
17 Frosina, G., Fortini, P., Rossi, O., Carrozzino, F., Raspaglio, G., Cox, L. S., Lane, D.P., Abbondandolo, A. and Dogliotti, E. (1996) Two pathways for base excision repair in mammalian cells. J. Biol. Chem. 271, 9573–9578.
18 Kim, K., Biade, S. and Matsumoto, Y. (1998) Involvement of flap endonuclease 1 in base excision DNA repair. J. Biol. Chem. 273, 8842–8848.
19 Klungland, A. and Lindahl, T. (1997) Second pathway for completion of human DNA base excision-repair: Reconstitu-

tion with purified proteins and requirement for DNase IV (FEN1). EMBO J. 16, 3341–3348.
20 Petermann, E., Ziegler, M. and Oei, S. L. (2003) ATP-dependent selection between single nucleotide and long patch base excision repair. DNA Repair (Amst) 2, 1101–1114.
21 Caldecott, K. W., McKeown, C. K., Tucker, J. D., Ljungquist, S. and Thompson, L.H. (1994) An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. Mol. Cell Biol. 14, 68–76.
22 Matsumoto, Y., Kim, K. and Bogenhagen, D. F. (1994) Proliferating cell nuclear antigen-dependent abasic site repair in *Xenopus laevis* oocytes: an alternative pathway of base excision DNA repair. Mol. Cell Biol. 14, 6187–6197.
23 Wu, X. T., Li, J., Li, X. Y., Hsieh, C. L., Burgers, P. M. J. and Lieber, M. R. (1996) Processing of branched DNA intermediates by a complex of human FEN-1 and PCNA. Nucleic Acids Res. 24, 2036–2043.
24 Almeida, K. H. and Sobol, R. W. (2007) A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification. DNA Repair (Amst) 6, 695–711.
25 Hegde, M. L., Hazra, T. K. and Mitra, S. (2008) Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res. 18, 27–47.
26 Klungland, A. and Bjelland, S. (2007) Oxidative damage to purines in DNA: role of mammalian Ogg1. DNA Repair (Amst) 6, 481–488.
27 Duncan, B. K. and Miller, J. H. (1980) Mutagenic deamination of cytosine residues in DNA. Nature 287, 560–561.
28 Slupphaug, G., Markussen, F. H., Olsen, L.C., Aasland, R., Aarsaether, N., Bakke, O., Krokan, H. E. and Helland, D. E. (1993) Nuclear and mitochondrial forms of human uracil-DNA glycosylase are encoded by the same gene. Nucleic Acids Res. 21, 2579–2584.
29 Slupphaug, G., Eftedal, I., Kavli, B., Bharati, S., Helle, N. M., Haug, T., Levine, D. W. and Krokan, H. E. (1995) Properties of a recombinant human uracil-DNA glycosylase from the UNG gene and evidence that UNG encodes the major uracil-DNA glycosylase. Biochemistry 34, 128–138.
30 Dizdaroglu, M., Karakaya, A., Jaruga, P., Slupphaug, G. and Krokan, H. E. (1996) Novel activities of human uracil DNA N-glycosylase for cytosine-derived products of oxidative DNA damage. Nucleic Acids Res. 24, 418–422.
31 Haushalter, K. A., Todd Stukenberg, M. W., Kirschner, M. W. and Verdine, G. L. (1999) Identification of a new uracil-DNA glycosylase family by expression cloning using synthetic inhibitors. Curr. Biol. 9, 174–185.
32 An, Q., Robins, P., Lindahl, T. and Barnes, D. E. (2005) C→T mutagenesis and gamma-radiation sensitivity due to deficiency in the Smug1 and Ung DNA glycosylases. EMBO J. 24, 2205–2213.
33 van der Kemp, P. A., Thomas, D., Barbey, R., de, O. R. and Boiteux, S. (1996) Cloning and expression in *Escherichia coli* of the OGG1 gene of *Saccharomyces cerevisiae*, which codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine. Proc. Natl. Acad. Sci. USA 93, 5197–5202.
