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REPAIR AND GENETIC CONSEQUENCES OF ENDOGENOUS DNA BASE DAMAGE IN MAMMALIAN CELLS

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■ **Abstract** Living organisms dependent on water and oxygen for their existence face the major challenge of faithfully maintaining their genetic material under a constant attack from spontaneous hydrolysis and active oxygen species and from other intracellular metabolites that can modify DNA bases. Repair of endogenous DNA base damage by the ubiquitous base-excision repair pathway largely accounts for the significant turnover of DNA even in nonreplicating cells, and must be sufficiently accurate and efficient to preserve genome stability compatible with long-term cellular viability. The size of the mammalian genome has necessitated an increased complexity of repair and diversification of key enzymes, as revealed by gene knock-out mouse models. The genetic instability characteristic of cancer cells may be due, in part, to mutations in genes whose products normally function to ensure DNA integrity.

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INTRODUCTION

Endogenous DNA damage, largely as a consequence of unavoidable hydrolysis and oxidation, is a problem faced particularly by aerobic organisms. Modification or loss of DNA bases can alter coding specificity leading to mutations, a vital source of genetic variation but also a significant cause of human disease. Thus, an effective mechanism, base-excision repair (BER), has evolved to deal with endogenous DNA base damage and maintain genome stability necessary for the survival of the individual and the species. Some key BER enzymes are highly conserved from microorganisms to humans, and the association of mutator phenotypes with repair-deficient microbial mutants demonstrates the threat of endogenous DNA damage to genetic integrity. The mammalian genome would be expected to present a larger target for mutagenesis, and the similarity of mutation rates among lineages with vastly different generation times points to a major contribution of replication-independent processes to overall mutation rate in mammalian genomes (77). We consider the major forms of endogenous DNA base damage in turn, as well as the enzymes that act on these lesions to initiate base-excision repair in the mammalian system. The later essential catalytic steps of BER, to synthesize and ligate a repair patch following damage-excision, are briefly covered. Much of this work has been guided by knock-out mouse models and we have focused on these, rather than on the biochemistry and structure of the enzymes involved, in assessing the genetic consequences of endogenous DNA base damage in mammalian cells.

HYDROLYTIC CYTOSINE DEAMINATION AND THE PRESENCE OF URACIL IN DNA

Hydrolytic deamination of cytosine in DNA generates the aberrant base, uracil. Such deamination occurs at least 100-fold more rapidly in single-stranded regions of DNA where cytosine residues are not protected by the complementary strand, e.g., at replication forks and transcription bubbles. If unrepaired, U:G mispairs would give rise to C:G → T:A transition mutations upon DNA replication. Excision of deaminated cytosine by the action of uracil-DNA glycosylase initiates BER

to counter this promutagenic event, and this class of enzymes is widely distributed in nature. The greatly increased frequency of spontaneous C:G → T:A transition mutations in *Escherichia coli* and *Saccharomyces cerevisiae ung*$^-$ mutants deficient in uracil-DNA glycosylase indicates that removal of deaminated cytosine is relevant in vivo (27, 67). Compared with microbial genomes, increased size, slower replication, and the need to repair frequently transcribed DNA would make the mammalian genome a much bigger target for mutagenesis and considerably more susceptible to deamination of cytosine. DNA uracil arises by hydrolytic deamination ~100–500 times per human cell per day, and the rate of deamination of 5-methylcytosine (5-meC) is threefold higher than that of unmethylated cytosine (86, 132). Furthermore, uracil can also appear as a noncanonical base in DNA by misincorporation of low levels of dUTP during DNA replication. Although uracil is not miscoding in this context, substitution of thymine with uracil through use of the dUTP pool is a major source of endogenous DNA abasic (AP) sites in *S. cerevisiae* (51) and may also mediate the cytotoxicity of certain cancer chemotherapeutic drugs. Recent developments have also highlighted the occurrence of enzymatic DNA deamination, specific to mammalian cells, where targeting to endogenous or foreign DNA mediates the diversification of antibody genes and immunity to retroviral infection, respectively (101).

UNG Uracil-DNA Glycosylase: Dedicated Roles in Mammals

Enzymes that excise uracil from DNA are ubiquitous in bacteria, archaea, and eukaryotes. The UNG uracil-DNA glycosylase is present in most species (7) and is the antimutator responsible for the repair of uracil in U:G mispairs in *E. coli* and yeast (27, 67). UNG is extraordinarily highly conserved from *E. coli* to human (55% amino acid identity in the catalytic domain; 110). The catalytic properties of the mammalian UNG enzyme and its ability to complement an *ung*$^-$ *E. coli* mutant presupposed that it would fulfill the same functional roles as the *E. coli* enzyme, namely excising uracil from dUMP misincorporated opposite A during DNA synthesis and from premutagenic U:G mispairs due to deamination of cytosine in situ. Surprisingly, UNG-deficient mice showed only a slight increase in frequency of genome-wide mutation, indicating that UNG is not the major enzyme removing premutagenic uracil from DNA in mammals (105). However, there was a ~100-fold increased steady-state level of uracil in the genome of dividing *Ung* null cells, due to slow removal of uracil from misincorporated dUMP. Thus, the UNG protein retains one major role in mammalian cells, counteracting U:A base pairs formed by occasional use of dUTP during DNA synthesis. It is facilitated in this role by interactions with PCNA and RPA, targeting UNG to sites of DNA replication in proliferating cells, and, exceptionally among the DNA glycosylases, a high turnover number consistent with a role at progressing replication forks (115). But the mammalian UNG enzyme does not appear to serve as an efficient antimutator acting on deaminated cytosine damage, at least not in the mammalian genome as a whole. In a special niche, UNG does have a role at U:G base pairs but surprisingly,

in a DNA-processing context, specifically resolving deaminated cytosines targeted to immunoglobulin loci during diversification of the immune system.

The "DNA deamination" model for antibody diversification, in which deamination of cytosine to uracil by the AID enzyme (activation-induced cytidine deaminase) provides an initiating DNA lesion, has recently gained prominence in the immunology field. Direct support was provided by the observation that the pattern of somatic hypermutation in immunoglobulin variable genes is dramatically shifted toward transitions at C:G base pairs and isotype switching is inhibited in UNG-deficient mice, indicating that UNG is the major DNA glycosylase processing the programmed U:G lesions and that class switch recombination largely proceeds via formation of an abasic site (121). Furthermore, UNG-deficient mice develop B-cell lymphomas, suggesting that UNG normally modifies DNA in germinal center B cells and the U:G lesions are indeed mutagenic if not removed; this was the first example of spontaneous tumorigenesis in the mouse due to deficiency in a DNA glycosylase (106). The secondary genetic changes that lead to B cell lymphomas in *Ung* null mice may reflect aberrant hypermutation of bystander oncogenes and/or their transcriptional deregulation via translocations into the immunoglobulin locus (45); these changes are currently under investigation. Mutations in the human *UNG* gene have been correlated with impaired class switch recombination in a subgroup of hyper-IgM syndrome patients (66); it has yet to be established if UNG-deficiency is associated with human B cell malignancies.

