Critical Reviews in Biochemistry and Molecular Biology, 43:239–276, 2008

ISSN: 1040-9238 print / 1549-7798 online
DOI: 10.1080/10409230802309905

informa
healthcare

Base Excision Repair and its Role in Maintaining Genome Stability

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For all living organisms, genome stability is important, but is also under constant threat because various environmental and endogenous damaging agents can modify the structural properties of DNA bases. As a defense, organisms have developed different DNA repair pathways. Base excision repair (BER) is the predominant pathway for coping with a broad range of small lesions resulting from oxidation, alkylation, and deamination, which modify individual bases without large effect on the double helix structure. As, in mammalian cells, this damage is estimated to account daily for 10^4 events per cell, the need for BER pathways is unquestionable. The damage-specific removal is carried out by a considerable group of enzymes, designated as DNA glycosylases. Each DNA glycosylase has its unique specificity and many of them are ubiquitous in microorganisms, mammals, and plants. Here, we review the importance of the BER pathway and we focus on the different roles of DNA glycosylases in various organisms.

Keywords DNA glycosylase, DNA repair, DNA damage, mutagenesis

BASE EXCISION REPAIR—GENERAL OVERVIEW

Base Excision Repair Pathway

The base excision repair (BER) pathway fixes lesions in bases that are similar in size and shape to the normal bases. These base lesions include deaminated cytosine, 5-methylcytosine, and adenine, but also oxidation products of all four bases and some types of base alkylation. Typically, only a small region (1 to 13 nucleotides) around the damaged base is removed and replaced during BER, in contrast to some other excision repair mechanisms, such as mismatch repair (MMR). BER happens in several steps (Figure 1): first, damage-specific recognition and removal of the base lesion or mismatched base, followed by cleavage of the sugar–phosphate backbone, excision of the abasic (apurinic–apyrimidinic, [AP]) site, DNA gap filling, and rejoining. Damage recognition depends on DNA glycosylases that remove the damaged base from the sugar–phosphate backbone, resulting in an AP site (Figure 1). This AP site is processed either by intrinsic 3′ AP lyase activity of the so-called bifunctional DNA glycosylases or by separate AP endonucleases after base lesion removal by a monofunctional DNA glycosylase. AP lyases and AP endonucleases cleave the sugar–phosphate backbone at the AP site, producing different types of "unconventional" DNA ends: AP endonucleases generate a 3′ OH and a 5′ deoxyribose–phosphate moiety (5′dRP) at the termini, whereas AP lyases form a 5′ phosphate and a 3′ blocking lesion, for instance 3′ α,β-unsaturated aldehyde after β-elimination (Figure 2). To allow gap filling by DNA polymerase and rejoining by DNA ligase, these unconventional ends have to be restored to the conventional 3′ OH and 5′ phosphate ends. The 5′dRP moiety generated by the AP endonuclease can be removed by the 5′dRPase activity of DNA polymerase β (Pol β) (Matsumoto and Kim, 1995; Deterding *et al*., 2000), whereas the intrinsic 3′ diesterase activity of AP endonucleases is able to remove the 3′ blocking lesion left by AP lyases (Izumi *et al*., 2000).

Once the 3′ terminus has been properly processed, gap filling and rejoining can continue by either of two sub-pathways: short-patch or long-patch BER, whereby only one or 2–13 nucleotides are replaced, respectively (Figure 1). In the short-patch BER sub-pathway, nucleotides are incorporated into the DNA by the mammalian Pol β or the *Escherichia coli* Pol I (Singhal *et al*., 1995; Sobol *et al*., 1996) and the resulting nick is ligated by a complex of mammalian XRCC1 and LigIIIα or bacterial LigI (Cappelli *et al*., 1997; Nash *et al*., 1997). XRCC1 is a scaffold protein, interacting with most components of the short-patch pathway, and plays a role in BER coordination (reviewed by Fortini and Dogliotti, 2007). During long-patch repair in mammals, Pol β probably also incorporates the first nucleotide (Podlutsky *et al*., 2001), but when the 5′ end cannot be processed, the additional elongation and strand displacement are

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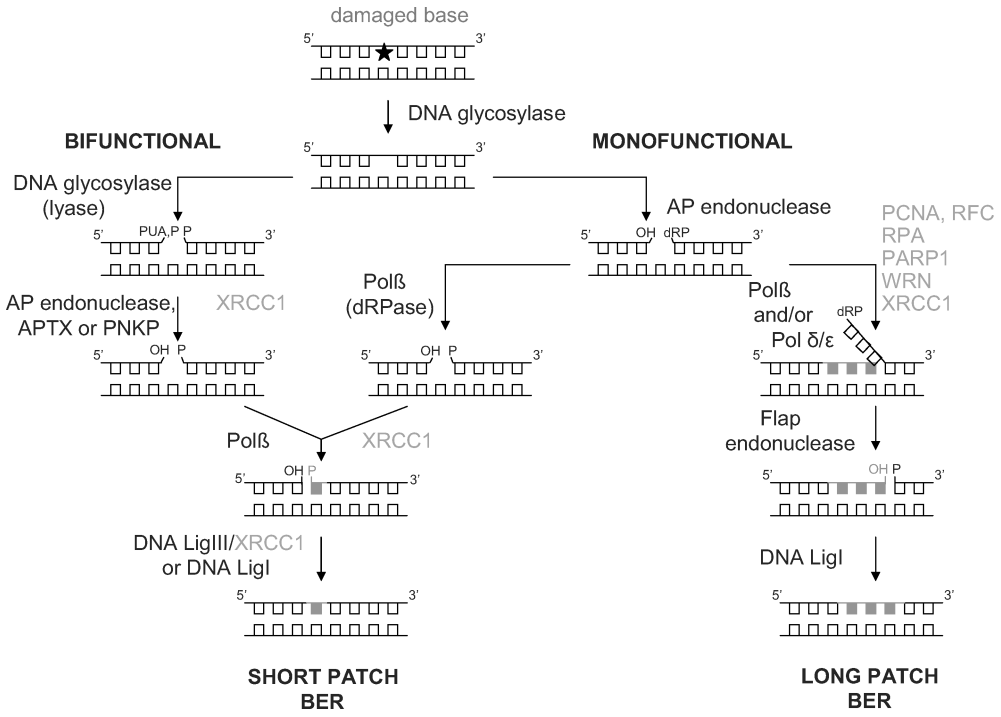

FIG. 1. Schematic BER pathway and different sub-pathways in mammals. BER starts with the recognition and removal of a lesion (star) by a DNA glycosylase. Only bifunctional DNA glycosylases are able to cleave the sugar–phosphate backbone and create a 5′ phosphate (P) and a 3′ phosphate or 3′ polyunsaturated aldehyde (PUA), depending on the DNA glycosylase. After removal of the damaged base by monofunctional DNA glycosylases, strand scission is exerted by AP endonuclease, creating 3′ hydroxyl (OH) and 5′ deoxyribose-phosphate (dRP). These unconventional termini have to be restored to 3′ OH and 5′ P to allow further repair through deoxyribose-phosphatase diesterase (dRPase) activity of Pol β (5′ dRP), diesterase activity of AP endonuclease (3′ PUA), phoshatase activity of polynucleotide kinase phosphatase (PNKP) (3′ P), or phosphatase activity of aprataxin (APTX) (3′ P). Repair then proceeds via short-patch or long-patch repair. During short-patch repair, Pol β incorporates one nucleotide, followed by nick ligation by the XRCC1/LigIIIα complex (predominantly) or LigI. If the 5′ lesion is refractory to cleavage by Pol β, the long-patch branch of BER is taken. Pol β and/or Pol δ/ε accomplish strand displacement by incorporating multiple nucleotides, followed by removal of the DNA flap containing the 5′ refractory moiety by Flap endonuclease and ligation of the resulting nick by LigI. Supportive BER proteins are indicated in gray. For more details, see text.

carried out by Pol δ or Pol ε, both replicative DNA polymerases (Fortini *et al*., 1998; Stucki *et al*., 1998). The resulting "flap" structure is then removed by the endonuclease FEN1 via a single-stranded break (SSB) and, subsequently, the nick is sealed by LigI (Levin *et al*., 1997). Additional players in long-patch repair are replication factor C (RFC) and poly(ADP-ribose)polymerase (PARP). The former is required to load the sliding clamp proliferating cell nuclear antigen (PCNA) onto DNA (Kelman and Hurwitz, 1998) to enhance the DNA polymerase activity (Gary *et al*., 1999; Matsumoto, 1999), whereas PARP1 and, to a lesser extent, PARP2 bind both SSBs and double-stranded breaks (DSBs). This binding triggers their activation toward poly-ADP ribosylation of specific nuclear proteins(Molinete *et al*., 1993; Amé *et al*., 1999). PARP1 binding to SSBs is believed to protect them from converting into DSBs, thus preserving the substrate for BER (Woodhouse *et al*., 2008). In addition, PARP1 is needed for stimulation of DNA synthesis and strand displacement, which may facilitate the repair of longer DNA stretches (Prasad *et al*., 2001). Pol δ and Pol ε require replication protein A (RPA) for DNA synthesis (Coverly *et al*., 1991), and Pol β needs the Werner syndrome protein (WRN) for stimulation of strand displacement synthesis in a helicase-dependent manner. In addition, WRN provides the lacking proofreading activity via its 3′→5′ exonuclease activity (Harrigan *et al*., 2006).

In *E. coli*, long-patch repair is initiated by Pol I that displaces and cleaves the dRP-containing strand by its 5′→3′ exonuclease activity (Xu *et al*., 1997). The nick is sealed by LigI, as in short-patch repair.

Additional Sub-Pathways

Besides the short-patch and long-patch repair pathways, over the past few years extra sub-pathways have been described that use BER proteins for repair of specific types of base damage. In

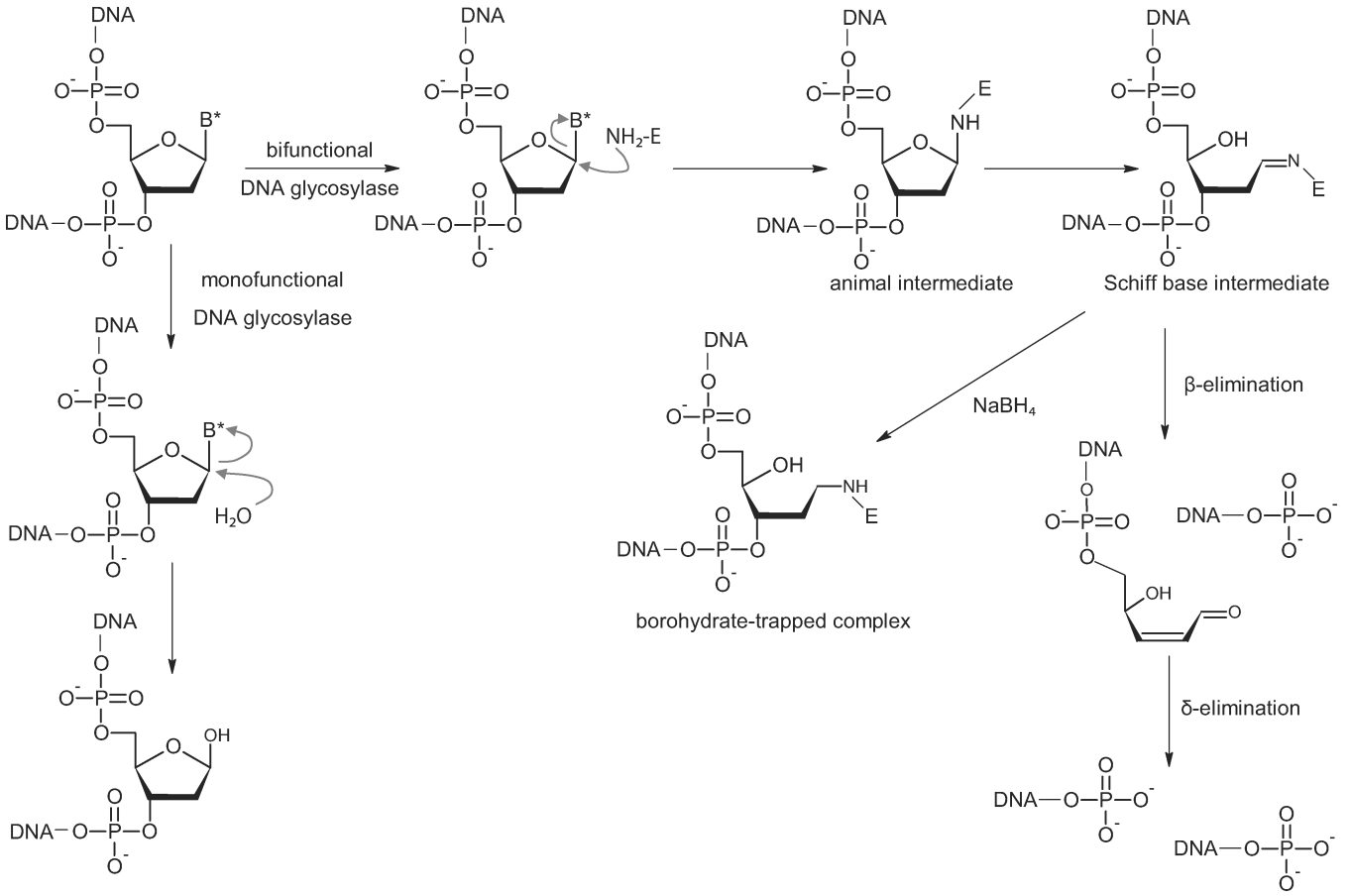

FIG. 2. Overview of the reactions catalyzed by monofunctional and bifunctional DNA glycosylases. Single-stranded DNA is presented. Monofunctional glycosylases remove the lesion in one step to generate an abasic site by using an activated water molecule. Bifunctional glycosylases catalyze reactions via the attachment of an active site amine moiety. A Schiff base intermediate is formed that can be trapped by $NaBH_4$. β-elimination generates the conventional 5′ phosphate and the 3′ blocking lesion 3′ α,β-unsaturated aldehyde, possibly followed by δ-elimination, generating 3′ and 5′ phosphates.

nucleotide incision repair, AP endonucleases repair oxidatively damaged DNA (predominantly 5-hydroxycytosine), independently of DNA glycosylases, while completion of the nucleotide incision repair happens probably predominantly through the long-patch repair pathway (Ischenko and Saparbaev, 2002; Gros *et al*., 2004). Given the importance of repair of oxidative DNA damage, postulated to be the major type of endogenous damage, nucleotide incision repair is proposed as an alternative or back-up method for BER.

The bifunctional endonuclease VIII or Nei-like DNA glycosylases NEIL1 and NEIL2 (see below) repair oxidized bases by means of an AP endonuclease-independent pathway (reviewed by Hazra *et al*., 2007). NEIL proteins process the 3′ unsaturated aldehyde by a β,δ-elimination mechanism that results in 3′ phosphate and 5′ phosphate at the termini. The 3′ phosphate is removed by polynucleotide kinase phosphatase (PNKP) rather than by AP endonuclease that has extremely weak 3′ phosphatase activity (Wiederhold *et al*., 2004). Aprataxin (APTX), the gene product mutated in the neurological disorder ataxia-ocular apraxia 1, is distantly homologous to PNKP (Moreira *et al*., 2001) and functions also in the removal of 3′ and 5′ blocking ends, such as 3′ phosphate groups (Ahel *et al*., 2006; Takahashi *et al*., 2007). NEIL-mediated repair is further processed through the short-patch repair pathway (Das *et al*., 2006).

AP sites occur as a consequence of both non-enzymatic and enzymatic hydrolysis of base-sugar bonds in DNA. They are highly mutagenic and cytotoxic because DNA replication can result in either misincorporation or in DSBs (Lindahl, 1993). Not only DNA glycosylases generate this lesion during the BER process, but also reactive oxygen species (ROS) and some alkylating agents promote the formation of AP sites due to destabilization of the glycosidic bond (Guillet and Boiteux, 2003). The total number of AP sites in a mammalian cell is higher than 10^4 events per day (Lindahl and Barnes, 2000). Repair starts with the recognition of these sites by an AP endonuclease followed by short-patch or long-patch BER.