34 Michaels, M. L. and Miller, J. H. (1992) The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). J. Bacteriol. 174, 6321–6325.
35 Radicella, J. P., Dherin, C., Desmaze, C., Fox, M. S. and Boiteux, S. (1997) Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 94, 8010–8015.
36 RoldanArjona, T., Wei, Y. F., Carter, K. C., Klungland, A., Anselmino, C., Wang, R. P., Augustus, M. and Lindahl, T. (1997) Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. Proc. Natl. Acad. Sci. USA 94, 8016–8020.
37 Bjoras, M., Luna, L., Johnsen, B., Hoff, E., Haug, T., Rognes, T. and Seeberg, E. (1997) Opposite base-dependent reactions of a human base excision repair enzyme on DNA containing 7,8-dihydro-8-oxoguanine and abasic sites. EMBO J. 16, 6314–6322.
38 Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeberg, E., Lindahl, T. and Barnes, D. E. (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc. Natl. Acad. Sci. USA 96, 13300–13305.
39 Girard, P. M., D'Ham, C., Cadet, J. and Boiteux, S. (1998) Opposite base-dependent excision of 7,8-dihydro-8-oxoadenine by the Ogg1 protein of *Saccharomyces cerevisiae*. Carcinogenesis 19, 1299–1305.
40 Jensen, A., Calvayrac, G., Karahalil, B., Bohr, V. A. and Stevnsner, T. (2003) Mammalian 8-oxoguanine DNA glycosylase 1 incises 8-oxoadenine opposite cytosine in nuclei and mitochondria, while a different glycosylase incises 8-oxoadenine opposite guanine in nuclei. J. Biol. Chem. 278, 19541–19548.
41 Samson, L., Derfler, B., Boosalis, M. and Call, K. (1991) Cloning and characterization of a 3-methyladenine DNA glycosylase cDNA from human cells whose gene maps to chromosome 16. Proc. Natl. Acad. Sci. USA 88, 9127–9131.
42 Chen, J., Derfler, B. and Samson, L. (1990) *Saccharomyces cerevisiae* 3-methyladenine DNA glycosylase has homology to the AlkA glycosylase of *E. coli* and is induced in response to DNA alkylation damage. EMBO J. 9, 4569–4575.
43 Berdal, K. G., Bjoras, M., Bjelland, S. and Seeberg, E. (1990) Cloning and expression in *Escherichia coli* of a gene for an alkylbase DNA glycosylase from *Saccharomyces cerevisiae*; a homologue to the bacterial alkA gene. EMBO J. 9, 4563–4568.
44 Berdal, K. G., Johansen, R. F. and Seeberg, E. (1998) Release of normal bases from intact DNA by a native DNA repair enzyme. EMBO J. 17, 363–367.
45 Leiros, I., Nabong, M. P., Grosvik, K., Ringvoll, J., Haugland, G. T., Uldal, L., Reite, K., Olsbu, I. K., Knaevelsrud, I., Moe, E. et al. (2007) Structural basis for enzymatic excision of N1-methyladenine and N3-methylcytosine from DNA. EMBO J. 26, 2206–2217.
46 Huffman, J. L., Sundheim, O. and Tainer, J. A. (2005) DNA base damage recognition and removal: new twists and grooves. Mutat. Res. 577, 55–76.
47 Lau, A. Y., Wyatt, M. D., Glassner, B. J., Samson, L. D. and Ellenberger, T. (2000) Molecular basis for discriminating between normal and damaged bases by the human alkyladenine glycosylase, AAG. Proc. Natl. Acad. Sci. USA 97, 13573–13578.
48 Lau, A. Y., Scharer, O. D., Samson, L., Verdine, G. L. and Ellenberger, T. (1998) Crystal structure of a human alkylbase-DNA repair enzyme complexed to DNA: mechanisms for nucleotide flipping and base excision. Cell 95, 249–258.
49 Daniels, D. S., Mol, C. D., Arvai, A.S., Kanugula, S., Pegg, A. E. and Tainer, J. A. (2000) Active and alkylated human AGT structures: a novel zinc site, inhibitor and extrahelical base binding. EMBO J. 19, 1719–1730.