SMUG Uracil-DNA Glycosylase: Devolution of Function to a Recently Evolved Family Member

Gene-targeted mice deficient in the evolutionarily conserved UNG uracil-DNA glycosylase lacked the expected mutator phenotype characteristic of bacterial and yeast ung^- mutants, but at the same time revealed a complementary uracil-DNA glycosylase activity that was detected in $ung^{-/-}$ cells and tissues as the new prime candidate responsible for the repair of premutagenic U:G mispairs resulting from genome-wide hydrolytic deamination of cytosine residues in vivo (105). This major uracil-excising activity in UNG-deficient mice was due to the SMUG1 uracil-DNA glycosylase (103), originally identified in *Xenopus laevis* by an *in vitro* expression cloning strategy (56). SMUG1 shares extremely limited amino acid sequence homology (less than 8% amino acid identity) with the UNG protein, restricted to residues required for substrate binding and catalysis. However, SMUG1 retains the core fold common to the uracil-DNA glycosylase superfamily; as it is present only in insects and vertebrates, SMUG1 represents a relatively recent branch of this family tree (7, 103). Unlike UNG, SMUG1 is present at similar levels in cell nuclei of nonproliferating and proliferating tissues, and does not contain a consensus PCNA binding motif, indicating a replication-independent role in DNA repair. Although UNG could deal with U:G lesions in addition to U:A mispairs at the replication fork, the low K_m of SMUG would give this enzyme a clear kinetic advantage at low substrate concentrations and favor a DNA repair role, efficiently detecting rare deaminated cytosines throughout the mammalian genome. This compartment model for

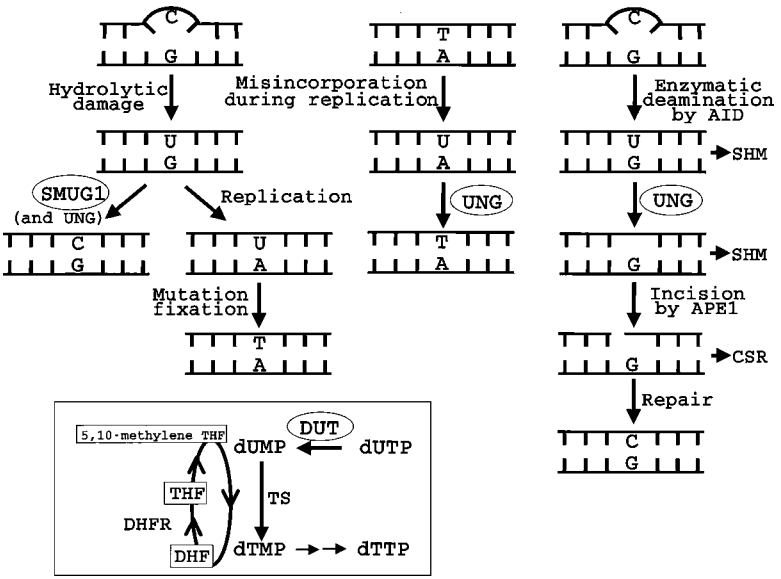

Figure 1 Compartment model for the repair of uracil residues in the mammalian genome. DNA regions temporarily present in single-stranded form due to replication or transcription are susceptible to hydrolytic deamination of cytosine residues. The SMUG1 uracil-DNA glycosylase can recognize these rare, weak U:G mispairs present throughout the genome. If unrepaired, they give rise to U:A during subsequent replication, generating C → T transition mutations. The UNG uracil-DNA glycosylase is mainly targeted to the replisome where it operates during replication to detect and excise uracil from U:A base pairs, arising through occasional use of dUTP in place of TTP during DNA synthesis. The DUT-encoded dUTPase minimizes dUTP in precursor pools. Inhibition of thymidylate synthase (TS), or depletion of the tetrahydrofolate (THF) methyl donor cofactor generated by dihydrofolate reductase (DHFR), leads to elevation of the dUTP versus TTP pool. Enzymatic deamination of C residues in antibody variable (V) genes by AID (activation induced deaminase) occurs in activated B cells, in DNA regions single-stranded as a consequence of transcription of the complementary strand. The resultant U:G mispairs are specifically processed by UNG. Somatic hypermutation (SHM) occurs upon replication of the U-containing template (phase 1A; transitions only) or following excision of the U by UNG (phase 1B; transitions and transversions); the major pathway of class-switch recombination also proceeds via formation and incision of the AP site.

the repair of uracil is shown in Figure 1. Ongoing genetic and biochemical studies with null mice and cell lines depleted for SMUG1, in both an UNG-proficient and -deficient background, should clarify the strategies used to counteract the mutagenic threat to the mammalian genome from cytosine deamination.

SMUG1 was rediscovered in a different guise as the activity initiating BER of 5-hydroxymethyluracil (5-OH-meU), a modified base generated by oxidation

of thymine, or oxidation and deamination of 5-meC, in mammalian cells (12). Thus, similarly to uracil in DNA, 5-OH-meU can arise opposite adenine or be miscoding opposite G. The 3D structure of the SMUG1 enzyme has revealed a novel and subtle mechanism that governs the remarkable ability of the enzyme to bind 5-OH-meU (C5-CH_2OH) as well as uracil (C5-H) as a substrate, while still discriminating against the normal base, thymine (C5-CH_3) (149). It has yet to be determined if SMUG1 is able to excise other potentially mutagenic oxidized cytosine derivatives, such as 5-hydroxycytosine, 5-hydroxyuracil, and uracil glycol (76), which are substrates for distinct DNA glycosylases that normally act on such oxidized pyrimidines (see below). The overlapping phylogenetic distribution of SMUG1 with both use of 5-meC as a regulator of gene expression and the dedication of UNG to roles in the replisome/immune system has led to speculation as to whether SMUG1 evolved to deal with oxidized, deaminated 5-meC, or deaminated cytosine (7, 72, 103, 105). Initial observations of an alternatively spliced transcript, transcriptional regulation of expression, and accumulation of SMUG1 in nucleoli are intriguing (30, 72). In vitro enzyme assays and also overexpression of human SMUG1 in an *ung*$^-$ mutant of *S. cerevisiae* (which does not have a SMUG1 orthologue) fail to discriminate whether 5-OHmeU:G or U:G is the major substrate of SMUG1 (31, 72). However, it remains clear from the phenotype of *Ung* null mice that the mammalian SMUG1 uracil-DNA glycosylase activity cannot replace UNG's replication-associated and immune-specific roles, but can suffice as an antimutator at deaminated cytosine residues in vivo. As C → T transition mutations at methyl-CpG sequences are the most frequent point mutation in human cancers, as well as in the germline giving rise to genetic disease, a null mouse model would address the biological relevance of the SMUG1 5-OHmeU-DNA glycosylase activity to spontaneous mutagenesis at 5-meC:G base pairs. It has also been proposed that SMUG1 might act in an orchestrated sequence of 5-meC oxidation, deamination, and excision in the effective demethylation of silenced DNA (12); the presence of SMUG1 but not 5-meC in *Drosophila* might argue against this corollary (7).