SSBs are not only generated during the BER pathway, but also through endogenous oxidative metabolism and environmental

agents, such as ionizing radiation, creating 3′ and 5′ blocking lesions. SSB repair starts with recognition and binding of PARP1 and is followed by recruitment of the scaffold protein XRCC1. The typically unconventional termini are then converted by PNKP, APTX, or AP endonuclease, dependent on the type of DNA ends that flank the gap, so that short-patch or long-patch repair can follow. Because of their similarity, the SSB repair pathway is often considered part of the BER pathway.

Different factors determine the choice of one of the different sub-pathways (reviewed by Fortini and Dogliotti, 2007). First of all, the type of lesions and, as a result, the type of DNA termini generated during BER are determinants in the sub-pathway selection (Klungland and Lindahl, 1997; Dianov *et al*., 1998; Fortini *et al*., 1999; Bennett *et al*., 2001; Ho and Satoh, 2003). For instance, 5′ lesions refractory to Pol β cleavage are repaired via the long-patch repair pathway (Fortini *et al*., 1999). Also, bifunctional DNA glycosylases are expected to act as monofunctional DNA glycosylases under physiological conditions, because AP endonucleases stimulate turnover of DNA glycosylases bound to an abasic site (Hill *et al*., 2001; Vidal *et al*., 2001; Yang *et al*., 2001; Marenstein *et al*., 2003). Binding to abasic sites is a common feature of most DNA glycosylases, probably to avoid exposure of the mutagenic lesion (Miao *et al*., 1998; Petronzelli *et al*., 2000b; Hill *et al*., 2001; Nilsen *et al*., 2001; Krusong *et al*., 2006). So, bifunctional DNA glycosylases that act as monofunctional ones will influence the subsequent sub-pathway choice (Figure 1).

A second factor determining the sub-pathway choice is the local concentration of BER components and protein–protein interactions: BER is a highly coordinated, stepwise process, in which every repair intermediate is transferred from one protein (complex) to the next (Wilson *et al*., 2000; Almeida and Sobol, 2007), avoiding in this manner the exposure of possibly cytotoxic lesions. So, relative changes in concentration of each repair factor can influence the sub-pathway selection. For instance, excess of PARP1 inhibits long-patch repair, while excess of AP endonuclease promotes this sub-pathway, probably by controlling the Pol β activity (Sukhanova *et al*., 2005).

Thirdly, the cell state may determine which sub-pathway is followed. It has been suggested that different pathways may be involved in dividing and non-dividing cells. For instance, several lines of evidence indicate that long-patch repair is more frequent in replicating than in non-replicating DNA (reviewed by Fortini and Dogliotti, 2007).

Regulation of the Different Base Excision Repair Steps

The intermediate steps in the BER pathway generate products that are often far more toxic to the cell than the targets of the repair pathway, the base lesions themselves, as is illustrated by the generation of knockout mice for particular BER components. In general, mutations in DNA glycosylase genes do not display overt developmental abnormalities (Schärer and Jiricny, 2001), possibly because of the often overlapping substrate specificities of these enzymes or because of the presence of back-up repair pathways. In contrast, null mice for *APE1*, the major mammalian AP endonuclease, show an early embryo-lethal phenotype (Xanthoudakis *et al*., 1996). Also, yeast mutants unable to repair AP sites are not viable (Guillet and Boiteux, 2002) and *E. coli* strains devoid of both *Xth* (exonuclease III) and *Nfo* (endonuclease IV) are hypersensitive to alkylating and oxidative agents (Demple *et al*., 1983; Cunningham *et al*., 1986). The embryo-lethal phenotype of *Pol* β and *XRCC1* null mice (Sobol *et al*., 1996; Tebbs *et al*., 2003) reveal that lethality is induced by the inability to remove an abasic site or gapped DNA rather than by the presence of a specific modified base. Thus, a tight coordination of the different steps in BER is necessary to avoid mutagenesis (Allinson *et al*., 2004). Indeed, overproduction of BER enzymes may result in a mutator phenotype and in tumorigenesis (Coquerelle *et al*., 1995; Canitrot *et al*., 1998; Glassner *et al*., 1998; Bergoglio *et al*., 2002), probably because of reduced BER efficiency. Furthermore, BER is not only regulated through the formation of protein complexes at the site of the lesion, but also through posttranslational modifications that change binding affinities, turnover rates, and subcellular localization (reviewed by Fan and Wilson, 2005; Almeida and Sobol, 2007). Moreover, proteins involved in the BER pathway not only form protein complexes with each other, but also with proteins involved in other DNA transaction pathways, such as DNA replication and recombination, coordinating BER and these other pathways (Fan and Wilson, 2005; Kovtun and McMurray, 2007).

Base Excision Repair in Plants

In plants, the BER pathway and DNA repair, in general, are not studied as well as in mammals. However, from the moment the sequences of the entire genome of the dicot *Arabidopsis thaliana* and of the monocot *Oryza sativa* (rice) had been released (Arabidopsis Genome Initiative, 2000; Sasaki *et al*., 2002; Kikuchi *et al*., 2003), a remarkable similarity between human and plant DNA repair proteins became clear. The most striking difference is the lack of Pol β in any plant genome analyzed until now, whereas homologs for most of all other major players have been identified, at least *in silico* (Britt, 2002). In plants, the role of Pol β has been proposed to be carried out by DNA Pol λ, because *in vitro* the Pol λ of rice shows dRPase activity and is upregulated after treatment with DNA-damaging agents (Uchiyama *et al*., 2004). As no homologs of DNA LigIII have been found in plants, the LigIII function is possibly taken over by DNA ligase I and/or IV. Alternatively, plant BER might rely completely on the long-patch branch of the pathway, because the existence of the short-patch BER is still not proven in plants. In addition, plant XRCC1 lacks the mammalian domains that are responsible for interaction with Pol β and LigIIIα, although the interaction domain with PARP1 is conserved (Doucet-Chabeaud *et al*., 2001; Uchiyama *et al*.,

2004). Indeed, no direct *in vivo* interaction between rice XRCC1 and Pol λ could be detected, but that between rice XRCC1 and PCNA was confirmed (Uchiyama *et al*., 2008). The hypothesis that PCNA mediates complex formation between XRCC1 and Pol λ, which would corroborate a function for this polymerase in plant BER, remains to be investigated.

The conservation of the PARP interaction domain in XRCC1 suggests that homologs play a role in BER. Both PARP1 and PARP2 homologs are present in plants and, as their mammalian counterparts, are activated by DNA damage (Babiychuk *et al*., 1998). Inhibition of PARP activity results in increased stress tolerance by blocking stress-induced cell death. This observation has been correlated with a reduced NAD^+ breakdown and, thus, with lower energy consumption in stress situations (De Block *et al*., 2005). It has been speculated that PARP detects and signals the level of DNA damage, most likely DNA breaks: under mild stress conditions, PARP would help repair the damage, whereas under more severe stress situations, PARP activity would result in NAD^+ depletion, followed by cell death (Scovassi and Diederich, 2004). Thus, the increased stress tolerance as a consequence of low PARP levels offers possibilities for future crop development with higher yields under stress conditions. Another application in mammalian cells is also currently under debate, namely the use of PARP inhibitors in cancer therapy, given that PARP inhibition sensitizes tumor cells to several chemotherapeutics (reviewed by Ratnam and Low, 2007).

DNA GLYCOSYLASES – SEEKING AND REMOVING LESIONS

DNA glycosylases initiate BER by recognition and excision of a modified base, resulting in an abasic site, and they have different, sometimes overlapping, substrate specificities. As the efficiency of the BER process strongly depends on the detection of the substrate, a long-standing focus has been to gain insight into how these enzymes detect modified bases when embedded in millions of normal base pairs (Verdine and Bruner, 1997; Zharkov and Grollman, 2005).

Recognition of Lesions by DNA Glycosylases

Structural and biophysical studies have revealed that the different DNA glycosylases recognize and remove DNA damage in a similar manner, described as the "pinch-push-plug-pull" mechanism (Stivers, 2004). Therefore, BER enzymes possess a "reading head" that is inserted into the DNA helix at the position of the lesion, resulting in kinking of the DNA, extrusion of the damaged nucleotide from the interior of the DNA helix, and cleavage of the extruded base in a base-specific binding pocket. First, the DNA damage is recognized by bending of the DNA double helix, constituting the pinch. Then, base extrusion follows through active pushing and plugging by the "reading head" that inserts into the DNA minor groove, and, finally, pulling by hydrogen-bounding groups that interact with the extrahelical base in the binding pocket, where the base is removed (Parikh *et al*., 2000; Hollis *et al*., 2001; Stivers and Jiang, 2003; Fromme *et al*., 2004). Substrate specificity is attained because shape, hydrogen bounding, and electrostatic potential of the extruded base has to match the active site of the DNA glycosylase (Kavli *et al*., 1996).

Although monofunctional and bifunctional DNA glycosylases recognize base lesions with the same mechanism, the removal is accomplished by other chemical interactions (Figure 2). On the one hand, monofunctional DNA glycosylases cleave the glycosidic bond between N and C1′ to generate an abasic site by using an activated water molecule as nucleophile to attack C1′ of the target nucleotide. Their bifunctional counterparts, on the other hand, use an active site amine moiety, thereby forming a Schiff base intermediate (O′Brien, 2006), which makes it possible to discriminate experimentally between monofunctional and bifunctional DNA glycosylases (Dodson *et al*., 1994) (Figure 2).

How DNA glycosylases search for lesions in the large pool of undamaged DNA is not completely understood, although the "pinch-push-plug-pull" mechanism for recognition and removal of lesions is well described (Parikh *et al*., 2000; Hollis *et al*., 2001; Stivers and Jiang, 2003; Fromme *et al*., 2004). Several hypotheses have been proposed. A first option is that every base is actively extruded from the DNA helix and presented into the active site. However, taking into account the energetic demands, this mechanism seems rather unlikely. A second hypothesis is that only lesions that have undergone spontaneous extrusion from the DNA helix are recognized. Until now, only uracil DNA glycosylase (UNG) has been shown to use this mechanism to select the damaged lesions by stabilizing the open conformation formed during DNA breathing (Cao *et al*., 2004; Parker *et al*., 2007). A third possible mechanism is that the lesion is recognized intrahelically by an intercalating probe that destabilizes the target base pair. In this manner, structure and energetics of base pairs are tested, while a lesion is searched for (Banerjee and Verdine, 2006; David *et al*., 2007) and recognized, because most lesions show reduced stability and helix distortion (Yang, 2006). Recently, the extremely fast movement of 8-oxoguanine DNA glycosylase 1 (OGG1) along a normal DNA duplex has been visualized (Blainey *et al*., 2006), suggesting that the imprecision of the searching process is compensated by speed, allowing repeated chances to find a damaged base (Banerjee and Verdine, 2006). Using this mechanism, DNA glycosylases can minimize the time-consuming extrusion of every non-lesion base pair in normal DNA.

Accumulating data reveal that the substrate specificity of most DNA glycosylases is broad, although it might be expected to be narrow from the often very subtle differences between normal and damaged DNA (Table 1). As most repair proteins can recognize multiple substrates, it can be questioned whether normal DNA is always excluded. Indeed, some BER proteins are able to act on normal undamaged DNA (Berdal *et al*., 1998; Connor and Wyatt, 2002; O′Brien and Ellenberger, 2004), termed gratuitous repair (Branum *et al*., 2001; Hanawalt, 2001; Sancar and Reardon, 2004; Reardon and Sancar, 2005). This repair is

TABLE 1
Substrate specificity of DNA glycosylases, classification in four structural superfamilies, and occurrence in different organisms

Acronym	DNA glycosylase	Substrate[d]	Organism	References
Helix-two turn-helix				
MDB	Methyl-CpG-binding domain 4[a]	G:T, G:U, 5FU:G, 5IU:G, Tg:G, O^6-meG:T, ...	Vertebrates	Petronzelli *et al*. (2000b), Turner *et al*. (2006)
Mig.Mth[a]		G:T	Bacteria, Archaea	Horst and Fritz (1996)
UDG	Uracil DNA glycosylase (6)[a,c]	ssU, U:T, U:G, U:A, U:G > 8-oxoG, ...	Bacteria, Archaea	Chung *et al*. (2003)
ROS1	Repressor of silencing 1[b]	5-meC	Plants	Zhu *et al*. (2007)
DME	DEMETER[b]	5-meC	Plants	Gehring *et al*. (2006)
TAG	3-meA DNA glycosylase I[a]	3-meA>3-meG, 7-meG	Bacteria, plants	Bjelland and Seeberg (1987), Bjelland *et al*. (1993)
AlkA/MAGI	3-meA DNA glycosylase II[a]	3-meA, 7-meG, 3-meG, 7-meA, εA, HX, 5-forU, ...	Bacteria, Archaea, yeast	Bjelland *et al*. (1994), Bjelland and Seeberg (1996)
MGPII	3-meA DNA glycosylase II[a]	7-meG, 3-meA	Bacteria, Archaea	Begley *et al*. (1999)
MAGIII	3-meA DNA glycosylase IV[a]	3-meA, εA	*Helicobacter pylori*	Eichmann *et al*. (2003)
Nth/NTH1	Endonuclease III[b]	5-OH-C, Tg, 5-OH-U, FapyG, ...	Bacteria, yeast, vertebrates	Hazra *et al*. (2007)
MutY/MUTYH	MutY/MUTYH[b]	8-oxoG:A, G:A>>C:A, 2-OH-A, ...	Bacteria, vertebrates	Au *et al*. (1989), Ohtsubo *et al*. (2000)
OGG1	8-oxoG DNA glycosylase 1[b]	8-oxoG:C, FapyG:C>>8-oxoG:T>8oxoG:G, ...	Vertebrates, plants, Archaea	Tchou *et al*. (1991)
Helix-two turn-helix				
Fgp/MutM	Formamidopyrimidine DNA glycosylase [b]	8-oxoG:C, FapyG, FapyA, 5-OH-C, 5-OH-U, Tg, 5-ForU, ...	Bacteria, plants	Karahalil *et al*. (1998)
Nei/NEIL	Endonuclease VIII[b]	8-oxoG, Tg, Ug, 5-OH-C, 5-OH-U, FapyG, FapyA, 5-guanidinohydantoin, spiroiminodihydantoin, ...	Bacteria, vertebrates	Hazra *et al*. (2002a), Hailer *et al*. (2005)
UDG				
UNG	Uracil DNA glycosylase (1)[a,c]	ssU>U:G, U:A>5-FU, oxidized pyrimidines	Bacteria, yeast, vertebrates	Dizdaroglou *et al*. (1996), Krokan *et al*. (2001)
MUG/TDG	Mismatch specific UDG/thymine DNA glycosylase (2)[a,c]	U:G> εC:G>(T:G), O^6-meG:T, 5FU:G, 5-OH-meU, 5-forU:G, ...	Bacteria, yeast, vertebrates, insects	Hardeland *et al*. (2003), Cortázar *et al*. (2007)
SMUG1	Single-strand-selective monofunctional UDG 1 (3)[a,c]	ssU>U:G, U:A, oxidized pyrimidines, εC:G, 5FU, 5ForU, ...	Vertebrates, insects, prokaryotes	Nilsen *et al*. (2001)
UDGa	Uracil DNA glycosylase a (4)[a,c]	ssU,U:G>U:A	Archaea	Sartori *et al*. (2002)
UDGb	Uracil DNA glycosylase b (5)[a,c]	U:G, εC:G, 5-OH-me-U>U:A, ...	Bacteria, Archaea	Sartori *et al*. (2002)

TABLE 1
Substrate specificity of DNA glycosylases, classification in four structural superfamilies, and occurrence in different organisms *(continued)*

Acronym	DNA glycosylase	Substrate[d]	Organism	References
AAG				
AAG/ANPG/MPG	Alkyladenine DNA glycosylase/alkylpurine DNA glycosylase/N-methylpurine DNA glycosylase[a]	3-meA, 7-meA, 7-meG, ... > εA, HX, ...	Vertebrates, bacteria, plants	Samson *et al*. (1991), Dosanjh *et al*. (1994), Saparbaev and Laval (1994)

[a]Monofunctional DNA glycosylases.
[b]Bifunctional DNA glycosylases.
[c]The numbers in parentheses refer to the numbering of the different UDG families.
[d]Substrate abbreviations: 5FU, 5-fluorouracil; 5IU, 5-iodouracil; Tg, thymineglycol; O^6-meG, O^6-methylguanine; ssU, single-stranded uracil; 8-oxoG, 7,8-dihydro-8-oxoguanine; 5-meC, 5-methylcytosine; 3-meA, 3-methyladenine; 3-meG, 3-methylguanine; 7-meG, 7-methylguanine; 7-meA, 7-methyladenine; εA, ethenoadenine; HX, hypoxanthine; 2-OH-A, 2-hydroxyadenine; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; Ug, uracilglycol, 5-OH-C, 5-hydroxycytosine; 5-OH-C, 5-hydroxyuracil; FapyA, 4,6-diamino-5-formamidopyrimidine; εC, ethenocytosine; 5-OH-meU, 5-hydroxymethyluracil; 5-ForU, 5-formyluracil.

not necessarily mutagenic, because it is expected to restore the original sequence. Thus, although gratuitous repair may seem energetically wasteful, it is a consequence of the ability of a single enzyme to repair a whole set of lesions. In this manner, the necessary number of repair enzymes is reduced, compensating for the energetic waste of unnecessary repair (O′Brien, 2006). In addition, recognition of damaged bases must be sufficiently fast to repair all damage, unavoidably resulting in a certain level of gratuitous repair.