50 Sundheim, O., Vagbo, C. B., Bjoras, M., Sousa, M. M., Talstad, V., Aas, P. A., Drablos, F., Krokan, H. E., Tainer, J. A. and Slupphaug, G. (2006) Human ABH3 structure and key residues for oxidative demethylation to reverse DNA/RNA damage. EMBO J. 25, 3389–3397.
51 Bruner, S. D., Norman, D. P. and Verdine, G. L. (2000) Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. Nature 403, 859–866.
52 Doublie, S., Bandaru, V., Bond, J. P. and Wallace, S. S. (2004) The crystal structure of human endonuclease VIII-like 1 (NEIL1) reveals a zincless finger motif required for glycosylase activity. Proc. Natl. Acad. Sci. USA 101, 10284–10289.
53 Wallace, S. S., Bandaru, V., Kathe, S. D. and Bond, J. P. (2003) The enigma of endonuclease VIII. DNA Repair (Amst) 2, 441–453.
54 Kavli, B., Sundheim, O., Akbari, M., Otterlei, M., Nilsen, H., Skorpen, F., Aas, P. A., Hagen, L., Krokan, H. E. and Slupphaug, G. (2002) hUNG2 is the major repair enzyme for

removal of uracil from U:A matches, U:G mismatches, and U in single-stranded DNA, with hSMUG1 as a broad specificity backup. J. Biol. Chem. 277, 39926–39936.
55 Slupphaug, G., Mol, C. D., Kavli, B., Arvai, A. S., Krokan, H. E. and Tainer, J. A. (1996) A nucleotide-flipping mechanism from the structure of human uracil-DNA glycosylase bound to DNA. Nature 384, 87–92.
56 Parikh, S. S., Walcher, G., Jones, G. D., Slupphaug, G., Krokan, H. E., Blackburn, G. M. and Tainer, J. A. (2000) Uracil-DNA glycosylase-DNA substrate and product structures: conformational strain promotes catalytic efficiency by coupled stereoelectronic effects. Proc. Natl. Acad. Sci. USA 97, 5083–5088.
57 Wibley, J. E., Waters, T. R., Haushalter, K., Verdine, G. L. and Pearl, L. H. (2003) Structure and specificity of the vertebrate anti-mutator uracil-DNA glycosylase SMUG1. Mol. Cell 11, 1647–1659.
58 Hendrich, B., Hardeland, U., Ng, H. H., Jiricny, J. and Bird, A. (1999) The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. Nature 401, 301–304.
59 Wu, P., Qiu, C., Sohail, A., Zhang, X., Bhagwat, A. S. and Cheng, X. (2003) Mismatch repair in methylated DNA. Structure and activity of the mismatch-specific thymine glycosylase domain of methyl-CpG-binding protein MBD4. J. Biol. Chem. 278, 5285–5291.
60 Hardeland, U., Bentele, M., Lettieri, T., Steinacher, R., Jiricny, J. and Schar, P. (2001) Thymine DNA glycosylase. Prog. Nucleic Acid Res. Mol. Biol. 68, 235–253.
61 Baba, D., Maita, N., Jee, J. G., Uchimura, Y., Saitoh, H., Sugasawa, K., Hanaoka, F., Tochio, H., Hiroaki, H. and Shirakawa, M. (2005) Crystal structure of thymine DNA glycosylase conjugated to SUMO-1. Nature 435, 979–982.
62 Krokan, H. E., Standal, R. and Slupphaug, G. (1997) DNA glycosylases in the base excision repair of DNA. Biochem. J. 325 (Pt 1), 1–16.
63 Aravind, L. and Koonin, E. V. (2000) The alpha/beta fold uracil DNA glycosylases: a common origin with diverse fates. Genome Biol. 1, RESEARCH0007.
63a Katoh, K., Kuma, K., Toh, H. and Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33, 511–518.
63b Clamp, M., Cuff, J., Searle, S. M. and Barton, G. J. (2004) The Jalview Java alignment editor. Bioinformatics 20, 426–427.