Thymine-DNA Glycosylases and Deamination of 5-Methylcytosine

The eponymous UNG or SMUG uracil-DNA glycosylases hydrolyze the glycosyl bond between the aberrant uracil base and the deoxyribose sugar moiety in single- or double-stranded DNA. By contrast, the TDG and MBD4 DNA glycosylases excise the normal base thymine but only from T:G mispairs that arise from deamination of 5-meC in vivo and, as long as it is mispaired with guanine, can also excise uracil (see Table 1); these two mismatch DNA glycosylases do not account for the backup activity on U:G mispairs in *Ung* null mice (105). TDG can additionally act on mutagenic alkylated bases such as ethenocytosine (2), formed by reaction of endogenous lipid peroxidation products with DNA and responsible for the carcinogenicity of chemicals such as vinyl

TABLE 1 Mammalian DNA glycosylases

Enzyme	Chromosomal location (human)	Cellular localization (nuclear or mitochondrial)	Major or significant substrates[a]
UNG	12q23–24.1	N and M	U in single- or double-strand DNA
SMUG1	12q13.3–11	N	U in single- or double-strand DNA, 5-OH-meU
TDG	12q24.1	N	T, U or ethenoC opposite G (preferably CpG sites)
MBD4	3q21–22	N	T or U opposite G at CpG, T opposite O^6-meG
MYH	1p32.1–34.3	N and M	A opposite 8-oxoG, 2-OH-A opposite G
OGG1	3p26.2	N and M	8-oxoG opposite C, fapyG
NTH1	16p13.3	N and M	Tg, DHU, fapyG, 5-OHU, 5-OHC
NEIL1	15q22–24	N	As NTH1; also fapyA, 5S, 6R Tg isomer, 8-oxoG
NEIL2	8p23	N	Overlap and some differences with NTH1/NEIL1
NEIL3	4q34.2	N	To be determined
MPG	16p13.3	N	3-meA, hypoxanthine, ethenoA

For comprehensive updated information see: http://www.cgal.icnet.uk/DNA_Repair_Genes.html

[a]For abbreviations, see the text.

chloride. Inactivation of the orthologue, MUG, in *E. coli* has no effect on C → T or 5meC → T mutations (91, 111); the effect of mammalian TDG on mutagenesis in vivo has not been assessed but could be compounded by its interaction with the basal transcription machinery. Interaction of mammalian TDG with the XP-C protein (involved in the global nucleotide excision repair pathway for the correction of helix-distorting DNA lesions such as UV-induced pyrimidine dimers) appears to augment enzymatic turnover of TDG and might suppress mutagenesis at nondipyrimidinic CpG sites in Xeroderma pigmentosum group C patients (133). In contrast to TDG, the MBD4 protein comprises a glycosylase domain but also an amino terminal methyl-CpG binding domain. Analysis of MBD4-deficient mice showed a ~threefold increase in CpG → TpG mutations that correlated with tumorigenesis but only in a cancer-susceptible $Apc^{Min/+}$ background (97, 154).

MBD4 also interacts with the proapoptotic Fas-associated death domain protein (FADD) and the mismatch repair (MMR) protein MLH1, and exhibits thymine-DNA glycosylase activity on O^6-meG:T mismatches (23). Thus, like MMR-defective cell lines, *Mbd4* null cells are tolerant to the cytotoxic effects of antitumor drugs that methylate the O^6 position of guanine. MBD4-deficient mice

do not exhibit microsatellite instability, but mutation of MBD4 is a frequent occurrence secondary to MMR dysfunction in human tumors with clinical implications for drug resistance. TDG can also act on O^6-meG:T mismatches in vitro but, significantly, does not appear to mediate cytotoxicity of methylating agents. Given the diverse roles of MBD4 (117), the rather modest increase in CpG mutability that does not in itself initiate carcinogenesis in MBD4-deficient mice (97, 154), together with the strong preference of mammalian TDG for CpG sites in the excision of both T and ethenoC (2), attention must surely refocus on TDG as the major antimutator at 5-meC residues in mammalian cells.

Other Enzymes for the Excision or Bypass of Uracil in DNA

Interestingly, a TDG orthologue has been described in *Drosophila* and the fission yeast *Schizosaccharomyces pombe* (55), but appears to be a recent loss from *S. cerevisiae* (7). With SMUG making a much later evolutionary appearance in insects and mammals, *E. coli* and yeast, but *S. cerevisiae* in particular, are poor model systems for studying the genetic consequences of uracil in the mammalian genome. Conversely, the apparent lack of the ubiquitous UNG in *Drosophila* and singular presence of UNG alone in *Caenorhabditis* have yet to be exploited in developmental studies of these multicellular organisms. A fourth paralogous family of uracil-DNA glycosylases only occurs in eubacteria, and as the sole activity in archaea. Interestingly, the archaea have adopted a novel additional safeguard, as their DNA polymerases can "read ahead" and stall at unrepaired uracil in a template-strand, avoiding mutagenic incorporation of adenine and providing a rationale for the close coupling of uracil excision with DNA replication (41, 47). It then becomes tempting to speculate whether in mammalian cells, where UNG has been sequestered to the replisome, the replicative DNA polymerases might also discriminate against a template uracil, and what the consequences of polymerase pausing would be in conditions of elevated dUTP or UNG-deficiency. Although most DNA polymerases are constrained by Watson-Crick base-pairing, members of the Y-family of DNA polymerases operate at lower fidelity, often in the interests of efficient lesion bypass. Specific misinsertion of G opposite U by the error-prone mammalian DNA polymerase iota (Polι), which is targeted to the replisome and has also been implicated in somatic hypermutation of immunoglobulin genes, was proposed to decrease the mutagenicity of deaminated cytosine (145). However, deficiency in this polymerase did not affect somatic hypermutation or lymphomagenesis in *Ung* null mice (106).

Uracil Coding in Place of Thymine

It has been argued that the only advantage to replacing U with T in the ancestral genome would have been to aid discrimination and repair of deaminated cytosine, although the problem reappeared with the advent of cytosine methylation; deamination of 5-meC once again generates a normal DNA base (120). But why bother to correct occasional uracils in the DNA genome back to thymine when

in a perfectly good U:A base pair, where they would also not be easy to detect? Replicative DNA polymerases discriminate poorly between TTP and dUTP, an intermediate in the de novo biosynthesis pathway for TTP, and dUMP will be incorporated into newly synthesized DNA in proportion to the size of the dUTP pool. Even though the U:A base pair is neither miscoding nor cytotoxic, increased dUMP incorporation into DNA is associated with lethality in dUTPase null mutants of *E. coli* and *S. cerevisiae* (34, 44). The received wisdom is that futile or inefficient UNG-initiated repair leads to persistent DNA strand breaks, although UNG deficiency does not rescue microbial null mutants totally deficient in dUTPase. This is also thought to be the mode of action of cancer chemotherapeutic drugs such as 5-fluorouracil and methotrexate, which inhibit thymidylate synthase (TS), directly or indirectly (through the folate cofactor), respectively (Figure 1). Analogously, a mutant hamster cell line deficient in SMUG1 activity was isolated on the basis of its resistance to 5-hydroxymethyl-2′-deoxyuridine (11). There are differences in dUTP metabolism between various microorganisms and mammals, and uracil substitution has other effects, on DNA binding factors and transcription, independent of DNA repair. Furthermore, UNG expression does not appear to have a major effect in modulating cellular sensitivity to TS inhibition in mammalian cells (148) and, surprisingly, overexpression of TS may also be correlated with neoplastic transformation (121a). Given the diversification in numbers and specialization in function of the mammalian uracil-DNA glycosylases, there is clearly a need for further investigation in this area, especially as modulation of dUMP incorporation versus uracil excision is looking increasingly important to both rational chemotherapy (for review, see 3) and developmentally programmed cell death.