Structural Families

In general, DNA glycosylases are small proteins that usually contain fewer than 400 amino acids and maximum two structural domains. No cofactors are required for activity. Although DNA glycosylases utilize the same mechanism to recognize and remove damaged bases, three-dimensional structure determinations have revealed that they belong to four different structural superfamilies: helix-hairpin-helix, helix-two turn-helix, UDG, and alkyladenine DNA glycosylase (AAG) (recently reviewed by Hitomi *et al*., 2007). Within one superfamily, the three-dimensional fold is conserved and often the active site location and the identity of the key catalytic residues as well. However, the primary amino acid sequences have diverged beyond detectable sequence similarity (O′Brien, 2006). Table 1 gives an overview of the different DNA glycosylases identified to date, with the superfamily they belong to.

Helix-Hairpin-Helix Superfamily

The helix-hairpin-helix motif is a sequence-independent DNA-binding motif that is also found in a number of DNA-binding proteins in addition to both monofunctional and bifunctional DNA glycosylases (Thayer *et al*., 1995; Doherty *et al*., 1996). This group of DNA glycosylases is the most diverse, with divergent substrate specificities. The core fold consists of four N-terminal and six to seven C-terminal α-helices, linked by a type-II β-hairpin. The hairpin loop, important for the sequence-independent DNA binding, has a strong sequence conservation (L/F–P/K/H–G–V/I–G–K/R/T) (Doherty *et al*., 1996). A conserved aspartic acid is responsible for cleavage of the lesion (except in 3-methyladenine DNA glycosylase I [TAG]), by activating the nucleophile for attack of the glycosylic bond (Huffman *et al*., 2005). In various DNA glycosylases of this superfamily, additional functional domains serve specialized biological roles: an iron–sulfur cluster, involved in recognition of DNA lesions through redox chemistry (Kuo *et al*., 1992; Guan *et al*., 1998; Mol *et al*., 2002; Fromme and Verdine, 2003; Lukianova and David, 2005), a β-sheet (Hollis *et al*., 2000), a MutT-like domain (Bruner *et al*., 2000; Kwon *et al*., 2003), a zinc-binding domain (Kwon *et al*., 2003), and a methyl-CpG-binding domain (Hendrich *et al*., 1999).

Helix-Two Turn-Helix Superfamily

The helix-two turn-helix motif has a function comparable to that of the helix-hairpin-helix motif and is also found in DNA-binding proteins other than DNA glycosylases (Hosfield *et al*., 1998). This superfamily is defined by two DNA glycosylases, formamidopyrimidine-DNA glycosylase (Fpg) and Nei, both involved in the repair of oxidative damage. The helix-two turn-helix proteins consist of N- and C-terminal domains that create a DNA-binding cleft. The difference from the helix-hairpin-helix is the presence of β-sheets in both C- and N-terminal domains; the N-terminal domain contains a two-sheet

TABLE 2
Overview of the phenotypes in DNA glycosylase-deficient backgrounds

Knockout gene(s)	Organism	Phenotype
ung	*E. coli*	Strongly increased frequency of C-to-T transitions
UNG	Mouse	Slightly increased mutation frequency
		100-fold increased steady state level of uracil in the genome
		No overt developmental defects
		Increased incidence of B-cell lymphomas in aging mice
		No hypersensitivity to γ-irradiation
	Human	Hyper IgM syndrome
SMUG	Mouse	Slightly increased mutation frequency
		No overt developmental defects
		No hypersensitivity to γ-irradiation
$UNG^{-/-}$ $SMUG^{-/-}$	Mouse	Moderately increased mutation frequency (additive effect)
		Hypersensitive to γ-irradiation
MBD4	Mouse	2- to 3-fold increased mutation frequency
		No overt developmental defects
		Hyperresistant to cisplatin, 5-FU, . . .
		Increased tumorigenesis in $APC^{-/-}$ tumor-susceptible background
	Human	Increased cancer incidence?
TDG	Mouse	Embryo lethal
alkA tag	*E. coli*	Hypersensitive to alkylating agents
		Overexpression results in hypersensitivity to MMS
AAG	Mouse	No overt developmental defects
		Hypersensitive to alkylating agents
		Overexpression results in hypersensitivity to MMS
mutT	*E. coli*	Strongly increased mutation frequency
mutM	*E. coli*	Slightly increased mutation frequency
mutY	*E. coli*	Slightly increased mutation frequency
mutM mutY	*E. coli*	Strongly increased mutation frequency
nth	*E. coli*	Small mutator phenotype
nei	*E. coli*	No mutator phenotype
nth nei	*E. coli*	Slightly enhanced mutator effect
		Hypersensitive to ionizing radiation and H_2O_2
OGG1	Mouse	No overt developmental defects
		Accumulation of 8-oxoG in specific tissues
		Modest increase in mutation frequency
		Higher incidence of adenoma and carcinoma only in aging mice
	Human	Increased cancer incidence?
MUTYH	Mouse	No overt developmental defects
		Modest increase in mutation frequency
		Higher incidence of tumor formation only in aging mice
	Human	Colorectal tumor formation
$OGG1^{-/-}$ $MUTYH^{-/-}$	Mouse	High accumulation of 8-oxoG
		Strong increase in tumor predisposition
MTH1	Mouse	No overt developmental defects
		Increased tumorigenesis
		No increase in mutation frequency
		Shift in mutation spectrum
	Human	Increased cancer incidence?
NTH1	Mouse	No aberrant phenotype
		Slower repair kinetics of thymineglycol
		No increased sensitivity to γ-irradiation and ROS
NEIL1	Mouse	Increased sensitivity to γ-irradiation
		Linked to metabolic syndrome?

antiparallel β-sandwich flanked by helices, while the C-terminal domain consists of the helix-two turn-helix motif and zinc-finger motif (Gilboa *et al*., 2002; Serre *et al*., 2002; Fromme and Verdine, 2002; 2003; Zharkov *et al*., 2003; Coste *et al*., 2004).

UDG Superfamily

The structural UDG superfamily contains at least five different families (Table 1) based on conserved active site residues and specificity (Aravind and Koonin, 2000; Pearl, 2000). In addition, a sixth family of uracil-recognizing DNA glycosylases has been found with more structural homology to the helix-hairpin-helix superfamily (Chung *et al*., 2003). The different families show limited sequence similarity, but they possess a common core fold, consisting of a central four-stranded parallel twisted β-sheet encompassed by at least two α-helices from each side (Aravind and Koonin, 2000; Huffman *et al*., 2005). Divergent N- and C-terminal domains can often be found within one family that can account for differences in substrate specificity, substrate interaction, and kinetics of base release (Gallinari and Jiricny, 1996; Hardeland *et al*., 2003; O′Neill *et al*., 2003; Steinacher and Schär, 2005). In the UDG superfamily, monofunctional DNA glycosylases occur that are specific for the recognition of mismatched uracil and thymine and several types of derived pyrimidines that have been deaminated, oxidized, or even alkylated.

AAG Superfamily

The monofunctional AAGs are not structurally related to one of the other three superfamilies, but recognize and release their substrate in a comparable manner (Lau *et al*., 1998; Berti and McCann, 2006). Members are compact single-domain proteins consisting of an antiparallel β-sheet surrounded by α-helices similar to the methionyl-tRNA$_f^{Met}$ formyltransferase C-terminal domain.

DNA GLYCOSYLASES—MUTATION AVOIDANCE

A wide range of endogenous and exogenous agents can cause DNA damage that affects individual bases. BER constitutes the primary defense against these lesions. However, survival of species not only relies on preservation of genome integrity, but also simultaneously on generation of genetic diversity. DNA repair pathways, such as BER, limit mutations, but do not completely avoid them, illustrating the role of DNA repair in both processes. Here, we discuss the role for BER and, primarily, the role of DNA glycosylases. DNA glycosylases can be classified based on three-dimensional structures, cleavage mechanism (mono- or bifunctional), and substrate preference (see above). The major forms of individual base lesions and the enzymes that recognize them to initiate BER will be considered. The observed phenotypes in DNA glycosylase-deficient backgrounds is presented in Table 2.

Deamination Damage—Uracil and Thymine

Uracil can appear in DNA by misincorporation of low levels of dUMP during replication and by hydrolytic deamination of cytosine in DNA (Figure 3), either spontaneously or enzymatically (Sousa *et al*., 2007). The incorporation of uracil during replication is probably the most important source for its presence in DNA (Andersen *et al*., 2005b). The resulting U:A base pairs are not mutagenic by themselves (Figure 3B), but uracil removal due to DNA glycosylase activity generates abasic sites that are strongly mutagenic and cytotoxic after replication because of misincorporations and DSB formation (El-Hajj *et al*., 1992; Guillet and Boiteux, 2003; Auerbach *et al*., 2005). Misincorporation of dUMP in DNA can be enhanced above normal levels by the presence of cytostatic drugs, such as 5-fluorouracil and 5-fluorodeoxyuridine that interfere with pyrimidine metabolism (Ingraham *et al*., 1982).

The emergence of uracil in DNA because of deamination is, in contrast to misincorporation of dUMP, *de facto* mutagenic (Figure 3B). From biological and chemical measurements, spontaneous hydrolytic deamination of cytosine has been estimated to take place daily at a rate of 60–500 events per human genome (Bockrath and Mosbaugh, 1986; Frederico *et al*., 1990; Krokan *et al*., 2002; Barnes and Lindahl, 2004). This relatively large variation in deamination rates is attributed to the at least 100-fold higher occurrence in single-stranded than in double-stranded DNA, whose ratio because of replication, transcription, and "breathing" in DNA (Bjursell *et al*., 1979) might vary in different tissues and is currently unknown (Lindahl, 1993; Kavli *et al*., 2007). Certain chemicals, such as bisulfite (Sono *et al*., 1973) and N_2O_3 (Caulfield *et al*., 1998; Dedon and Tannenbaum, 2004), may induce deamination of cytosine, but their *in vivo* contribution remains probably rather limited (Dong and Dedon, 2006). Ultraviolet (UV) irradiation is also a common source of uracil in DNA, because cytosine deamination is greatly accelerated within cyclopyrimidine dimers (Tessman *et al*., 1994), one of the major products formed after UV irradiation. Uracil in DNA can also be generated by γ-irradiation, because of cytosine deamination (An *et al*., 2005), but this is probably not the most mutagenic and cytotoxic lesion formed (Kavli *et al*., 2007).

In addition, cytosines can be deaminated enzymatically by members of the apolipoprotein B-editing enzyme 1/activation-induced cytidine deaminase (APOBEC1/AID) family (Harris *et al*., 2002; Franca *et al*., 2006). For instance, AID deaminates cytosine to uracil during antibody diversification, a process in which also UNG is involved (Di Noia and Neuberger, 2002). Another class of enzymes that can convert cytosine to uracil is the group of cytosine-5-methyltransferases that transfer a methyl group from the methyl donor *S*-adenosyl-L-methionine (SAM) to cytosine. During the methylation process, intermediates, such as dihydrocytosine, are formed that are prone to rapid deamination (Shen *et al*., 1992).

G:T mismatches can arise as a consequence of replication errors and as the result of deamination of 5-methylcytosine (Figure 3A), whose deamination rate is 3- to 5-fold higher than

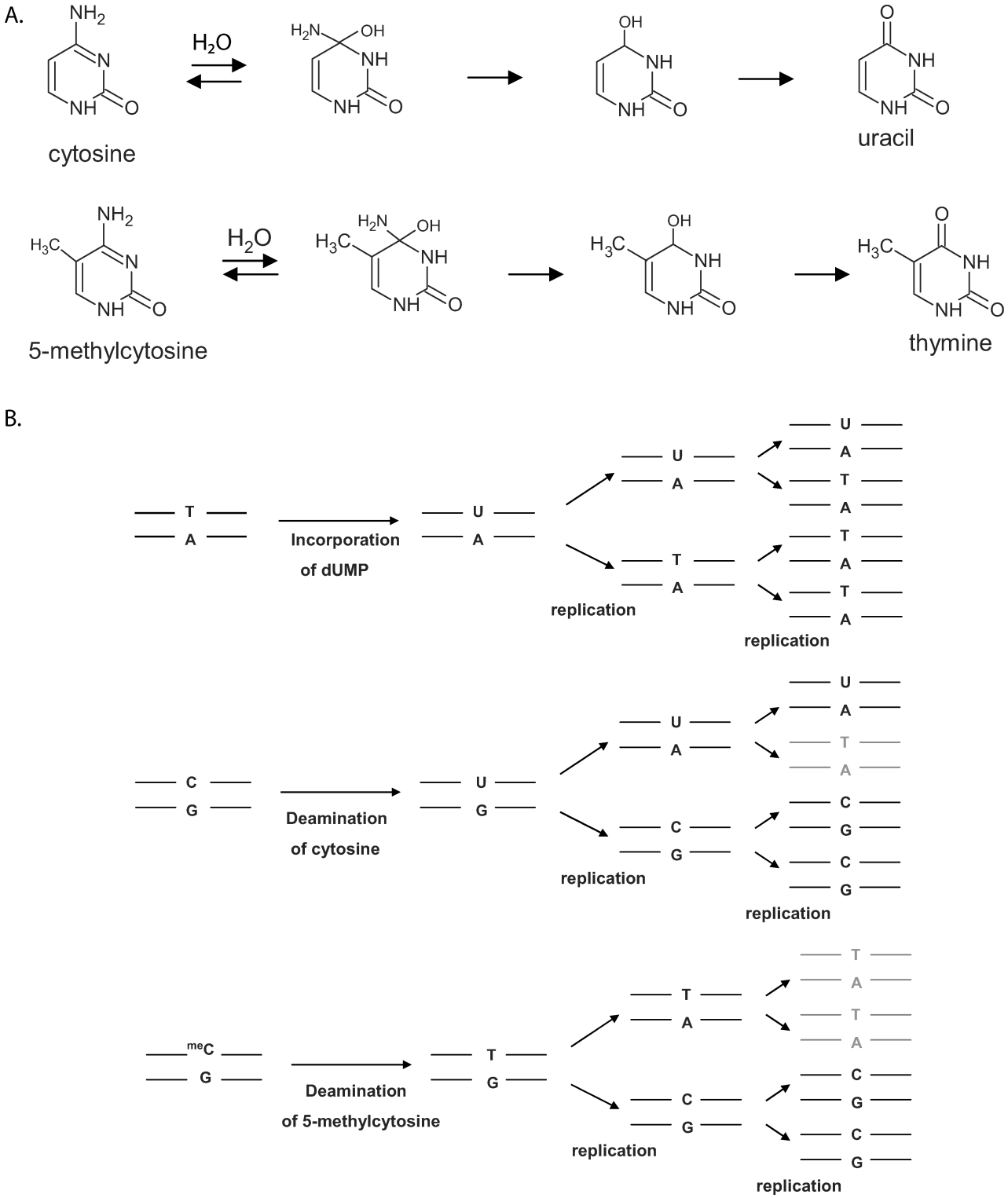

FIG. 3. Formation of uracil and mismatched thymine in DNA and consequences for mutagenicity. A. Reaction pathways for hydrolytic deamination of cytosine and 5-methylcytosine, resulting in the formation of uracil and thymine, respectively. B. Outcome of misincorporation of dUMP (upper), deamination of cytosine (middle), and deamination of 5-methylcytosine (lower). Misincorporation of dUMP does not result in mutations. In subsequent rounds of replication, the U:A base pair is further diluted. In contrast, deamination of cytosine and 5-methylcytosine fixes of mutations when not repaired correctly. Mutations are shown in gray. Adapted from Sousa *et al*. (2007).