64 Aravind, L., Wallace, R. and Koonin, E. V. (1999) Conserved domains in DNA repair proteins and evolution of repair systems. Nucleic Acids Res. 27, 1223–1242.
65 Larsen, E., Meza, T. J., Kleppa, L. and Klungland, A. (2007) Organ and cell specificity of base excision repair mutants in mice. Mutat. Res. 614, 56–68.
66 Friedberg, E. C. and Meira, L. B. (2004) Database of mouse strains carrying targeted mutations in genes affecting biological responses to DNA damage (Version 6). DNA Repair (Amst) 3, 1617–1638.
67 Nilsen, H., Rosewell, I., Robins, P., Skjelbred, C. F., Andersen, S., Slupphaug, G., Daly, G., Krokan, H. E., Lindahl, T. and Barnes, D. E. (2000) Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication. Mol. Cell 5, 1059–1065.
68 Nilsen, H., Stamp, G., Andersen, S., Hrivnak, G., Krokan, H. E., Lindahl, T. and Barnes, D. E. (2003) Gene-targeted mice lacking the Ung uracil-DNA glycosylase develop B-cell lymphomas. Oncogene 22, 5381–5386.
69 Rada, C., Jarvis, J. M. and Milstein, C. (2002) AID-GFP chimeric protein increases hypermutation of Ig genes with no evidence of nuclear localization. Proc. Natl. Acad. Sci. USA 99, 7003–7008.
70 Imai, K., Slupphaug, G., Lee, W.I., Revy, P., Nonoyama, S., Catalan, N., Yel, L., Forveille, M., Kavli, B., Krokan, H. E. et al. (2003) Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat. Immunol. 4, 1023–1028.
71 Minowa, O., Arai, T., Hirano, M., Monden, Y., Nakai, S., Fukuda, M., Itoh, M., Takano, H., Hippou, Y., Aburatani, H. et al. (2000) Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. Proc. Natl. Acad. Sci. USA 97, 4156–4161.
72 Xie, Y., Yang, H., Cunanan, C., Okamoto, K., Shibata, D., Pan, J., Barnes, D. E., Lindahl, T., McIlhatton, M., Fishel, R. et al. (2004) Deficiencies in mouse Myh and Ogg1 result in tumor predisposition and G to T mutations in codon 12 of the K-ras oncogene in lung tumors. Cancer Res. 64, 3096–3102.
73 Michaels, M. L., Cruz, C., Grollman, A. P. and Miller, J. H. (1992) Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA. Proc. Natl. Acad. Sci. USA 89, 7022–7025.
74 Elder, R. H., Jansen, J. G., Weeks, R. J., Willington, M. A., Deans, B., Watson, A. J., Mynett, K. J., Bailey, J. A., Cooper, D. P., Rafferty, J. A. et al. (1998) Alkylpurine-DNA-N-glycosylase knockout mice show increased susceptibility to induction of mutations by methyl methanesulfonate. Mol. Cell Biol. 18, 5828–5837.
75 Engelward, B. P., Dreslin, A., Christensen, J., Huszar, D., Kurahara, C. and Samson, L. (1996) Repair-deficient 3-methyladenine DNA glycosylase homozygous mutant mouse cells have increased sensitivity to alkylation-induced chromosome damage and cell killing. EMBO J. 15, 945–952.
76 Prasad, A., Wallace, S. S. and Pederson, D. S. (2007) Initiation of base excision repair of oxidative lesions in nucleosomes by the human, bifunctional DNA glycosylase NTH1. Mol. Cell Biol. 27, 8442–8453.
77 Beard, B. C., Wilson, S. H. and Smerdon, M. J. (2003) Suppressed catalytic activity of base excision repair enzymes on rotationally positioned uracil in nucleosomes. Proc. Natl. Acad. Sci. USA 100, 7465–7470.
78 Mostoslavsky, R., Chua, K. F., Lombard, D. B., Pang, W. W., Fischer, M. R., Gellon, L., Liu, P., Mostoslavsky, G., Franco, S., Murphy, M. M. et al. (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 124, 315–329.
79 Barnes, D. E. and Lindahl, T. (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu. Rev. Genet. 38, 445–476.