OXIDATIVE DNA BASE DAMAGE

Reactive oxygen species arise as by-products of normal aerobic metabolism and, despite the confinement of respiration to mitochondria, can damage the nuclear genome, giving rise to oxidized bases, AP sites, and strand breaks. The major mutagenic base damage is due to modification at the C8 position of guanine to form 7, 8-dihydro-8-oxoguanine (8-oxoG), also called 8-hydroxyguanine in the alternative tautomeric form (107). 8-oxoG is strongly mutagenic, able to base pair with adenine and cause G:C $\rightarrow$ T:A transversion mutations in repair-deficient bacteria and yeast (96, 142). Bacteria mount a three-pronged attack to prevent spontaneous mutagenesis at 8-oxoG; the Fpg (MutM) DNA glycosylase excises the oxidized base from 8-oxoG:C base pairs, the MutY DNA glycosylase excises adenine where it has been misincorporated opposite unrepaired 8-oxoG during replication, and the MutT 8-oxoGTPase prevents incorporation of 8-oxoG into nascent DNA (92, 96). MutY and MutT orthologues occur in mammals but not in *S. cerevisiae*, whereas in yeast and mammalian cells, the OGG1 DNA glycosylase is the major activity excising 8-oxoG from DNA, performing this function in place of an orthologue of bacterial Fpg. An unanticipated elaboration of

repair pathways for oxidative base damage has emerged from mammalian genetic models, with complex outcomes for mutagenesis and tumorigenesis, which are addressed here. Possible tolerance of oxidative base damage by translesion synthesis is beyond the scope of this review. Efficient and accurate replication by DNA polymerase eta (Polη) could be particularly relevant (54), especially with regard to defective excision-repair of chemically stable, bulky oxidative lesions (78, 122).

Function of the OGG1 8-oxoG-DNA Glycosylase

Although there is little sequence similarity between OGG1 and the bacterial Fpg/MutM protein, OGG1 has a high specificity for 8-oxoG:C base pairs (Table 1) and can complement the mutator phenotype of an *fpg*$^-$ mutant of *E. coli*. Ablation of the enzyme function in *Ogg1* null mice leads to an accumulation of 8-oxoG in DNA associated with a modest ($\sim$threefold) increase in spontaneous mutation frequency (75, 99). Although no other 8-oxoG-DNA glycosylase activity was detected in *Ogg1* null extracts, there was still slow excision of the lesion in *Ogg1* null cells. This "backup" activity required the CSB gene product (114), which normally acts in transcription-coupled nucleotide excision repair of UV-induced pyrimidine dimers. CSB is apparently involved in OGG1-independent transcription-coupled repair of 8-oxoG from the transcribed strand of active genes (82). However, the backup activity in *Ogg1* null cells was entirely dependent on CSB but not transcription (114), in an unknown mechanism possibly related to the reduced incision at both 8-oxoG and 8-oxoA observed in CSB-deficient cells (26, 144). CSB-deficient cells are sensitive to oxidative DNA damage (25). Combined deficiency of OGG1 and CSB results in a pronounced accumulation of 8-oxoG in various tissues (114). However, in the absence of OGG1, the CSB-dependent activity together with dilution of the lesion by cell division, appeared to suffice to maintain a low steady state of the lesion in proliferating cells (75). Thus, accumulation of 8-oxoG in *Ogg1* null mice was tissue specific as well as age related and largely restricted to organs with high oxidative metabolism and low cell turnover, such as liver (113).

Unexpectedly, marked accumulation of potentially miscoding 8-oxoG lesions in the genome gave rise to a relatively low spontaneous mutation frequency and was not associated with an increase in tumorigenesis in the liver, even in old *Ogg1*$^{-/-}$ mice (>2 years) or after exposure to chronic oxidative stress (6, 75, 99). It is not known if the 8-oxoG residues in *Ogg1*$^{-/-}$ liver cells are randomly distributed, or if selective repair of open reading frames occurs, with harmless accumulation of the lesion in "junk" DNA. A confounding report of a statistically weak association of OGG1-deficiency with lung tumors, which was apparently suppressed by deletion of MTH1 despite an increased load of 8-oxoG, is difficult to evaluate, not least because the MTH-deficient mice remained tumor-free but were previously reported to develop lung and liver tumors (129). As (mis)replication is required for mutagenesis at 8-oxoG, stimulating proliferation in quiescent liver by partial

hepatectomy enhanced the mutation frequency in *Ogg1* null mice (5). It is possible that misincorporation opposite 8-oxoG in vivo is less than predicted by extrapolation from in vitro assays, and may be modulated by the particular specificities of nonprocessive error-prone polymerases in lesion bypass, as well as the sequence context of 8-oxoG lesions in target genes. Notably, the ~tenfold greater increase in G → T mutagenesis in OGG1-deficient yeast versus human cells may be due to the lack of the MutY DNA glycosylase in *S. cerevisiae* (142). The excision of adenine misincorporated opposite 8-oxoG by the MutY homologue (MYH; Table 1) could suppress mutagenesis due to 8-oxoG in proliferating mammalian tissues.

The MYH DNA Glycosylase: Excision of Adenine from Mutagenic 8-oxoG:A Mispairs

The antimutagenic potential of the MYH enzyme depends on its ability to discriminate and specifically excise adenine in an 8-oxoG:A mispair among the vast excess of normal A:T base pairs, but not excise cytosine opposite 8-oxoG, which would instead be promutagenic; the structural basis of how MYH achieves this has recently been clarified (42). Similarly to the *Ogg1* null, MYH-deficiency alone does not lead to a marked increase in tumorigenesis in mice (157) and there is a modest ~twofold increase in spontaneous mutation frequency in $Myh^{-/-}$ cells (62). Strikingly, $Ogg1^{-/-}Myh^{-/-}$ mice deficient in both these DNA glycosylases show a marked increase in tumor predisposition, most notably lung, ovary, lymphoma, and small intestine (157). Thus, accumulation of unrepaired 8-oxoG contributes to tumorigenesis with a synergy of OGG1- and MYH-deficiency paralleling the situation in bacteria; there is a ~100-fold increase in spontaneous G → T mutations in a $mutM^{-}mutY^{-}$ double mutant but a much weaker mutator phenotype for the respective single gene mutations alone (96, 157).

Unexpectedly, in the first example of a causal link between BER and human genetic disease, inherited mutations of h*MYH* were shown to be associated with colorectal tumors (4, 70, 134). Germline mutations of *MYH* lead to an increase in somatic G → T mutations of the familial adenomatous polyposis *APC* gene, with a remarkable "footprint" of *APC* mutations at GAA sequences (4, 70); GGT in codon 12 of the K-ras gene is also frequently targeted (87). Mutant *MYH* alleles affect both recognition and excision at A:8-oxoG in a sequence-dependent manner (20). Unlike MMR-deficient hereditary nonpolyposis colorectal cancer in humans, microsatellite instability is not seen in human MYH-associated polyposis, despite interaction of hMYH with MMR proteins (49). Furthermore, small-intestinal tumors are seen in MMR-defective mice (147), but only in MYH-deficient mice that also lack OGG1 (157). Although lung tumors predominate in $Myh^{-/-}$ $Ogg1^{-/-}$ mice, codon 12 of the murine K-ras gene is again mutated (in 75% of lung tumors) and malignancy is enhanced with combined heterozygosity of the *Msh2* mismatch repair gene (157). The mean age of tumor diagnosis is ~55 years in the limited number of cases so far described for the human MYH-associated disease

(87); *Myh* null mice clearly show no tumor predisposition at 17 months (157) but MYH-deficiency might yet prove to be associated with tumorigenesis beyond 18 months of age. Note that in contrast to the $Myh^{-/-}$ null mouse, the human MYH-associated disease is due to point mutations that affect catalytic activity; a mutant protein may still mediate protein:protein interactions or binding at 8-oxoG:A base pairs. Despite the redundancy of enzymes acting as antimutators at 8-oxoG, an alternative repair function(s) is clearly limiting in proliferating cells of the human lower gastrointestinal tract such that they are particularly sensitive to reduced MYH activity.