that of unmethylated cytosines (Lindahl and Nyberg, 1974; Ehrlich *et al*., 1986; Shen *et al*., 1994). Methylation of cytosine is the most important postreplicative modification of DNA in eukaryotes and occurs at the 5′ position of the pyrimidine ring (Chan *et al*., 2005; Vanyushin, 2006). CpG dinucleotides, which are often methylated in vertebrate genomes, are hot spots for mutations. Of all germline mutations responsible for genetic diseases, 23% occur at CpG positions and 90% are C-to-T and G-to-A transitions (Krawczak *et al*., 1998). For instance, 25% of the cancer-associated mutations in the *p*53 tumor suppressor gene are C-to-T transitions located at CpG sites (Greenblatt *et al*., 1994). As a consequence of these elevated mutation rates, CpGs are present only at approximately 20% of the expected random frequency in mammalian genomes (Sved and Bird, 1990). Moreover, CpGs are distributed non-randomly: the genome is mostly CpG poor, but so-called CpG islands exist that are CpG rich and almost always free of methylation (Cross and Bird, 1995).

Some chemicals can promote deamination of 5-methylcytosine. For example, the rate of C-to-T transitions in *Salmonella typhimurium* (Wink *et al*., 1991) is increased by nitric oxide, but whether as a consequence of stimulated deamination is unclear (Schmutte *et al*., 1994; Felley-Bosco *et al*., 1995). Another example is glyoxal, which directly deaminates both cytosine and 5-methylcytosine (Murata-Kamiya *et al*., 1997; Kasai *et al*., 1998). Deamination of cytosine and 5-methylcytosine can be enhanced by compounds that intercalate into the DNA double helix, creating regions of single-stranded DNA with high deamination rates (Pfeifer, 2006), and can be enhanced by exposure to UV light, as a consequence of high pyrimidine dimer formation of methylated cytosines (Tommasi *et al*., 1997). In addition, some enzymes, such as AID and APOBEC homologs, might be able to deaminate 5-methylcytosine, although current data are contradictory (Morgan *et al*., 2004; Larijani *et al*., 2005).

Repair of Uracil in DNA—UNG and SMUG1

More than 30 years ago, Tomas Lindahl (1974) discovered UNG, the first DNA glycosylase in *E. coli*. Now, enzymes that excise uracil from DNA are known to be ubiquitous in bacteria, Archaea, and eukaryotes. The major uracil DNA glycosylases are UNG1, UNG2 (Lindahl, 1974; Nilsen *et al*., 1997), and the single-strand-selective monofunctional UDG1 (SMUG1) (Haushalter *et al*., 1999). However, also thymine DNA glycosylase (TDG) (Neddermann and Jiricny, 1993) and methyl-CpG-binding domain (MBD) 4 protein (Hendrich *et al*., 1999) recognize uracil. They differ by their action in specific sequence contexts, in single- versus double-stranded DNA, and in various cell cycle phases.

Repair of U:A and U:G

UNG enzymes all belong to the UDG (1) superfamily (Table 1) and have been identified in bacteria, yeast, vertebrates, and plants, but not in Archaea (Eisen and Hanawalt, 1999). Their substrate spectrum is broad: they preferably recognize lesions in single-stranded DNA, but uracil in double-stranded DNA as well (Krokan *et al*., 2001). Lesions that result from oxidative damage to uracil, such as alloxan, isodialuric acid, and 5-hydroxyuracil, are also repaired, although not as efficiently as uracil (Dizdaroglu *et al*., 1996).

The human *UNG* gene encodes both mitochondrial UNG1 and nuclear UNG2. The proteins differ in their N-terminal sequences because of alternative promoters and splicing (Nilsen *et al*., 1997). The mitochondrial UNG1 is ubiquitously produced in human tissues, with the highest levels in mitochondria-rich tissues, whereas the production of nuclear UNG2 is the highest in proliferating tissues. UNG2 is also cell cycle regulated with the highest levels of mRNA observed during late G_1-to-S transition (Haug *et al*., 1998; Muller-Weeks *et al*., 2005), suggesting that its major role is to counteract U:A base pair formation due to misincorporation of dUMP during replication (Nilsen *et al*., 2000; Barnes and Lindahl, 2004). This hypothesis is supported by the co-localization of a fraction of nuclear UNG2 with replication foci and its interaction with PCNA and RPA, targeting UNG2 to DNA replication sites (Otterlei *et al*., 1999). However, in *E. coli* and in *Saccharomyces cerevisiae*, UNG is also responsible for repair of U:G base pairs formed after deamination of cytosine, as shown by the greatly increased frequency of spontaneous C:G-to-T:A transitions in mutants (Duncan and Weiss, 1982; Impellizzeri *et al*., 1991). Surprisingly, UNG-deficient mice showed only a moderate mutator phenotype (Nilsen *et al*., 2000; An *et al*., 2005), despite a 100-fold increased steady-state level of uracil in the genome of these mutant mice (Nilsen *et al*., 2000; Andersen *et al*., 2005b), consistent with the postulated role of UNG in U:A repair. This modest mutator phenotype can be attributed to the complementary UDG activity in UNG-deficient mice that is encoded by *SMUG* (Haushalter *et al*., 1999; Nilsen *et al*. 2000, 2001). The designation of SMUG1 is misleading because it prefers double-stranded DNA in the presence of APE1 (Nilsen *et al*., 2001). Only recently, SMUG1 has been shown to occur not only in vertebrates and insects, but also in Proteobacteria and Planctomycetes and in marine non-vertebrates (e.g., sea urchin [*Strongylocentrotus purpuratus*] and sea squirt [*Ciona intestinalis*]; see Pettersen *et al*., 2007). Seemingly, non-vertebrate organisms possess an enzyme of either the SMUG1 or the UNG family, while vertebrates have both (Pettersen *et al*., 2007), implying additional roles for both proteins in these organisms. In murine *SMUG* knock-down cells, the mutator phenotype is rather weak and the mutation frequency is only slightly more than an additive effect in cells deficient in both SMUG1 and UNG (An *et al*., 2005), hinting at non-redundant roles in preventing mutagenesis at C:G base pairs. However, the mutational spectra and substrate specificities are consistent with both UNG and SMUG1 acting on deaminated cytosine rather than on different substrates (An *et al*., 2005; Kavli *et al*., 2007). Therefore, it is speculated that the non-redundancy results from differences in localization and expression patterns of both enzymes

(Pettersen *et al*., 2007). In contrast to UNG2, SMUG1 is constitutively produced, although at low levels, in cell nuclei of non-proliferating and proliferating tissues (Nilsen *et al*., 2001). Thus, both UNG2 and SMUG1 are expected to repair U:G base pairs generated by cytosine deamination, but their activity spectrum depends on the state of the genome, i.e., whether cells divide or not or whether damage occurs in transcriptionally active or inactive sequences (An *et al*., 2005; Pettersen *et al*., 2007). A model proposed by Kavli *et al*. (2007) is presented in Figure 4.

There is also some evidence that SMUG1 and UNG2 play a role in the repair of oxidative lesions, namely isodialuric acid, alloxan, and 5-hydroxyuracil (Figure 4) (Dizdaroglu *et al*., 1996; An *et al*., 2005). In addition, SMUG1 recognizes 5-fluorouracil, 5-hydroxymethyluracil, and 5-formyluracil (Boorstein *et al*., 2001; Masaoka *et al*., 2003; An *et al*., 2007), lesions that, if base paired with guanine, are also recognized by MBD4 and TDG (Liu *et al*., 2003; Turner *et al*., 2006). In the repair of oxidative damage caused by γ-irradiation, SMUG1 and UNG2 act redundantly, because double knockout cells are hypersensitive to γ-irradiation and the single knockouts are not (An *et al*., 2005).

For some time, BER proteins are known to be often post-translationally modified to coordinate the process (reviewed by Fan and Wilson, 2005; Almeida and Sobol, 2007). UNG2 is phosphorylated in the N-terminal domain (Muller-Weeks *et al*., 1998) and this equilibrium between phosphorylated

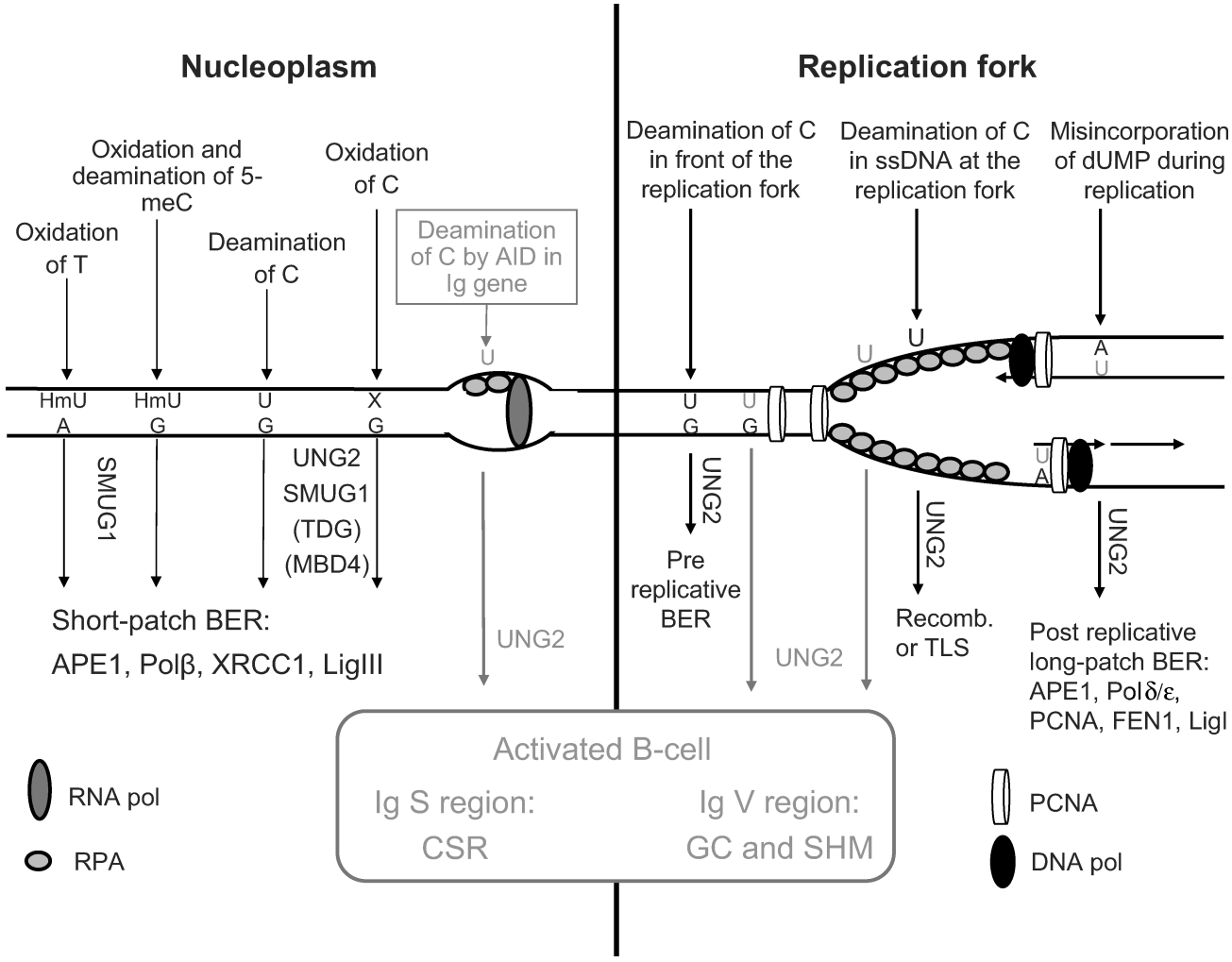

FIG. 4. Model for potential processing pathways of uracil and uracil analogs, originating from various sources, in different genomic contexts. Uracil present at the site of the replication fork, due to either cytosine deamination or dUMP incorporation, is primarily repaired by UNG2. In addition, UNG2, but also SMUG1, MBD4, and TDG, are involved in repair of deamination and oxidation damage in non-replicating DNA. Finally, UNG2 also plays a role in somatic hypermutation, gene conversion, and in class-switch recombination in activated B-cells (indicated in gray). Abbreviations: HmU, 5-hydroxymethyluracil; X, oxidized cytosine (alloxan, isodialuric acid, and 5-hydroxyuracil). Adapted from Kavli *et al*. (2007).

and non-phosphorylated is cell cycle regulated and can be influenced by exogenous stress (Lu *et al*., 2004; Kavli *et al*., 2007; Hagen *et al*., 2008).

UNG and Immunoglobulin Gene Diversification

Besides its role in repair of U:A and U:G base pairs, UNG is also involved in immunoglobulin (Ig) gene diversification in mammals during the acquired immune response (Figure 4) (recently reviewed by Di Noia and Neuberger, 2007). Three processes are responsible for this diversification: gene conversion (GC), somatic hypermutation (SHM), and class switch recombination (CSR). A first step in the diversification of Ig is deamination of cytosines in particular sequence contexts to form uracil by the AID enzyme (Muramatsu *et al*., 1999); uracil is then removed by UNG and abasic sites are created. Replication beyond these abasic sites results in mutations, either because of GC as the stalled replication fork triggers homologous recombination or SHM as a result of translesion synthesis, which is the bypassing of an abasic site by specialized polymerases. Also other repair factors, such as MutS homolog (MSH) 2 and MSH6, have a comparable function in inducing GC and SHM (Rada *et al*., 2004). UNG too has a role in CSR, because UNG deficiency profoundly impairs the isotype switch from the primary immunoglobulin IgM to the other isotypes (Imai *et al*., 2003). In an UNG-deficient background, both SHM and CSR are not fully abolished (Begum *et al*., 2004), suggesting that also other UDGs play a role in these processes. Production of SMUG1 decreases during B-cell activation (Di Noia *et al*., 2006), while SHM and CSR remain normal in MBD4-deficient mice (Bardwell *et al*., 2003). However, TDG would be a good candidate, because its expression is upregulated upon B-cell activation *in vitro*, like that of UNG (Imai *et al*., 2003; Cortázar *et al*., 2007). The function of UNG in Ig diversification is illustrated also by the generation of B-cell lymphomas late in life of UNG-deficient mice, indicating that U:G lesions are mutagenic if not removed (Nilsen *et al*., 2003; 2005; Andersen *et al*., 2005a). Humans lacking UNG2 are prone to recurrent infections and lymphoid hyperplasia and, in addition, have elevated IgM and reduced IgG, IgA, and IgE levels due to defective SHM and CSR (Kavli *et al*., 2007). Nevertheless, knockout mice are viable, develop normally, are fertile, and young animals have no overt phenotypes. All these data imply that UNG not only plays a role in normal BER, but also in the controlled generation of mutations in Ig genes.