Tumorigenesis in $Ogg1^{-/-}$ $Myh^{-/-}$ mice has been correlated with increased levels of 8-oxoG residues in the DNA of lung and small intestine, consistent with accumulation of unrepaired oxidized bases in DNA contributing to neoplastic transformation (127). There was no build-up of the lesion in these tissues from mice deficient in either Ogg1 or Myh alone. As Myh does not excise 8-oxoG, Myh-deficiency would not be expected to affect levels of the lesion in DNA, only its mutagenic consequences. Thus, accumulation of the lesion in tissues from $Ogg1^{-/-}$ $Myh^{-/-}$ but not $Ogg1^{-/-}$ mice demonstrates that deficiency of Ogg1 affects the accumulation of the lesion but only in the absence of Myh. This would in turn indicate that 8-oxoG:A mispairs due to replication of lesions unrepaired by Ogg1 are processed by the concerted action of Myh and a second 8-oxoG-DNA glycosylase. Indeed, similarly to the cooperative interaction of Fpg and MutY in *E. coli*, *in vitro* studies with *Ogg1* null cells indicated the 8-oxoG:A base pair is repaired in a coordinated two-step reaction with sequential removal of A (presumably by Myh) and then 8-oxoG by a DNA glycosylase distinct from Ogg1 (24), possibly the Fpg homologue, NEIL1 (see below). Furthermore, the accumulation of 8-oxoG in liver DNA of both $Ogg1^{-/-}$ and $Myh^{-/-}$ mice, and the additive effect on age-dependent accumulation of the lesion in the $Ogg1^{-/-}$ $Myh^{-/-}$ double mutant, indicates that this other DNA glycosylase activity might be limiting in nonreplicating tissues. Interestingly, expression of NEIL1 is increased in S phase (61), and although it is highly expressed in the liver, an S phase-specific role may be compromised. However, liver-specific effects could simply be due to the high oxidative metabolism of this organ.

Incorporation and Repair of 8-oxoG from Contaminated dNTP Pools

The *MutT* encoded hydrolase prevents misincorporation of 8-oxoG by removing 8-oxo-dGTP from the dNTP pool and, in contrast to MutM and MutY, inactivation of MutT alone is sufficient to produce a greatly elevated (>100-fold) mutator phenotype in *E. coli*. The fact that dGTP invariably constitutes the smallest of the DNA precursor pools in cells may reflect its unique vulnerability to mutagenic oxidation, necessitating rapid use of dGTP after biosynthesis. 8-oxodGMP can be incorporated opposite C or A and, depending on the subsequent action of MutM and MutY, can cause not only G → T but also A → C transversions; it is the

latter class of mutation, presumably due to the action of MutY, that predominates in *MutT*$^{-}$ cells (138). Surprisingly, there was no significant increase in spontaneous mutagenesis in *Mth1* null mice (143), apparently due to the presence of a second MutT homologue MTH2 in mammalian cells (15), as well as a novel backup 8-oxo-dGDPase activity due to the *NUDT5* gene product (69). Nevertheless, *Mth1* null mice were reported to show increased tumorigenesis in lung, liver, and stomach (143). However, although the overall mutation frequency was not increased, the mutation spectrum in *Mth1*$^{-/-}$ cells was significantly shifted toward single-base frameshifts at mononucleotide runs, indicative of the action of MMR in the mutagenesis at oxidized bases (29). By modulating MTH expression, it has now been shown that the dNTP pool is a significant source of both steady-state and oxidant-induced 8-oxoG in DNA, and furthermore contributes to the genetic instability of MMR-defective cells (126).

Deficiency of OGG1 or an MMR component (MSH2 or MLH1) had similar, but independent and additive, effects on the accumulation of 8-oxoG in DNA in mammalian cells, indicating that MMR reduces the burden of 8-oxoG by a mechanism distinct from OGG1-initiated BER (21). However, both 8-oxoG:C and 8-oxoG:A are poor substrates for the human MMR proteins (80), whereas they are the preferred substrates for the OGG1 and MYH DNA glycosylases, respectively. OGG1 is inactive at 8-oxoG:A, consistent with the need to remove the oxidized guanine from the DNA template before replication generates an 8-oxoG:A mispair that should not be converted to T:A. Similarly, although MYH appears to be targeted to replication-associated repair via interaction with PCNA and the MMR machinery (57), its adenine-DNA glycosylase activity would actually be promutagenic at A:8-oxoG pairs arising through misincorporation of 8-oxo-dGMP. The so-called OGG2 enzyme has been identified as a second 8-oxoG-DNA glycosylase in yeast with a preference for 8-oxoG:A rather than 8-oxoG:C base pairs, and yeast *ogg2*$^{-}$ cells show elevated A:T $\rightarrow$ C:G rather than G:C $\rightarrow$ T:A mutations, consistent with mutagenesis at misincorporated 8-oxoG (14). A distinct DNA glycosylase with the specificity of OGG2 would appear to be best suited to dealing with 8-oxoG:A base pairs arising through misincorporation from 8-oxo-dGTP but conversely would be promutagenic where dAMP had been incorporated opposite unrepaired 8-oxoG. An OGG2 activity has been described in mammalian cells (60) but, puzzlingly, an ORF has still to be identified and its possible relationship to NEIL1 clarified (61).

Clearly, there is much we do not yet understand about the repair of 8-oxoG:A pairs arising from oxidation of guanine in DNA versus the precursor pool, and its coordination with replication. However, repair of 8-oxoG in mammalian cells appears to depend on several distinct DNA glycosylases, as shown in Figure 2. Furthermore, human MTH1 but not *E. coli* MutT hydrolyzes 2-hydroxy-dATP (43), and MYH but not MutY can excise 2-OH-A from DNA (109). 2-OH-dAMP is particularly promiscuous in its base-pairing properties and, unlike 8-oxoA incorporated into DNA, 2-OH-A is highly mutagenic (71, 155). Thus, it appears that both the incorporation of 2-OH-dAMP and 8-oxo-dGMP account for the