UDG in Plants

In plants, the BER process has been shown first in carrot (*Daucus carota*) cells, in which both UDG and AP endonuclease activity were identified (Talpaert-Borlé and Liuzzi, 1982). Meanwhile, UDG activity has been found in *Allium cepa* (onion) (Maldonado *et al*., 1985), in wheat (*Triticum aestivum*) germ (Blaisdell and Warner, 1983), and in pea (*Pisum sativum*) chloroplasts (Wang *et al*., 1999). In *Arabidopsis* and in rice, an UNG homolog has been found *in silico* (Kimura and Sakaguchi, 2006), but, to our knowledge, no SMUG1 homologs have yet been identified in plants, suggesting that primarily misincorporated uracil is removed from the genome and, only to a lesser extent, uracil originating from cytosine deamination. Until now, the impact of deamination damage on plant genome stability is unclear. It would be interesting to know if and by which DNA glycosylases deamination damage is repaired in plants.

Conclusions

UNG2 in mammals can be assumed to be the major enzyme for the repair of U:A base pairs formed by misincorporation of dUMP during replication. Together with SMUG1, it also counteracts U:G base pairs formed after hydrolytic deamination of cytosines. UNG2 is localized in replication foci and acts either pre-replicative (U:G) or post-replicative (U:A), while SMUG1 repairs deaminated cytosines in non-replicating chromatin. SMUG1 and UNG complement each other in the repair of oxidative damage formed after γ-irradiation. Finally, UNG is involved in Ig gene diversification and plays a role in SHM, GC, and CSR.

Deamination of 5-Methylcytosine—MBD4 and TDG

Two additional DNA glycosylases able to excise uracil are MBD4 and TDG. In contrast to UNG and SMUG1, they also remove the normal thymine base when paired with guanine. As TDG and MBD4 recognize both G:T and G:U base pairs, they are supposed to play a role in the defense against genetic mutation through spontaneous deamination of 5-methylcytosine and cytosine. The potential to remove a perfectly normal base, even mispaired, is rather exceptional among DNA glycosylases. TDG and MBD4 belong to a different superfamily and have co-evolved with other UDG proteins in the same organisms (Table 1; Figure 5), suggesting highly coordinated non-redundant biological functions (Hardeland *et al*., 2007).

Substrate Spectrum

TDG-related genes have been detected in bacteria, yeast, insect, and vertebrate genomes (Hardeland *et al*., 2003; Cortázar *et al*., 2007) and the family has been named after the ortholog mismatch-specific UDG (MUG) of *E. coli* (Gallinari and Jiricny, 1996). All MUG proteins have very broad substrate specificity and a strong opposite G preference (Barrett *et al*., 1998; Waters and Swann, 1998; Hardeland *et al*., 2003; O'Neill *et al*., 2003; Cortázar *et al*., 2007). For the human TDG, it was shown that specific contacts are made with the opposite base, explaining this preference (Schärer *et al*., 1997). Substrate spectra are not only broad, but can vary considerably between orthologs of different origins, although G:U and ethenocytosines (εCs) are commonly the most efficiently processed substrates (Waters and Swann, 1998; Hardeland *et al*.,

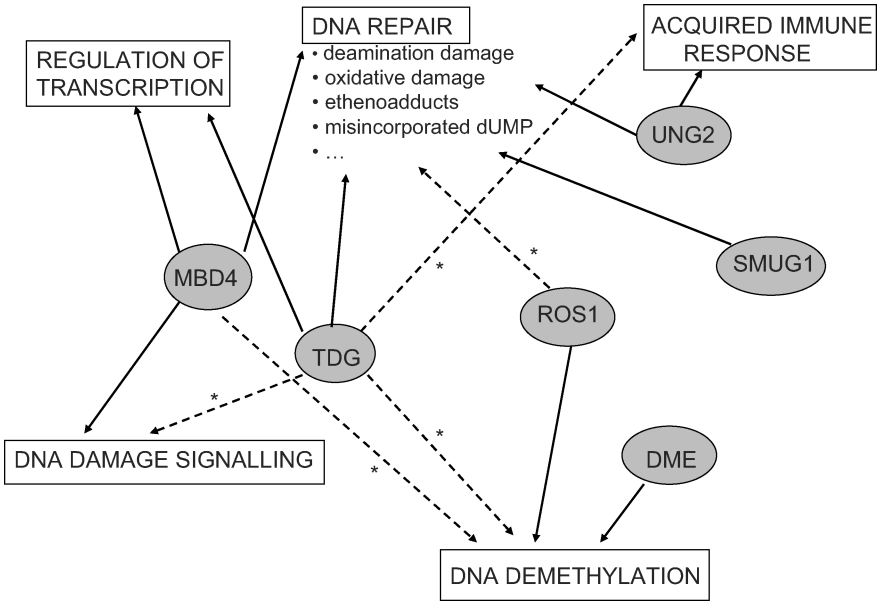

FIG. 5. Representation of the biological processes in which the DNA glycosylases UNG2, SMUG1, MBD4, TDG, ROS1, and DME are involved. Functions still under debate are indicated by a asterisk and a dotted line.

2003). A striking difference with vertebrate and insect counterparts is that bacterial and yeast MUG enzymes are not able to excise T opposite G. Obviously, organisms that do not methylate their genome, such as *S. pombe* (Antequera *et al*., 1984), do not need a repair system for G:T mismatches originating from deaminated 5-methylcytosine (Hardeland *et al*., 2003). The substrates that are recognized by MUG can be divided in three groups: damaged cytosine bases (e.g., uracil, 5-bromouracil, 5-fluorouracil, 5-hydroxyuracil, and εC); damage derived from 5-methylcytosine (e.g., thymine and 5-hydroxymethyluracil); and purine derivatives (e.g., hypoxanthine and 3,N^6-ethenoadenine (εA)) (Gallinari and Jiricny, 1996; Hang *et al*., 1998; Saparbaev and Laval, 1998; Waters and Swann, 1998; Hardeland *et al*., 2003; O'Neill *et al*., 2003; Hang and Guliaev, 2007; Morgan *et al*., 2007). Based on their *in vitro* substrate preferences, it can be postulated that the primary function of MUG orthologs is to protect the genome from mutations arising from base deamination and/or oxidation. However, MUG orthologs can also process other base modifications, such as lipid peroxidation products (ethenoadducts), illustrating that MUG proteins have a broader function in the maintenance of genome stability than only the repair of deamination damage.

In contrast to the other uracil-recognizing DNA glycosylases, MBD4 belongs to the helix-hairpin-helix superfamily and, thus, has evolved from another ancestor. A MBD4 homolog has been identified in mammals, *Gallus gallus* (chicken), sea squirt, *Xenopus laevis* (frog), and *Danio rerio* (zebrafish) (Hendrich and Tweedie, 2003). It is worth noting that the designation MBD4 is based on the presence of the MBD and not on the glycosylase domain, so it might be confusing for homologs of species that have retained the glycosylase domain only. Indeed, the mammalian MBD4 homolog consists of two domains, an N-terminal MBD and a C-terminal DNA glycosylase domain (Hendrich *et al*., 1999) and the N-terminal domain of human MBD4 is not retained in non-mammalian MBD4 homologs, as that of chicken, sea squirt, or frog. The substrate specificity of MBD4 is rather broad: besides G:T and G:U, also G:5-fluorouracil and G:5-iodouracil (Petronzelli *et al*., 2000b; Turner *et al*., 2006), G:thymineglycol (resulting from oxidative deamination of 5-methylcytosine) (Yoon *et al*., 2003), and O^6-methylG:T (Cortellino *et al*., 2003; Turner *et al*., 2006) are recognized as well as a weak catalytic activity against 3,$N^4-\varepsilon$C:G (Petronzelli *et al*., 2000a). Thus, MBD4 distinguishes a wide range of DNA damages, including deamination, oxidation, and alkylation, although binding occurs with varying affinity (Zhu *et al*., 2000a; Turner *et al*., 2006; Balada *et al*., 2007) and its substrate spectrum largely overlaps with that of TDG. The affinity of MBD4 and TDG for a certain substrate depends on the sequence context; the cleavage rate is higher for base lesions in a CpG context than in a non-CpG context (Griffin *et al*., 1994; Sibghat-Ullah and Day, 1995; Sibghat-Ullah *et al*., 1996; Waters and Swann, 1998; Hendrich *et al*., 1999; Abu and Waters, 2003; Wu *et al*., 2003; Turner *et al*., 2006; Morgan *et al*., 2007). Moreover, in mammals, the methylation status can also influence the affinity of MBD4 for a certain substrate: a hemimethylated sequence context is preferred over a non-methylated or fully methylated one (Hendrich *et al*., 1999; Wu *et al*., 2003; Turner *et al*., 2006). Accordingly, MBD4 is targeted to regions of highly methylated DNA *in vivo* (Neddermann *et al*., 1996; Hendrich and Bird, 1998). In contrast, TDG recognizes base

mispairs in both unmethylated and methylated sequence contexts (Sibghat-Ullah and Day, 1995; Neddermann *et al*., 1996; Waters and Swann, 1998).

The preferred substrate for TDG is a G:U base pair (Hardeland *et al*., 2003). However, because at least three additional DNA glycosylases in humans have comparable activities, they should have non-redundant biological functions. Recently, TDG and UNG2 have both been shown to be cell cycle regulated, strictly inversely to one another: UNG2 expression peaks at the beginning of the S-phase and then gradually decreases, while TDG expression is undetectable during S-phase and then gradually increases (Otterlei *et al*., 1999; Fischer *et al*., 2004; Hardeland *et al*., 2007), excluding a replication-associated function and implying that TDG only repairs G:U mismatches originating from deamination of cytosine. In addition, G:T mismatch repair is strongly reduced in murine cell lines without *TDG* expression (Cortázar *et al*., 2007), further supporting a role for TDG in repair of deamination damage. Also, TDG interacts with DNMT3a, a *de novo* methyltransferase (Li *et al*., 2007): DNMT3a stimulates the glycosylase activity of TDG, while TDG inhibits methylation activity of DNMT3a *in vitro*. Probably, DNMT3a is necessary for remethylation of deaminated 5-methylcytosines that are first repaired by TDG. On the other hand, inactivation of *E. coli* MUG does not result in higher C-to-T or 5-methylcytosine-to-T transitions (Lutsenko and Bhagwat, 1999; O'Neill *et al*., 2003). Together with the fact that *Drosophila melanogaster* TDG is active during DNA replication (Hardeland *et al*., 2003; Cortázar *et al*., 2007), unlike its human counterpart, these latter observations illustrate that the biological functions of DNA glycosylase homologs in different organisms do not always completely overlap.

In addition to DNMT3a, TDG also interacts with xeroderma pigmentosum (XP) group C (XPC) protein, a component of the nucleotide excision repair (NER) pathway that recognizes structural abnormalities in double-stranded DNA. XPC stimulates TDG activity by promoting the dissociation of TDG from the AP site (Shimizu *et al*., 2003). This crosstalk is only one example of the coregulation and interaction between different DNA repair pathways, which is expected to be a common aspect of repair (Kovtun and McMurray, 2007).

TDG and MBD4 Null Phenotypes

The influence of mammalian TDG on *in vivo* mutagenesis has not been assessed, because homozygous *TDG* null embryos are not viable (Cortázar *et al*., 2007). This lethality can result from accumulation of mutations during early embryonal growth, but is in contrast to the knockouts in other DNA glycosylases often without overt phenotype (Engelward *et al*., 1997; Minowa *et al*., 2000; Nilsen *et al*., 2000; Millar *et al*., 2002; Takao *et al*., 2002b). Therefore, TDG is assumed to have other essential functions in growth and development (see below). In *E. coli*, the mutation frequency of *mug* mutants increases only moderately in non-dividing cells and not in dividing cells (Jurado *et al*., 2004). No role of TDG in tumor initiation or suppression can be ascertained, because no inactivating mutations in TDG have been identified in cancer tissues until now (Sard *et al*., 1997; Schmutte and Jones, 1998).

Inactivation of mouse MBD4 does not result in phenotypical abnormalities or reduction in the survival of mutant mice (Wong *et al*., 2002). However, loss of MBD4 function results in a 2- to 3-fold increase in mutation frequency mainly as a consequence of an increase in the incidence of C-to-T transition mutations at CpG sites (Millar *et al*., 2002; Wong *et al*., 2002). Furthermore, absence of MBD4 also increases tumorigenicity in the tumor-susceptible adenomatous polyposis coli ($APC^{-/-}$) background (Millar *et al*., 2002; Wong *et al*., 2002). Mutations in *APC* predispose mice to multiple intestinal neoplasia. In h*MBD4*, a naturally occurring frameshift mutation in a polynucleotide tract has been identified in different cancers (Bader *et al*., 1999; Riccio *et al*., 1999; Menoyo *et al*., 2001; Yamada *et al*., 2002). This frameshift introduces a premature stop codon with a truncated product without glycosylase domain as a result. The mutation has a dominant negative effect because the glycosylase activity of both hMBD4 and hUNG *in vitro* are inhibited and the mutation frequency is increased 2-fold in cell lines. Unexpectedly, the mutation spectrum of the truncated MBD4 does not specifically increase C-to-T transitions at methyl-CpG sites, but instead displays several types of base changes, probably due to an inhibitory activity of the truncated MBD4 on other DNA glycosylases and perhaps other DNA repair pathways (Bader *et al*., 2007).

Regulation of Gene Expression

The embryonic lethality in TDG-deficient mice can be the consequence of the accumulation of mutations (see above), but it can also be related to the inhibition of changes in CpG methylation during embryogenesis (Reik and Dean, 2001). Furthermore, in addition to its function in DNA repair, TDG also has a role in regulation of gene expression, illustrated by interactions with various transcription factors (especially with nuclear receptors) and also with chromatin-remodeling proteins (Chevray and Nathans, 1992; Um *et al*., 1998; Missero *et al*., 2001; Tini, *et al*., 2002; Chen *et al*., 2003; Lucey *et al*., 2005; Gallais *et al*., 2007).

Generally, TDG activates transcription and has been found only once as repressor of gene expression (reviewed by Cortázar *et al*., 2007). Mostly, the DNA glycosylase function is dispensable for transcriptional activation or repression. Posttranslational modifications of TDG have been reported and might reflect a molecular switch mechanism between the different functions of TDG. For instance, interaction of TDG with the histone acetyl transferase CBP results in TDG acetylation and this posttranslational modification inhibits its association with AP endonuclease, reducing the repair activity of TDG (Tini *et al*., 2002). Sumoylation of TDG decreases its binding rate to DNA and G:T mismatch repair (Hardeland *et al*., 2002). Possibly, the

DNA glycosylase activity of TDG is recruited to particular active genes by interaction with resident transcription factors, assuring that possible DNA damage would be corrected, resulting in proper transcription regulation. In this manner, DNA glycosylase activity can be reconciled with a role in gene regulation (Cortázar *et al*., 2007).

Recently, MBD4 has been demonstrated to be able to repress transcription, as do other MBD proteins, by binding hypermethylated promoters (Kondo *et al*., 2005; Fukushige *et al*., 2006; Majumder *et al*., 2006). Transcriptional repression of MBD4 depends on the interaction with two factors of the histone deacetylase-dependent complex (Kondo *et al*., 2005) and is enhanced upon its interaction with the RET finger protein, also involved in transcriptional repression (Fukushige *et al*., 2006). As for TDG, the glycosylase domain of MBD4 is dispensable for its function in transcription regulation (Kondo *et al*., 2005).

Both MBD4 and TDG have been shown to excise 5-methylcytosine opposite G (Zhu *et al*., 2000a; 2000b), which leads to the hypothesis that these proteins, or one of them, might act as active DNA demethylases (Zhu *et al*., 2000a; 2000b). A function of MBD4 in active demethylation is supported by the observation that MBD4 overproduction in $CD4^+$ T-cells from system lupus erythematosus patients coincides with global DNA hypomethylation (Balada *et al*., 2007). This questions whether MBD4, TDG, or both are involved in global DNA demethylation (Jost *et al*., 2001; Zhu *et al*., 2001) or whether they contribute to site-specific regulation of CpG methylation (Zhu *et al*., 2001). The latter would be in agreement with the observed TDG and MBD4 functions in transcription regulation. However, the excision efficiency of 5-methylcytosine *in vitro* is extremely low. Thus, whether excision of 5-methylcytosine by MBD4 and TDG is biologically relevant and whether these proteins are indeed active in DNA demethylation remain a matter for debate.

Signaling of DNA Damage

In addition to its role in DNA repair and gene regulation, MBD4 is also important in genomic surveillance and apoptosis by regulating cell cycle responses to DNA damage, a function comparable to that of the MMR components (Cortellino *et al*., 2003; Sansom *et al*., 2003). MMR-proficient cells are sensitive to the cytotoxic effects of DNA-damaging agents, because the damage elicits cell cycle checkpoint activation and subsequent apoptosis, whereas MMR-deficient cells can survive these cytotoxic effects. Similarly, unlike MBD4-proficient cells, MBD4-deficient cells fail to activate the G_2/M cell cycle checkpoint and to undergo apoptosis when treated with alkylating agents, such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and temozolomide, platinum compounds, such as oxaliplatin and cisplatin, γ-irradiation, 5-fluorouracil, and irinotecan (Cortellino *et al*., 2003; Sansom *et al*., 2003). A direct role in the signalization of DNA damage is supported by the interaction with the Fas-associated death domain, a protein involved in apoptosis by bridging death receptors with initiator caspases (Screaton *et al*., 2003).

TDG also recognizes O^6-methylguanine:T (Zhu *et al*., 2000b). Its interaction with the cell cycle checkpoint sensor Rad9-Rad1-Hus1 implies that TDG is involved in damage sensing to activate cell cycle control (Guan *et al*., 2007b).

Plant MBD4 and TDG Homologs

In plants, little is known about the impact on genome stability of spontaneous hydrolytic deamination and about the repair capacity of the generated damage. As in the human genome, CpG dinucleotides are underrepresented in the *Arabidopsis* genome, which is expected to be the consequence of 5-methylcytosine deamination (Tran *et al*., 2005). Given the relatively high frequency of 5-methylcytosine in plant DNA compared to human DNA, repair of deamination damage might also be essential in plants. In *Brassicca campestris* spp. *rapa* (turnip), the repair of G:T mismatches to G:C is biased (Riederer *et al*., 1992), supporting the postulated presence of a G:T base pair repair protein in plants. However, such bias has not been detected in *Nicotiana tabacum* (tobacco) cells (Inamdar *et al*., 1992).

No orthologs of TDG have been identified until now, but in *Arabidopsis*, rice, and poplar (*Populus trichocarpa*) homologs of MBD4 have been found. Like the other non-mammalian MBD4 homologs, the plant MBD4 homologs have no MBD, but only the DNA glycosylase domain.

Conclusions

The precise functions of both MBD4 and TDG are clearly not completely revealed yet. From the structural and biochemical data, interaction partners and mutant phenotypes, more than one function can be assumed (Figure 5). Also, how all members of both protein families are temporally and spatially coordinated in the cell remains to be resolved.

Plant-Specific DNA Glycosylases—DEMETER and ROS1

In 2002, two additional DNA glycosylases have been identified in plants without homologs in other species (Choi *et al*., 2002; Gong *et al*., 2002). These plant-specific DNA glycosylases, repressor of silencing 1 (ROS1) and DEMETER (DME), are not involved in DNA repair, but in active DNA demethylation (Figure 5), albeit in a completely different biological context (Agius *et al*., 2006; Gehring *et al*., 2006; Morales-Ruiz *et al*., 2006). It should be stressed that these DNA glycosylases demethylate certain 5-methylcytosine residues by initiating the BER process, thus by removing the methylated base, and not directly the methyl group, like, for instance, AlkB (see below). ROS1 and DME differ from the "conventional" DNA glycosylases in that they are much larger (1393 amino acids and 1729 amino acids, respectively). These bifunctional DNA glycosylases that are part of the helix-hairpin-helix superfamily, have an iron–sulfur cluster and encode an additional N-terminal domain with some similarity to the frog H1 linker histone (Choi *et al*., 2002; Gong *et al*., 2002; Morales-Ruiz *et al*., 2006). Two

consecutive steps (β,δ-elimination) produce a single nucleotide gap flanked by 5′ phosphate and 3′ phosphate groups. In DME, the aspartic acid conserved in all DNA glycosylases is also required for *in vivo* activity and indicates that the DNA glycosylase domain is essential for active demethylation (Choi *et al*., 2004).

DEMETER

DME is required for seed viability in *Arabidopsis* (Choi *et al*., 2002) because it regulates expression of the imprinted *MEDEA* (*MEA*) gene, necessary for proper female gametophyte and seed development (Grossniklaus *et al*., 1998). It does so by removing 5-methylcytosine from the maternal *MEA* allele in a BER-dependent process (Choi *et al*., 2002; Gehring *et al*., 2006). The resulting active *MEA* maternal allele is hypomethylated in specific 5′ and 3′ regions (Gehring *et al*., 2006). In addition, DME also regulates imprinting of the *FWA* and *FERTILIZATION-INDUCING SEED2* (*FIS2*) genes in a comparable manner (Kinoshita *et al*., 2004; Jullien *et al*., 2006). *DME* is expressed in the central cell of the female gametophyte (Choi *et al*., 2002), whereas the target genes *MEA*, *FWA*, and *FIS2* are expressed in the central cell before fertilization and in the endosperm from the maternal allele after fertilization (Kinoshita *et al*., 1999; Vielle-Calzada *et al*., 1999; Kinoshita *et al*., 2004; Jullien *et al*., 2006). In vegetative tissues, these target genes are generally methylated (Xiao *et al*., 2003; Kinoshita *et al*., 2004; Jullien *et al*., 2006). Ectopic expression of *DME* in pollen and stamen induces several genes, coding for DNA or RNA proteins, proteins with kinase activity, and transcription factors (Ohr *et al*., 2007). So, *DME* is an active DNA demethylase that is expressed in the central cell of the gametophyte and is necessary for genomic imprinting of different target genes.

ROS1

Demethylation activity of ROS1, a DME homolog also designated as DME-like 1 (DML1), has first been identified in transgenic plants harboring an *RD29A:luciferase* reporter gene and the endogenous *RD29A* gene (Gong *et al*., 2002; Agius *et al*., 2006; Morales-Ruiz *et al*., 2006). Loss-of-function mutations in *ROS1* result in DNA hypermethylation and transcriptional gene silencing of both the *RD29A*-controlled transgene and endogene. Overexpression of *ROS1* leads to more demethylation and, consequently, increased luciferase expression (Zhu *et al*., 2007). Recently, methylation levels of a representative set of transposable elements and of several genes have been determined in the *Arabidopsis* wild type and *ros1* mutants (Zhu *et al*., 2007). Methylation in the *ros1* mutant was increased primarily at the CpNpG and CpNpN sites and was associated with decreased expression (Zhu *et al*., 2007). In contrast, analysis of genome-wide methylation levels in the triple mutant *ros1 dml2 dml3* and in the corresponding single mutants has shown that an increase in methylation is generally not linked to a decrease in expression levels (Penterman *et al*., 2007). DML2 and DML3 are two additional members of the DME family that also excise 5-methylcytosine *in vitro* (Penterman *et al*., 2007). Comparison of triple mutants and the wild type indicate a methylation increase in 180 loci throughout the *Arabidopsis* genome in the mutants, of which some are demethylated by a particular DML and others by multiple DML proteins (Penterman *et al*., 2007). Demethylation occurs also at the 5′ and 3′ ends of genes (Penterman *et al*., 2007), probably because these regions are less likely to be methylated in the wild type, thereby preventing interference with transcription (Zhang *et al*., 2006; Zilberman *et al*., 2007). A role for DML proteins in genome-wide demethylation is inferred from the developmental abnormalities in some of the ROS1-defective plants after inbreeding for several generations (Gong *et al*., 2002). It now remains to be determined how the different DML proteins are targeted to specific loci and what their specific biological functions are. ROS1 has been proposed to play a role in DNA repair because ROS1-defective plants are hypersensitive to the genotoxic agents methyl methanesulfonate (MMS) and hydrogen peroxide (Figure 5) (Gong *et al*., 2002). In addition, ROS1 and DME also excise thymine when mispaired with guanine, besides 5-methylcytosine, both preferably in a CpG context. *In vitro* data reveal that G:5-methylcytosine is preferred over G:T base pairs (Agius *et al*., 2006; Gehring *et al*., 2006; Morales-Ruiz *et al*., 2006). To evaluate a possible function of DML proteins in DNA repair, the mutation frequency in *dml* mutant lines should be analyzed *in vivo*, for instance with mutation reporter lines (Van der Auwera *et al*., 2008).

DNA Glycosylases and Active Demethylation

The plant DML proteins indicate that DNA glycosylases can function in gene regulation, not only through interactions with other proteins, but also through immediate modification of the DNA methylation status (Kapoor *et al*., 2005). In mammals, it is unclear whether DNA glycosylases, such as MBD4 and TDG, act as active demethylases *in vivo*, given their weak activity on 5-methylcytosine (Zhu *et al*., 2000a; Hardeland *et al*., 2003). However, global demethylation after fertilization occurs by an active mechanism (Mayer *et al*., 2000; Oswald *et al*., 2000), implying the existence of enzymes that demethylate 5-methylcytosine either genome wide or in specific regions. Indeed, the growth-arrest and DNA-damage-inducible protein 45α (Gadd45α) promotes epigenetic gene activation by active DNA demethylation in frog (Barreto *et al*., 2007). The Gadd45α protein functions in numerous biological processes (Hollander and Fornace, 2002) and also in NER. Active demethylation by Gadd45α requires the endonucleases XPG and XPB, enzymes both involved in the NER process, suggesting that here too active demethylation depends on an excision repair pathway (Barreto *et al*., 2007).

Repair of Deaminated Bases in Thermophiles and Hyperthermophiles

Genes that encode uracil and T:G-recognizing enzymes have not only been found in eukaryotes, but also in a large variety

of Eubacteria and Archaea (Aravind and Koonin, 2000), which is not surprising, considering the high spontaneous hydrolytic deamination with increasing temperature (Lindahl and Nyberg, 1974). Thermophilic UDG proteins of Eubacteria and Archaea are classified in three structural families (Table 1). Two of them, UDGa (family 4) and UDGb (family 5), belong to the UDG superfamily, while the third is part of the helix-hairpin-helix superfamily (Chung *et al*., 2003). Characteristic for UDGa is the presence of an iron–sulfur cluster that does not occur in other DNA glycosylases of the UDG superfamily (Hinks *et al*., 2002). UDGa recognizes uracil in single-stranded and double-stranded DNA and prefers U:G over U:A (Sartori *et al*., 2002). UDGb has a much broader substrate specificity: uracil, 5-hydroxymethyluracil, and εC are equally well excised, while hypoxanthine is less well processed (Sartori *et al*., 2002). This UDGb family differs from all other five in that it is structurally related to the helix-hairpin-helix superfamily with an iron–sulfur cluster besides the helix-hairpin-helix motif. In addition to the removal of uracil from both single-stranded and double-stranded DNA, 7,8-dihydro-8-oxoguanine (8-oxoG) can also be excised, an exceptional activity for an UDG (Chung *et al*., 2003).

In thermophilic Eubacteria and hyperthermophilic Archaea, G:T mismatches can be removed, on the one hand, by TDG homologs, designated MUG (Begley and Cunningham, 1999; Begley *et al*., 1999; 2003; Fondufe-Mittendorf *et al*., 2002) and, on the other hand, by Mig.*Mth*, members of the helix-hairpin-helix superfamily (Horst and Fritz, 1996). Mig.*Mth* enzymes recognize G:T base pairs in a specific sequence context and contain a conserved iron–sulfur cluster as well as the helix-hairpin-helix motif (Horst and Fritz, 1996).

In conclusion, most organisms have evolved to possess a large set of DNA glycosylases for the repair of uracil in the genome and other deamination damages, emphasizing how important it is to remove this type of damage. A lot of DNA glycosylases have, besides DNA repair, additional functions, such as transcription regulation, and often they are themselves regulated by posttranslational modifications. Notwithstanding the large amount of information available for vertebrates and bacteria, almost nothing is known with respect to repair of deaminated bases in plants. This area still awaits further investigation.

DNA Alkylation Damage

Alkylation damage is another type of DNA damage that can be repaired by BER. In addition, cells have several other pathways to repair small alkyl adducts, whereas the large alkyl adducts are processed by NER (recently reviewed by Sedgwick *et al*., 2007). Alkylating agents are divided into two types, dependent on their reaction mechanism: S_N1-type agents can alkylate both nitrogen and oxygen species, while S_N2-type agents alkylate nitrogen in nucleic acids. Endogenous DNA alkylation sources are not well defined, except for SAM (Sedgwick 1997; 2004; Drabløs *et al*., 2004; Sedgwick *et al*., 2007). Of the environmental alkylating agents, the most important is MMS. Both SAM and MMS create covalent modifications at ring nitrogen residues of DNA bases, in particular 7-methylguanine (7-meG) and 3-methyladenine (3-meA) (Figure 6A) (Strauss *et al*., 1975). Whereas 7-meG appears to be relatively innocuous, 3-meA has a strong cytotoxic effect by blocking both replication and transcription, because of the aberrant protrusion of the methyl group in the

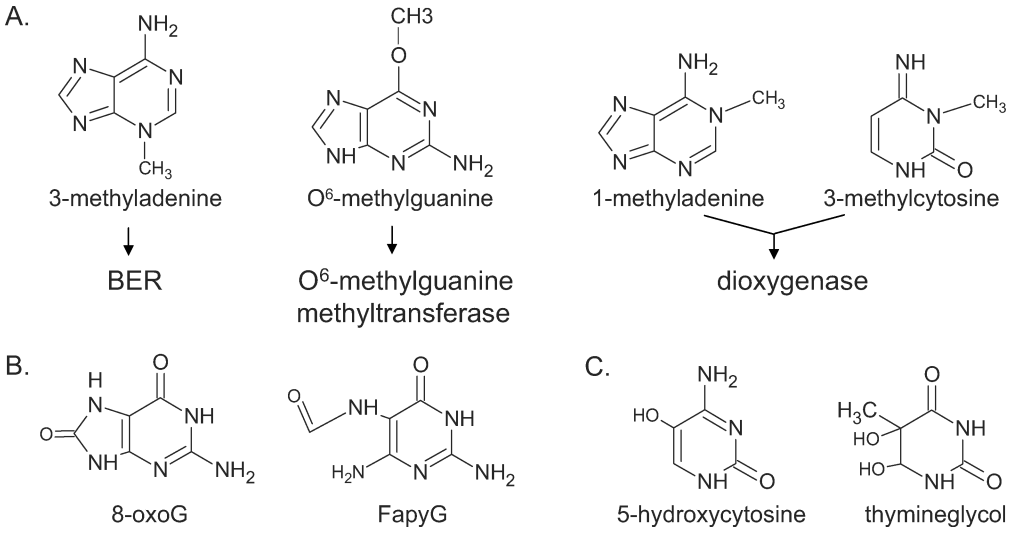

FIG. 6. Chemical structures of damaged bases. A. Major deleterious lesions formed by simple methylating agents in double-stranded (3-meA and O^6-meG) and single-stranded DNA (1-meA and 3-meC). Only 3-meA is repaired by the BER pathway; O^6-meG is directly demethylated by O^6-methylguanine DNA methyltransferase and the lesions 1-meA and 3-meC are demethylated by DNA dioxygenases. B. The two most abundant and best studied purine lesions, 8oxoG and FapyG, generated by oxidative stress. C. Thymineglycol and 5-hydroxycytosine, examples of oxidized pyrimidine bases.