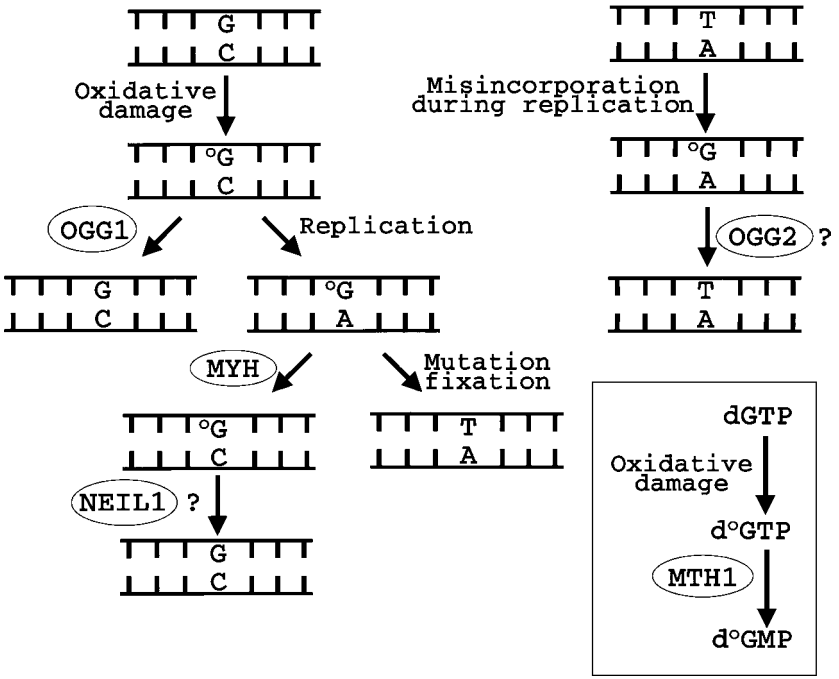

Figure 2 Counteracting the effects of 8-hydroxyguanine in the mammalian genome. The OGG1 DNA glycosylase excises oxidized guanine (°G) from °G:C base pairs. If unrepaired, °G can base-pair ambiguously with either C or A during DNA replication, giving rise to G → T transversions. The MYH DNA glycosylase excises A from such °G:A mispairs arising from inaccurate replication of unrepaired °G:C; correct insertion of C then regenerates a °G:C pair that is a substrate for OGG1, although a distinct DNA glycosylase, possibly NEIL1, may act in concert with MYH. Oxidation of dGTP can give rise to d°GTP, which, despite the sanitization of the nucleotide pool by the MTH1 hydrolase, can to some extent be misincorporated opposite A. In this instance, removal of the template A by MYH would be promutagenic, OGG1 does not act on °G:A, and instead, excision of °G from such a °G:A mispair must be achieved by a distinct enzyme. The "OGG2" DNA glycosylase activity is specific for °G:A but has not yet been assigned to an ORF.

genetic instability of MMR-defective cells and the ability of MTH1 to attenuate the mutator phenotype (126).

Oxidative Stress, Aging, and Cancer

In an expansive literature, 8-oxoG has been implicated in the etiology of degenerative diseases, aging, and cancer, prompting investigations into the use of antioxidants in dietary supplements and adjunct cancer therapy. In particular,

although nuclear DNA glycosylases acting on oxidative base damage are also sorted to the mitochondria (Table 1), mutagenesis due to constant oxidative stress might compromise mitochondrial integrity and cellular function with age. However, attributions of the biological consequences of 8-oxoG in DNA have often been based on inaccurate measurements of the lesion in vivo or small variations in repair capacity measured by in vitro assays. It is clear from the genetic models discussed here that there is a far from straightforward relationship between the incidence of a lesion, mutagenesis, and tumor formation, which is modulated by many factors including cellular environment, proliferative state, and the relative contributions of the various relevant repair activities. It would thus seem premature to extrapolate data from a limited number of nonsmall-cell lung cancer patients, with a <20% mean reduction in incision at 8-oxoG:C in protein extracts of peripheral blood cells, as a risk factor for tumorigenesis in the lung epithelium due to smoking (118). Moreover, differences in oxygen sensitivity of cells in culture (116) do not appear to correlate with observed cancer rates in mice versus humans deficient specifically in the repair of oxidative DNA base damage.

Oxidized Pyrimidine Bases in DNA

Although purines are particularly sensitive to oxidation, with 8-oxoG and 2-OH-A being the most relevant to mutagenesis in mammalian cells, a wide spectrum of oxidized pyrimidine derivatives is also formed. Thymine glycol (Tg) and 5,6-dihydrouracil (DHU) are not miscoding but can block DNA and RNA polymerases. However, 5-hydroxycytosine, 5-hydroxyuracil, and uracil glycol pair preferentially with A to cause C → T transitions (76). C → T rather than G → T is the most abundant change generated by oxidative damage as well as the most common base substitution in aerobic organisms. Oxidized pyrimidines are excised by a second major mammalian DNA glycosylase specific for oxidized bases, NTH1 (Table 1), which shares common structural elements and reaction mechanism with the OGG1 8-oxoG-DNA glycosylase. Ablation of NTH function did not have any obvious phenotypic consequences in the mouse (32, 108, 140). Like 8-oxoG, Tg can be repaired by transcription-coupled repair (TCR) dependent on the products of the *CS-B* and *XP-G* Cockayne syndrome genes (22). In addition, 8-oxoG is repaired in *Ogg1* null cells in a nontranscription-coupled pathway mediated by CSB (114), whereas XPG can directly modulate NTH1-initiated BER of Tg (74) and, of the factors involved in TCR of oxidative damage, CSB and XPG uniquely also slowed lesion removal in nontranscribed sequences (82). In the *Ogg1* null mouse, such repair, together with the antimutator effect of MYH, sufficed as a backup to prevent lesion accumulation and mutagenesis in most organs, with the notable exception of liver (75). Any in vivo relevance of the observed endonucleolytic nicking 5′ of polymerase-blocking oxidized base lesions in mammalian cell extracts has yet to be assessed (68). In the *Nth1* null, similarly to the *Ung* null mice (105), backup repair was effected by distinct DNA glycosylase activities, only readily detected in cell extracts devoid of the major NTH gene product (32, 108, 140).

To date, at least two novel DNA glycosylases acting on oxidized pyrimidines have been described in *Nth* null cells, and a third identified from the human genome sequence databases (Table 1). These so-called Nei-like or NEIL enzymes belong to a new glycosylase superfamily for the repair of oxidative damage, defined by the Fpg/MutM enzyme of *E. coli* and its paralogue, Nei, also called endonuclease VIII (for recent reviews, see 59, 146). NEIL1 (9, 61, 100, 139) and NEIL2 (58) have overlapping specificity with both NTH and OGG1, acting on 8-oxoG as well as oxidized pyrimidines, although published reports of their precise substrate specificity and expression patterns are somewhat contradictory (146). An S phase-specific increase in NEIL1 expression suggests an involvement in replication-associated repair of oxidized bases (61). None of these recently identified enzymes would appear to correspond to a hypothetical DNA glycosylase activity that might excise modified G residues opposite deaminated cytosine to account for the A:T phase of somatic hypermutation in immunoglobulin genes; OGG1 is not involved in this process (153).

Cells in which NEIL1 expression was significantly suppressed by short hairpin RNA-mediated RNA interference (RNAi) were sensitive to low levels of ionizing radiation, apparently due to inability to excise a stereoisomer of Tg or fapyA (an adenine-derived formamidopyrimidine), which are not excised by NTH1 or OGG1 (124). Furthermore, suppression of NEIL1 was not only carried out in cultured cells but, in an exciting development, by stable germline transmission of RNAi in the mouse, as the first example of heritable gene silencing (18). This has important applications as a complement to standard knock-out methodologies for tissue-specific, inducible, or developmental regulation of expression, and as a rapid means of assessing the consequences of suppressing one or more gene functions in vivo (98). Although the plethora of DNA glycosylases of overlapping specificity continue to be best tackled by conventional knock-outs, germline transmission of RNAi might allow the generation of viable hypomorphic mutants to study the later essential steps of BER.