DNA minor groove (Boiteux and Laval, 1982; Larson *et al*., 1985).

Repair of DNA Alkylation Damage

Alkylation DNA glycosylases can be divided into five gene classes (Table 1) that all repair 3-meA, indicating the need of repairing this lesion. Four of the five classes are part of the helix-hairpin-helix superfamily: 3-meA DNA glycosylase I (*TAG*) of *E. coli* (Karran *et al*., 1980); 3-meA DNA glycosylase (*MPGII*) of *Thermotoga maritima* (Begley *et al*., 1999); 3-meA glycosylase IV (*MAGIII*) of *Helicobacter pylori* (O'Rourke *et al*., 2000; Eichman *et al*., 2003); and 3-meA DNA glycosylase II (*AlkA*) of *E. coli* and of yeast (*MAGI*) (Karran *et al*., 1980; Chen *et al*., 1989). These gene classes are specific for bacteria, Archaea, and lower eukaryotes, such as yeast. However, *in silico* data reveal the presence of six and five homologs of *TAG* of *E. coli* in *Arabidopsis* (Britt, 2002) and rice (Kimura and Sakaguchi, 2006), respectively.

The fifth class of DNA glycosylases that recognize alkylation damage is AAG, also know as alkylpurine DNA glycosylase (ANPG) or as *N*-methylpurine DNA glycosylase (MPG) (Sansom *et al*., 1991). This monofunctional BER enzyme is found in mammals, plants, and some bacteria, for instance *Bacillus subtilis*. AAG has an unusual fold, not seen in other BER enzymes (Lau *et al*., 1998; 2000).

Substrate Spectrum

AlkA and AAG recognize a broad spectrum of damaged bases, including deamination (such as hypoxanthine), oxidation (such as 5-formyluracil), and cyclic etheno adduct products (such 1,N^6-εA), while the other three structural classes have a rather narrow substrate specificity, recognizing principally 3-meA (Bjelland and Seeberg, 1987; 1996; Bjelland *et al*., 1993; 1994; Mattes *et al*., 1996; Begley *et al*., 1999; Asaeda *et al*., 2000; Hollis *et al*., 2000; O′Rourke *et al*., 2000; Privezentzev *et al*., 2000; Gasparutto *et al*., 2002; Terato *et al*., 2002). As for TDG, the AAG activity is stimulated by its interaction with XPC, although not because of promotion of enzymatic turnover, but because of elevation of the excision rate of the alkylation damage (Miao *et al*., 2000).

Mutant Phenotypes

As expected, the double mutants *alkA tag* of *E. coli* are highly sensitive to DNA alkylation damage (Evensen and Seeberg, 1982; Clarke *et al*., 1984). Also murine embryonic stem cells deficient in AAG are hypersensitive to killing by MMS and other alkylating agents (Engelward *et al*., 1996). Surprisingly, *AAG* knockout mice show only marginal alkylation sensitivity, no increased cancer frequency, and no reduced fertility or reduced life span, although no back-up DNA glycosylase activity could be detected (Engelward *et al*., 1996). To explain the mild phenotype, it was postulated that a specific translesion DNA polymerase could bypass the cytotoxic lesions formed upon alkylation damage. Supporting this hypothesis, recent data in *E. coli* indicate that DNA polymerase IV (homolog of translesion synthesis DNA polymerase κ) performs an error-free bypass of DNA damage that accumulates in the *alkA tag* mutant background (Bjedov *et al*., 2007).

Unexpectedly, overexpression of *AlkA* in *E. coli* and of *AAG* in *Cricetulus griseus* (Chinese hamster) sensitizes cells to the cytotoxic effects of MMS (Kaasen *et al*., 1986; Coquerelle *et al*., 1995). Also, enhanced *MAGI* expression in yeast increases the *APN1* mutator phenotype that is reduced by suppressed *MAGI* (Xiao and Samson, 1993; Kunz *et al*., 1994), indicating that the DNA glycosylase:AP endonuclease ratio in cells is important to minimize the mutation rate. Probably, an excess of abasic sites is formed when *MAG1*, *AlkA*, or *AAG* are overexpressed, because DNA glycosylases with a broad substrate spectrum slowly excise undamaged bases (Berdal *et al*., 1998). Superfluous abasic sites are thought to be repaired by the error-prone translesion synthesis pathway rather than by the conventional BER pathway (Nelson *et al*., 1996). In addition, in some breast cancer and colon cancer cell lines, the expression of *AAG* is higher than in normal mammary cells (Vickers *et al*., 1993; Cerda *et al*., 1998), illustrating the importance of a highly coordinated BER process (Allinson *et al*., 2004).

In addition to the proposed six TAG and two AlkA homologs, *Arabidopsis* has also an AAG homolog that is able to complement the MMS-sensitive phenotype in the *alkA tag* genetic background of *E. coli* (Santerre and Britt, 1994). The gene is primarily expressed in meristematic tissues, linking the repair process to replication (Shi *et al*., 1997).

Regulation of Gene Expression

AAG plays not only a role in repair of alkylation damage, but seems also involved in transcription regulation (Watanabe *et al*., 2003; Likhite *et al*., 2004), like some other DNA glycosylases (see above). The involvement in transcription regulation has been suggested based on protein–protein interactions between AAG and the transcriptional repressor MBD1 (Watanabe *et al*., 2003; Likhite *et al*., 2004) and between AAG and the nuclear transcription factor estrogen receptor α (Likhite *et al*., 2004). It is unclear whether AAG is directly involved in transcription regulation or whether the interaction with, for instance, transcription factors targets the DNA repair to actively transcribed DNA to guarantee genome integrity of these sites. Posttranslational modification by acetylation has been proposed to coordinate the possibly different functions of AAG (Likhite *et al*., 2004).

Other Pathways Involved in Repair of DNA Alkylation Damage

Besides lesions recognized by DNA glycosylases of the BER pathway, additional lesions are repaired by other strategies (Figure 6A) (reviewed by Sedgwick *et al*., 2007). The

cytotoxic lesions 1-methyladenine and 3-methylcytosine in single-stranded DNA are fixed by a direct reversal mechanism that is catalyzed by the DNA dioxygenases AlkB in *E. coli* and the homologs ABH2 and ABH3 in humans (Aravind and Koonin, 2000; Duncan *et al*., 2002). This reaction proceeds through oxidative demethylation and requires Fe^{2+} as cofactor and 2-oxoglutarate.

The mutagenic and cytotoxic lesion O^6-methylguanine can be repaired not only by TDG and MBD4 when base paired by T, but also by direct reversal with transfer of the methyl group to a specific cysteine residue in the suicidal repair enzyme O^6-methylguanine DNA methyltransferase (reviewed by Mishina *et al*., 2006). Homologs of these proteins have been identified in bacteria and mammals, but until now not in plants, suggesting that plants might use other methods to counteract O^6-methylguanine mutagenicity and cytotoxicity.

Oxidative DNA Damage

ROS, such as hydrogen peroxide, superoxide, and hydroxyl radicals, are byproducts of the normal aerobic metabolism, but can also be produced after ionizing radiation, for instance (Gajewski *et al*., 1990). DNA bases are very susceptible to ROS-mediated oxidation, resulting in oxidized bases, formation of AP sites and strand breaks (Neeley and Essigmann, 2006). The most abundant lesions provoked after oxidative treatments are 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 8-oxoG (Figure 6B), which occur at a frequency of 10^{-6} per guanine. 8-oxoG is best studied and strongly mutagenic because of its preferred base pairing with adenine. Replicative DNA polymerases can bypass this lesion very efficiently, in contrast to many other types of DNA damage (Shibutani *et al*., 1991). So, when not repaired, 8-oxoG lesions result in G-to-T transversion mutations as well as FapyG lesions because of misincorporation of adenine, which is additionally cytotoxic (Wiederholt and Greenberg, 2002; Ober *et al*., 2003).

In cells under oxidative stress conditions, G-to-C transversions are observed that cannot be explained by the presence of 8-oxoG (Neeley and Essigmann, 2006). Therefore, other lesions must be responsible, possibly spiroiminodihydantoin and 5-guanidinohydantoin (Burrows *et al*., 2002), which result from the oxidation of G and 8-oxoG by a large number of oxidants, such as high-valent metal ions and ionizing radiation (Luo *et al*., 2001; Burrows *et al*., 2002). Opposite of these oxidative lesions, both dAMP and dGMP can be inserted (Kornyushyna *et al*., 2002; Kornyushyna and Burrows, 2003), implying that they are 100% mutagenic with G-to-C and G-to-T transversions as a consequence. Moreover, spiroiminodihydantoin strongly blocks replication, whereas 5-guanidinohydantoin can be more easily bypassed (Henderson *et al*., 2003; Delaney *et al*., 2007).

In addition to oxidized purines, a wide spectrum of oxidized pyrimidine derivates is formed after oxidation. Examples are thymineglycol (Tg) (Figure 6C) and 5,6-dihydrouracil, which are both not mutagenic, but able to block transcription and replication, and 5-hydroxycytosine (5-OH-C) (Figure 6C), 5-hydroxyuracil (5-OH-U), and uracilglycol (Ug), which cause C-to-T transitions (Kreutzer and Essigmann, 1998).

Repair of Oxidative DNA Damage

In bacteria, three proteins cooperate to prevent mutagenesis of 8-oxoG (GO repair pathway; Figure 7): MutM (also known as Fpg), which recognizes 8-oxoG:C base pairs and excises the oxidized base (Chetsanga and Lindahl, 1979; Boiteux *et al*., 1990; Tchou *et al*., 1991); MutY, which recognizes 8-oxoG:A base pairs and excises the A, generating a substrate for MutM (Au *et al*., 1989; Michaels *et al*., 1992; McGoldrick *et al*., 1995); and MutT, which recognizes free 8-oxodGTP and removes it from the nucleotide pool to prevent misincorporation into DNA (Mo *et al*., 1992). In human cells, three corresponding proteins have a comparable function: 8-oxoG DNA glycosylase 1 (OGG1) (van der Kemp *et al*., 1996), MutY homolog (MUTYH, formerly hMYH) (McGoldrick *et al*., 1995), and MutT homolog 1 (MTH1) (Bessho *et al*., 1993). In contrast to MUTYH and MTH1, which are orthologs of MutY and MutT, respectively, OGG1 does not share significant sequence identity with the bacterial MutM, but it also repairs oxidized purines and can complement the mutator phenotype of a *mutM mutY* mutant in *E. coli* (Radicella *et al*., 1997; Rosenquist *et al*., 1997). Recognition and repair of 8-oxoG:T depends on MMR, probably without any involvement of the BER machinery. Also, MMR interacts with OGG1 and MUTYH and thereby stimulates the activity of these DNA glycosylases (Kovtun and McMurray, 2007).

Substrate Spectrum

In addition to 8-oxoG, OGG1 and MutM can excise FapyG and 8-oxoA, whereas MutM can also excise 4,6-diamino-5-formamidopyrimidine (FapyA) (Tchou *et al*., 1991; Karahalil *et al*., 1998) and several oxidatively damaged pyrimidines, such as 5,6-dihydrothymine (D′Ham *et al*., 1998; Gasparutto *et al*., 2000), which are no substrates for OGG1 (Karahalil *et al*., 1998). So, despite their comparable functions in different organisms, the substrate specificity of OGG1 is narrower than that of MutM.

Bacterial MutY and its eukaryotic counterpart MUTYH act on DNA containing 8-oxoG:A, 8-oxoA:A, 2-OH-A:G (a substrate for MUTYH only and not for MutY), C:A, and G:A (Au *et al*., 1988; Michaels *et al*., 1992; Grollman and Moriya, 1993; Slupska *et al*., 1996; Lu and Fawcett, 1998; Boiteux and Radicella, 1999; Ohtsubo *et al*., 2000). To avoid mutagenicity, 8-oxoG:A lesions must be repaired by MutY, because removal of 8-oxoG from 8-oxoG:A base pairs results in T:A base pairs and G-to-T transversions. As expected from its function, the MUTYH activity is probably limited to replicating cells, an assumption supported by the finding that MUTYH interacts with RPA and PCNA (Parker *et al*., 2001). Yeast cells lack a MutY DNA glycosylase (Thomas *et al*., 1997), but most probably MMR proteins can act as functional analogs of this DNA glycosylase (Earley and Crouse, 1998; Ni *et al*., 1999).

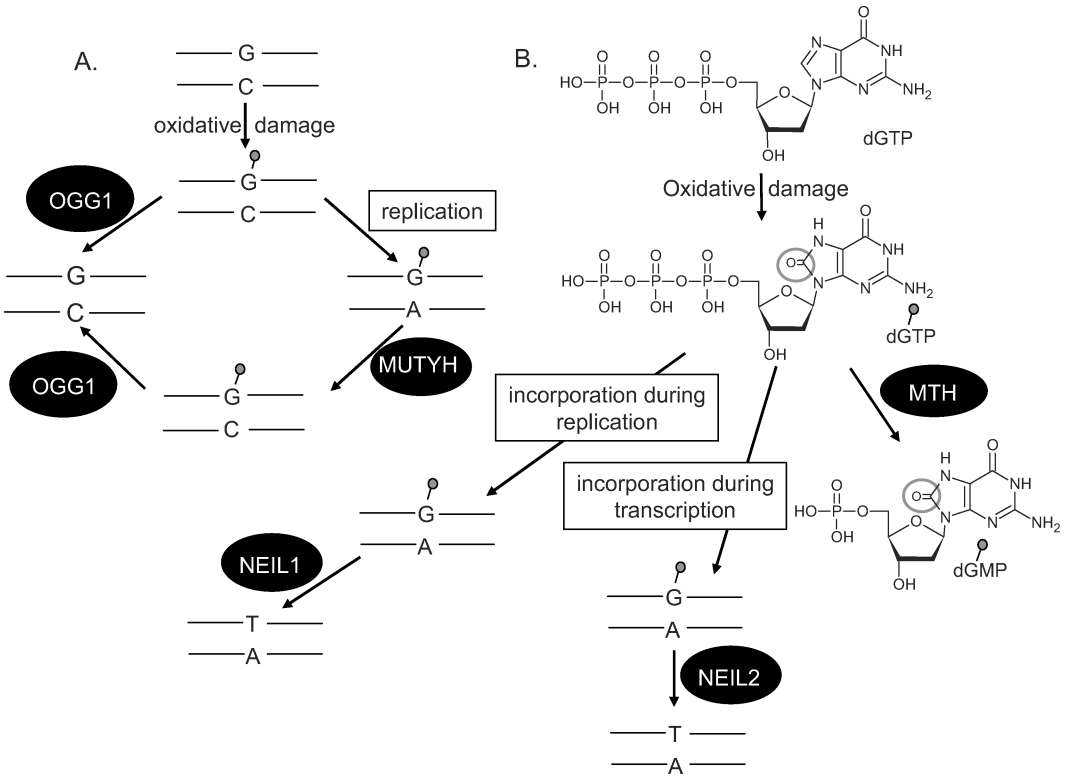

FIG. 7. Model for the repair of 8-oxoG in various genomic contexts. 8-oxoG:C in DNA is recognized and repaired by OGG1. When the damage is not repaired before replication, 8-oxoG can mispair with A. This 8-oxoG:A base pair is recognized by MUTYH, which removes the adenine and generates 8-oxoG:C. MTH removes 8-oxodGTP from the nucleotide pool to prevent misincorporation during replication. Despite this sanitization of the nucleotide pool, misincorporation opposite A during replication and transcription remains possible. Because MUTYH activity would be mutagenic in this instance, data suggest that NEIL1 and NEIL2 repair this type of damage, generated during replication and transcription, respectively. In addition, unrepaired oxidative damage occurring before replication or transcription takes place, is probably also repaired during these processes by NEIL1 or NEIL2, respectively. Oxidative damage is represented by a gray dot.