DNA ALKYLATION DAMAGE

Alkylating agents such as methyl methanesulphonate (MMS) as well as the intracellular methyl group donor S-adenosylmethionine generate covalent modifications at ring nitrogen residues of DNA bases, in particular 7-methylguanine and 3-methyladenine (3-meA). Whereas 7-methylguanine appears to be a harmless alteration, 3-meA is a cytotoxic lesion that blocks both replication and transcription due to the aberrant methyl group protruding into the minor groove of the double helix. The apparently universal occurrence of DNA repair of the 3-meA lesion among living organisms most likely reflects the fact that this is a common endogenous lesion. Mammalian cells have a single 3-meA-DNA glycosylase (MPG), which catalyzes the liberation of the alkylated base but can also remove 3-ethyladenine, deaminated adenine (miscoding hypoxanthine), and the $1,N^6$-ethenoadenine lesion (ethenoA) generated in DNA as a consequence of lipid peroxidation (Table 1;

for recent reviews, see 63, 130). However, in several bacteria, the orthologous enzyme lacks the latter two accessory activities, whereas the DNA glycosylase activity for 3-meA appears generally distributed, suggesting that the release of the methylated base is the main function of the enzyme. This is also in agreement with the physiological properties of *alkA*$^{-}$ DNA glycosylase mutants of *E. coli* unable to excise 3-meA, which are hypersensitive to killing by MMS but do not exhibit a spontaneous mutator phenotype.

The MPG 3-meA-DNA Glycosylase

Initial studies on *Mpg*$^{-/-}$ null murine embryonic stem (ES) cells showed that they were much more sensitive than control ES cells to killing by MMS and other alkylating agents, while they were normally resistant to UV light (35). These results seemed in good agreement with studies on repair-deficient microbial mutants. However, two groups then proceeded to construct *Mpg* null mice; these animals were viable, fertile, and had a normal life span, and fibroblasts derived from them were only moderately sensitive to alkylating agents, less so than had been expected from results with microbial model systems or ES cells (33, 36). No backup DNA glycosylase activity excising 3-meA has been detected in *Mpg* null cells. In agreement with the biochemical properties of the Mpg enzyme, the null mice were also deficient in clearing hypoxanthine and ethenoA from their DNA (36, 52). The mild phenotype of the *Mpg* null mice suggests the existence of an alternative repair or tolerance mechanism for the common DNA lesion, 3-meA. One possibility is that a specific translesion DNA polymerase can copy 3-meA residues in DNA, in a fashion analogous to the accurate bypass of cyclobutane thymine dimers by polη (93). Unfortunately, the chemical lability of the 3-meA-deoxyribose glycosyl bond has precluded the synthesis of oligodeoxyribonucleotides containing this lesion, which would be required for in vitro assays of translesion DNA synthesis. Although most *Mpg* null cells become sensitized to alkylating agents, myeloid cells from *Mpg*$^{-/-}$ mice are more resistant, and it has been proposed that imbalance between various BER enzymes in certain cells and tissues could make it deleterious to initiate normal repair (125). In support of this model, overexpression of MPG in nuclei or mitochondria can enhance MMS-induced cytotoxicity (40). However, recent results with RNAi-induced knockdown of the MPG enzyme in HeLa cells have shown substantial sensitization of the repair-deficient cells to killing by MMS (J. Paik, T. Lindahl, B. Sedgwick, manuscript in preparation).

Reversal of Alkylation Damage

The highly mutagenic alkylation lesion O^6-methylguanine (O^6-meG) occurs as a consequence of exposure of DNA to N-methylnitrosourea (MNU), and it is probably generated in small amounts endogenously by nitrosation of amines or amides (131). The ambiguously coding O^6-meG can pair with either T or C during replication. The lesion is repaired by a unique suicide mechanism in which the methyl group is transferred to a specific cysteine residue in the repair enzyme

O^6-meG-DNA methyltransferase (MGMT), which is consequently inactivated. The *MGMT* gene belongs to a small group of tumor-suppressor genes, also including the mismatch repair gene *MLH1*, which are under epigenetic control and are readily switched off by enzymatic hypermethylation of C residues in promoter CpG sequences (37).

Mgmt$^{-/-}$ null mice have been constructed (46, 73, 128). These mice appeared essentially normal but exhibited slightly retarded growth and low body weight. They are hypersensitive to alkylating agents, being susceptible to the lethal effects of MNU at 25-fold lower doses than are controls. Low sublethal doses of MNU, which were not detectably carcinogenic in control Mgmt-proficient mice, produced thymic lymphomas and lung adenomas in the *Mgmt* null mice. Moreover, *Mgmt* null ES cells are 100-fold more sensitive to killing by MNU than are control ES cells (128). There is no known backup mechanism for repair of O^6-meG in mammalian cells, but loss of the mismatch repair system causes increased resistance to this lesion in cells that do not express the Mgmt function (13). One possible explanation is that abortive processing of O^6-meG by mismatch repair is a cytotoxic event. *Mgmt*$^{-/-}$ *Mlh1*$^{+/-}$ mice are relatively resistant to killing by MNU but remain susceptible to tumor induction by the alkylating agent (73). The *Mgmt* expression status of human tumors can clearly be relevant with regard to chemotherapy using simple alkylating agents (38).

A different mode of reversal of cytotoxic DNA alkylation damage has recently been shown to be due to the mammalian DNA dioxygenases ABH2 and ABH3 (1, 28). These enzymes catalyze oxidative demethylation of 1-methyladenine and 3-methylcytosine in DNA, lesions generated in single-stranded stretches of DNA that then prevent normal base pairing. They can also slowly demethylate the minor lesion, 3-methylthymine (P. Koivisto, P. Robins, T. Lindahl & B. Sedgwick, submitted). The demethylation reaction requires the unusual cofactors Fe^{2+} and α-ketoglutarate, and the methyl moiety is released as formaldehyde with regeneration of the unmodified base residue in situ. Loss of the *AlkB* orthologue of *E. coli* renders cells hypersensitive to killing by MMS, and ongoing studies in different laboratories aim to establish whether this is also the case for mammalian cells deficient in ABH2 and/or ABH3. These two mammalian enzymes are localized to cell nuclei; ABH2 shows some preference for 1-methyladenine and ABH3 for 3-methylcytosine, and this simple difference in substrate specificity may explain the presence of two similar enzymes. They could also serve as backups for each other in repair of cytotoxic damage. In addition, ABH3 shows weak ability to demethylate MMS-treated RNA (1), but it is not known if this biochemical function is of any physiological relevance in cellular metabolism.

REPAIR OF ABASIC SITES IN DNA

DNA apurinic/apyrimidinic (AP) sites occur as a consequence of nonenzymatic hydrolysis of base-sugar bonds in DNA and are also generated by DNA glycosylases as reaction intermediates in the BER pathway, in particular due to removal

of uracil from misincorporated dUMP residues in DNA (51). The total load of AP sites in a mammalian cell from these sources is over 10,000 per day and is a challenge to the cellular base excision-repair system (85). Adenine and guanine are released from DNA at similar rates by hydrolytic depurination, leaving chemically identical and indistinguishable sugar-phosphate residues at the abasic sites. Thus, translesion synthesis by a DNA polymerase at AP sites would not distinguish if a dAMP or dGMP residue had been present previously, so would be a highly error-prone and mutagenic strategy for repair of a common lesion and probably does not occur to a significant extent in vivo. Moreover, due to the abundance and high turnover number of the mammalian AP endonuclease (APE1), the steady-state level of AP sites in the mammalian genome is very low and cannot be detected by current methodology (8). Scattered reports on the detection of large numbers of AP sites in mammalian cells are likely to be due to experimental artifacts analogous to spurious claims in the early 1990s of the presence of very high levels of DNA 8-oxoG in vivo. APE1 is a 35-kDa protein with several residues in the C-terminal two thirds of the protein shown by site-specific mutagenesis to be required for incision of AP sites in DNA. The 3D structure of the enzyme bound to its DNA substrate has been solved (for review, see 151). Attempts to construct *Ape1* null mice have demonstrated an early embryonic lethal phenotype (90, 95, 156). These results appear consistent with the finding that mutants of *Saccharomyces cerevisiae* unable to repair DNA abasic sites are inviable (50). Microorganisms with reduced levels of AP endonuclease activity are hypersensitive to single alkylating agents such as MMS because of the greatly increased occurrence of AP sites when 7-methylguanine and 3-meA residues are removed from DNA. Similarly, down-regulation of APE1 with antisense oligonucleotides conveyed increased sensitivity to MMS as well as therapeutically used alkylating agents in a human glioma cell line (135).