The catalytic activities of both OGG1 and MUTYH are regulated by posttranslational modifications, like for most BER enzymes, and are stimulated by phosphorylation of certain residues (Dantzer *et al*., 2002; Parker *et al*., 2003; Hu *et al*., 2005).

Bacterial MutT and the human ortholog MTH1 are not DNA glycosylases but hydrolases that prevent misincorporation of 8-oxoG into DNA by hydrolyzing 8-oxodGTP to 8-oxodGMP and pyrophosphate (Maki and Sekiguchi, 1992; Nakabeppu, 2001). Bacterial MutT hydrolyzes 8-oxo-dGTP and 8-oxo-GTP, whereas the human MTH1 has a broader substrate specificity, because it can hydrolyze 2-OH-dATP and 8-OH-dATP as well (Fujikawa *et al*., 1999).

Oxidized pyrimidines are usually not processed by MutM and its human counterpart OGG1, but by another set of DNA glycosylases. In bacteria, Nth and Nei (also referred to as EndoIII and EndoVIII, respectively) excise many damaged pyrimidines (Cunningham and Weiss, 1985; Bailly and Verly, 1987) and the orthologs NTH1, NEIL1, NEIL2, and NEIL3 occur in humans (Ikeda *et al*., 1998; Bandaru *et al*., 2002; Hazra *et al*., 2002a; 2002b; Morland *et al*., 2002). Homologs of Nth have been identified not only in vertebrates, such as mouse and human (Aspinwall *et al*., 1997; Hilbert *et al*., 1997; Ikeda *et al*., 1998; Sarker *et al*., 1998), but also in yeast (Eide *et al*., 1996; Roldán-Arjona *et al*., 1996), Archaea (Eisen and Hanawalt, 1999), and plants (Roldán-Arjona *et al*., 2000). Although the substrate specificity of Nth is very broad, DNA damage is only recognized in double-stranded DNA, as is also true for OGG1 (Breimer and Lindahl, 1984; Hatahet *et al*., 1994; Krokan *et al*., 1997; Speina *et al*., 2001) with Tg, 5-OH-U, and 5,6-dihydroxyuracil as preferred substrates (Hazra *et al*., 2007). The human NTH1 contains a putative nuclear localization signal (Aspinwall *et al*., 1997), but is transported both to the nucleus and the mitochondria (Takao *et al*., 1998), whereas in yeast, two functional homologs of NTH are found of which Ntg1p localizes primarily to mitochondria and Ntg2p to the nucleus (You *et al*., 1999).

Both NEIL1 and NEIL2 bind 5-guanidinohydantoin and spiroiminodihydantoin with higher affinity than other lesions

(Hailer *et al*., 2005; David *et al*., 2007). Like its bacterial counterpart Nei, NEIL1 has high affinity for FapyG, FapyA, and Tg, and weak affinity for 8-oxoG like NEIL2, which apparently prefers cytosine-derived lesions, such as 5-OH-U and 5-OH-C (Hazra *et al*., 2002a). Strikingly, NEIL1 and NEIL2 preferably excise DNA lesions in single-stranded DNA, including 'bubble' DNA, in contrast to most other DNA glycosylases (Dou *et al*., 2003). For NEIL3, no glycosylase activity could be detected until now.

Mutant Phenotypes

The proposed function of MutT is reflected in the greatly elevated mutator phenotype after inactivation of MutT in *E. coli* (Tajiri *et al*., 1995). In contrast, inactivation of MutM or MutY results only in a modest increase in mutator phenotype that is drastically enhanced (up to 100-fold) in the double mutant (Michaels *et al*., 1992). The ability of OGG1 to suppress the *mutM mutY* mutator phenotype in *E. coli* and the tissue-specific and age-related accumulation of abnormal levels of 8-oxoG in *OGG1* null mice (Klungland *et al*., 1999b; Minowa *et al*., 2000; Osterod *et al*., 2001), support the biochemical *in vitro* data. However, the small increase in mutation frequency in null mice and the remaining slow excision rate of 8-oxoG in null cells (Klungland *et al*., 1999b; Minowa *et al*., 2000) imply the existence of back-up systems. Indeed, the Cockayne syndrome group B gene, involved in transcription-coupled repair of UV-induced pyrimidine dimers, plays a role in repair of 8-oxoG both in transcribed and non-transcribed DNA regions (Osterod *et al*., 2002; de Waard *et al*., 2003). In addition, NEIL1 and NEIL2 recognize 8-oxoG in single-stranded DNA. As for deamination damage, the redundancy of the different DNA glycosylases that recognize oxidative DNA damage might be only partial, because increasing evidence points toward various preferences for different genomic contexts (Hazra *et al*., 2007). OGG1 and NTH repair oxidative damage exclusively in double-stranded DNA, whereas NEIL proteins do so in single-stranded DNA. Also, *NEIL1* has been reported to be expressed only in the S-phase and to interact with the sliding clamp PCNA, whereas NEIL2 levels are cell cycle independent (Hazra *et al*., 2002a; Dou *et al*., 2008). *OGG1*, whose expression is constant during the cell cycle, is essential only for repair of 8-oxoG:C in non-transcribed regions (Le Page *et al*., 2000). Thus, oxidative lesions in transcriptionally active sequences are probably repaired by NEIL proteins, whereas OGG1 and NTH are involved in more global repair of these lesions (Hazra *et al*., 2007) (Figure 7).

Similarly to *OGG1* deficiency in mice cells, the mutation frequency increases 2-fold in *MUTYH*$^{-/-}$ cells (Xie *et al*., 2004). *MUTYH* and *OGG1* knockout mice are viable and show no obvious phenotypes or increased tumorigenesis when compared to wild-type mice (Klungland *et al*., 1999b; Minowa *et al*., 2000; Xie *et al*., 2004). Even in old mice or after exposure to chronic oxidative stress, tumorigenesis does not augment in *OGG1*-deficient mice (Arai *et al*., 2002). However, formation of adenoma and carcinoma in the lungs of 18-month-old *OGG1*-deficient mice has been reported (Sakumi *et al*., 2003) as well as intensified tumor formation in different internal organs in 18-month-old *MUTYH*-deficient mice (Sakamoto *et al*., 2007). In yeast, the *OGG1* gene is not essential, because viability does not depend on this gene (Thomas *et al*., 1997). Comparably to the drastically induced mutation frequency in the *mutM mutY* genetic background in *E. coli*, mice deficient in both *OGG1* and *MUTYH* are strongly predisposed to tumorigenicity in lungs, ovary, lymphoma, and small intestine, correlated with increased levels of 8-oxoG in the DNA of these tissues (Xie *et al*., 2004).

In contrast to the elevated mutation frequency in *MutT*-deficient *E. coli* cells, spontaneous mutagenesis does not augment in *MTH1* null mice (Tsuzuki *et al*., 2001). However, tumorigenesis in lung, liver, and stomach is increased in these null mice and, despite no alteration in the mutation frequency, the mutation spectrum is shifted from A-to-C transversions toward 1-bp frameshift mutations at the mononucleotide runs (Egashira *et al*., 2002). Probably different factors might explain these results. First of all, 8-oxoG:A and 8-oxoG:C can be repaired by MUTYH and OGG1, respectively, in *MTH1* mutants. Secondly, MMR possibly participates in repair of oxidized purine lesions in mammals, in a manner comparable to that in yeast. The accumulation of 8-oxoG in the genome of MMR-deficient embryonic stem cells in mice hints at such a hypothesis (DeWeese *et al*., 1998). In addition, the MSH2/MSH6 complex physically interacts with MUTYH (Bertrand *et al*., 1998), suggesting that the more frequent occurrence of frameshift mutations in the *MTH1*$^{-/-}$ background is due to sequestering of MMR for the repair of the oxidative lesions (Egashira *et al*., 2002). Finally, an additional MutT homolog has been identified in mammalian cells, MTH2 (NUDT15) with an activity similar to that of MTH1 (Cai *et al*., 2003). Another mammalian counterpart of MTH1 is NUDT5 that efficiently hydrolyzes 8-oxodGDP to 8-oxodGMP and phosphate (Ishibashi *et al*., 2003).

E. coli nth mutants exhibit a weak mutator phenotype, whereas *nei* mutants do not. In *nth nei* double mutants, the *nth* mutator effect is slightly enhanced and cells are hypersensitive to ionizing radiation and hydrogen peroxide (Saito *et al*., 1997). Quadruple mutants lacking *Nth*, *Nei*, *MutY*, and *MutM* have strong synergistic effects, confirming an overlap in their substrate specificity (Blaisdell *et al*., 1999). *NTH1* null mice show no aberrant phenotype and unaltered sensitivity to ROS and irradiation (Takao *et al*., 2002b), but the repair kinetics of Tg are slow (Elder and Dianov, 2002; Ocampo *et al*., 2002; Takao *et al*., 2002a; 2002b). However, NEIL1 downregulation tremendously sensitizes cells to γ-irradiation (Rosenquist *et al*., 2003). NEIL1 mRNA expression can be upregulated upon treatment with ROS through activation of the transcription factors CREB/c-Jun (Das *et al*., 2005). NEIL2 activity is regulated through acetylation by p300 (Bhakat *et al*., 2004) and, dependent on the acetylation location, DNA repair activities are abrogated. For NTH, no posttranslational modifications have been described yet that regulate the repair capacities, but the activity can be stimulated

by interaction with the NER endonuclease XPG through promotion of damaged DNA binding (Klungland *et al*., 1999a). NEIL1 can be stimulated as well by interaction with the Rad9–Rad1–Hus1 complex (Guan *et al*., 2007a). This interaction might point to a link between BER, more specifically lesion recognition by NEIL1, and DNA damage signaling. In addition to NTH1, NEIL1, and NEIL2, SMUG1 and UNG2 might also act on oxidized pyrimidines, as illustrated *in vivo* by an elevated radiation sensitivity of mouse embryo fibroblasts with down-regulated *SMUG1* and *UNG2* genes (Dizdaroglu *et al*., 1996; An *et al*., 2005).

BER and Human Disease

The first clear link between BER and human disease was established by the discovery of inherited mutations in the h*MUTYH* gene associated with the prevalence of colorectal tumors (Al-Tassan *et al*., 2002; Jones *et al*., 2002; Sieber *et al*., 2003). Biallelic germline mutations in h*MUTYH* result in an increase in G:C-to-T:A transversions in the *APC* gene (Al-Tassan *et al*., 2002), which controls the proliferation of colon cells (Fearnhead *et al*., 2001). In addition, tumors from patients with mutations in h*MUTYH* often show G-to-T transversions in the oncogene *K-Ras* (Lipton *et al*., 2003), suggesting that other genes than *APC* and *K-Ras* are also mutated as a result of dysfunctional MUTYH.

In *MUTYH* null mice, data concerning tumor formation are contradictory: increased tumor formation in different internal organs, particularly in the intestine, has been reported (Sakamoto *et al*., 2007; Tsuzuki *et al*., 2007), whereas no difference with the wild-type mice has also been reported (Xie *et al*., 2004). Possible differences between mice and humans might be that, although disease-causing mutations in h*MUTYH* affect the catalytic activity (Chmiel *et al*., 2003; Wooden *et al*., 2004; Pope *et al*., 2005; David *et al*., 2007), protein–protein interactions and binding at 8-oxoG:A base pairs might still be mediated by the mutant protein but not in knockout mice (Barnes and Lindahl, 2004).

For hOGG1, a role in tumor suppression has been suggested, based on its location on chromosome 3, a region with frequent loss of heterozygosity in different tumors (Chevillard *et al*., 1998). Moreover, *OGG1* polymorphisms have been reported in a variety of cancers, such as prostate cancer and smoking-associated lung cancer (Goode *et al*., 2002; Trzeciak *et al*., 2004; Hung *et al*., 2005). Although no clear role has been found for MTH1 as for MUTYH in cancer predisposition, a single nucleotide polymorphism in the h*MTH* gene might be linked to a higher risk for cancer incidence (Oda *et al*., 1999; Kimura *et al*., 2004; Kohno *et al*., 2006).

Mutations in BER genes can result not only in increased cancer predisposition, but also in a higher risk for the prevalence of other diseases. For instance, a link has recently been established between BER and Alzheimer's disease that is characterized by the accumulation of oxidative damage in brain tissue and by increased mutations in *OGG1*, coinciding with reduced repair (Mao *et al*., 2007; Weissman *et al*., 2007). NEIL1 knockout mice and mice heterozygous for NEIL1 show symptoms comparable to those of the metabolic syndrome in humans (Vartanian *et al*., 2006). This disorder is linked to oxidative stress, possibly through disruption of energy homeostasis because of extensive mitochondrial damage or because of accumulation of oxidative lesions in nuclear DNA of some specific cell types in the absence of NEIL1 (Vartanian *et al*., 2006). In addition, *NEIL1*-inactivating mutations and the occurrence of human gastric cancer might be correlated (Shinmura *et al*., 2004).

Repair of Oxidative Damage in Plants

Unlike other organisms, plants have homologs for OGG1 and MutM, both able to excise 8-oxoG *in vitro* (Ohtsubo *et al*., 1998; Garcia-Ortiz *et al*., 2001; Murphy and George, 2005). In addition, AtOGG1 complements the *mutM mutY* mutator phenotype in *E. coli* (Dany and Tissier, 2001). Although it is unclear why plants possess homologs of two clearly redundant enzymes, they might be located in different organelles (for instance, nucleus and chloroplasts), occur in different parts of the plant, or have evolved different specificities. The latter hypothesis is supported by significantly different excision kinetics of AtOGG1 compared to other species (Morales-Ruiz *et al*., 2003). Seven splice variants of At*MMH* have been reported that are expressed differentially in various tissues (Ohtsubo *et al*., 1998; Gao and Murphy, 2001; Murphy and Gao, 2001). Although h*OGG1* is also alternatively spliced (Kohno *et al*., 1998; Dherin *et al*., 1999) with one form targeted to the mitochondria (Takao *et al*., 1998; Nishioka *et al*., 1999) and the other containing a nuclear localization signal (Nishioka *et al*., 1999), At*OGG1* is not (Dany and Tissier, 2001; Garcia-Ortiz *et al*., 2001). This gene is widely expressed, albeit at low levels (Garcia-Ortiz *et al*., 2001). Surprisingly, expression of both At*MMH* and At*OGG1* is not induced after treatment with hydrogen peroxide, paraquat, or γ-irradiation (Dany and Tissier, 2001). In addition to AtOGG1 and AtMMH, a homolog of NTH is expressed in *Arabidopsis*. *In vitro* analysis has revealed that this protein has a substrate spectrum comparable to that of the human homolog (Roldán-Arjona *et al*., 2000). Although the glycosylases involved in recognition of oxidative DNA damage are the best studied in plants until now, understanding of the importance of these proteins in stress tolerance awaits further investigation.

CONCLUSIONS

Repair of oxidative and other DNA lesions is obviously extremely complex, because of the wide range of base modifications, redundancy, regulation of the BER process, and posttranslational modifications. Additionally, *in vitro* data often oversimplify processes, while *in vivo* repair depends on efficient recognition of lesions in an excess of millions of normal base pairs. Moreover, a condensed chromatin structure might

complicate the repair process and protein interactions and modifications *in vivo* influence repair enzyme activity. The future challenge will be to unravel the regulation and coordination of the BER process and all proteins involved.

ACKNOWLEDGEMENTS

We are grateful to Geert Angenon for critical reading of the manuscript and for suggestions and Martine De Cock for help in preparing the manuscript. J.B. was indebted to the Institute for the Promotion of Innovation by Science and Technology in Flanders for a predoctoral fellowship.

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Editor: Michael M. Cox