The N-terminal region of APE1 appears to have a separate role in aiding reduction of oxidized cysteine residues in the Fos and Jun transcription factors (112, 151); only the reduced form of the Fos/Jun complex binds to DNA. A critical Cys residue (Cys 64 in mice, Cys 65 in humans) in APE1 was initially proposed as the key redox regulator. However, this particular residue is deeply buried in the 3D structure of the protein, making it an unlikely candidate for an active redox function. Recently, mice in which Cys 64 had been exchanged for Ala were constructed; in contrast to *Ape1* null mice, these animals were viable and had a normal life span. Possibly APE1 serves in a more indirect role in conjunction with a separate redox factor to maintain the reduced states of Fos and Jun. A small-molecule inhibitor of the redox activity of APE1 interfered with Fos/Jun-dependent transcription in human lung epithelial cells and was suggested as a useful therapeutic agent in the treatment of asthma (102). It now seems important to better define the molecular basis of the redox function of APE1.

The pivotal role of APE1 in the processing of abasic sites is followed in the BER pathway by DNA gap-filling, excision of the abasic sugar-phosphate residue, and ligation to restore the intact DNA structure (Figure 3). The short-patch versus long-patch mode of BER (Figure 3) has been studied in several laboratories, but

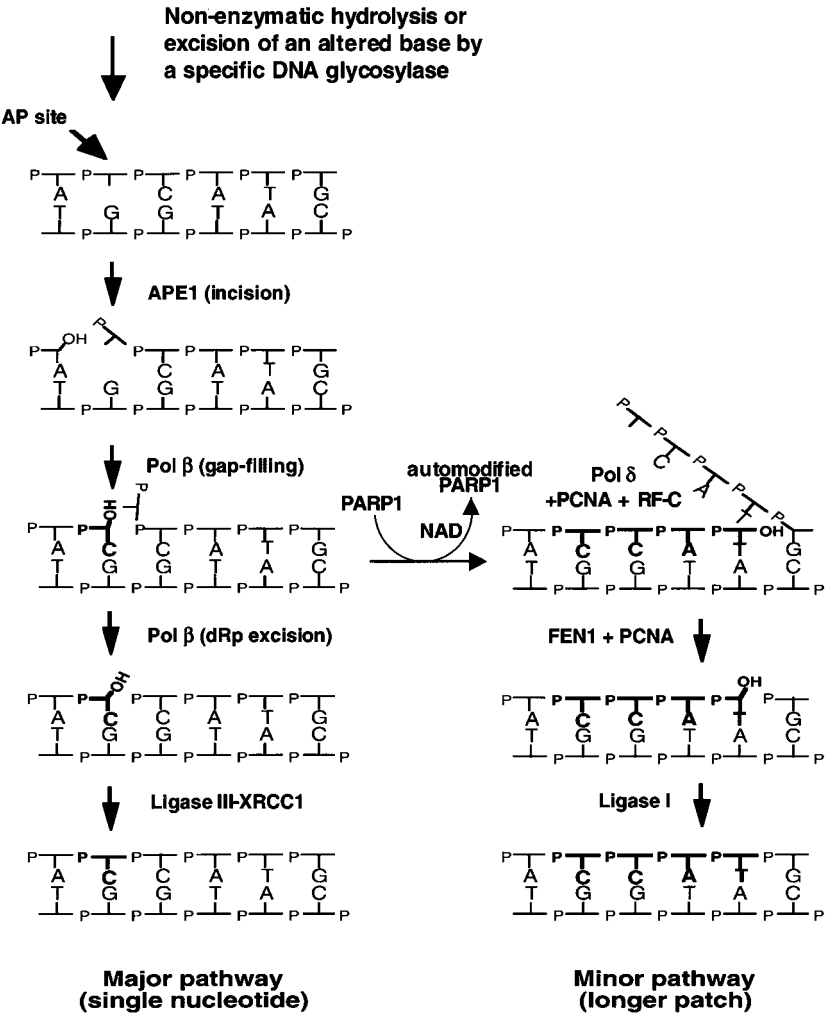

Figure 3 Postexcision catalytic steps of the base excision repair pathway. AP sites, arising spontaneously or following excision of an aberrant base by a DNA glycosylase, are incised 5′ of the AP site by the APE1 endonuclease. Filling-in of one nucleotide is achieved by polβ. In the major pathway, subsequent excision of the 5′ terminal deoxyribosephosphate (dRp) residue by the lyase domain of polβ and ligation by the DNA ligaseIII-XRCC1 heterodimer generates a single nucleotide repair patch. If polymerisation/dRp excision by polβ is blocked, a longer repair patch is generated by polδ, with cleavage of a displaced 5′ flap by FEN1 prior to ligation by DNA ligase I, in a PCNA-dependent minor pathway. Persistence of the transient single-strand interruption, for example if polβ were stalled, can serve to recruit PARP1 and activate poly(ADP-ribose) synthesis, which may indirectly facilitate repair.

two minor alternative strategies of repair have been described recently. In addition to the efficient recognition of abasic sites in double-stranded DNA, some AP endonucleases, including human APE1, can incise DNA directly at certain oxidized bases, such as α-deoxyadenosine and fragmented pyrimidines; this strategy obviates the need for a DNA glycosylase to remove the damaged base (48, 65).

DNA GAP-FILLING AND REJOINING DURING BASE EXCISION-REPAIR

The latter steps of the BER pathway have been investigated in detail and will only be briefly described here. Mammalian cell nuclei contain the 39-kDa DNA polymerase β (Polβ), which accounts for repair synthesis during BER (152). The protein is comprised of a 31-kDa polymerase domain that efficiently fills in single nucleotide gaps in DNA, and a separate 8-kDa dRP lyase domain for excision of the sugar-phosphate residue at the incised site. The latter activity is due to a highly basic N-terminal domain without a direct counterpart in the great majority of DNA polymerases. Thus, several low-fidelity polymerases that catalyze translesion synthesis, including Polι, Polη, and Polκ, appear to be excluded from a role in BER by their inability to remove the 5′-terminal sugar-phosphate residue, which also prevents single-nucleotide gap-filling (Figure 3). This arrangement makes physiological sense, because base substitution error rates by Polβ during BER are only about 10^{-4}, in spite of the lack of a Polβ editing function (94); gap-filling by a polymerase of distinctly lower fidelity than Polβ would be more promutagenic (53).

Attempts to construct Polβ null mice revealed an embryonic lethal phenotype (136). However, Polβ null fibroblast cultures could be established from midgestation embryos. These cells are hypersensitive to MMS, demonstrating that the alternative long-patch repair pathway utilizing replication factors (Figure 3) cannot completely substitute for the main short-patch pathway, at least at high levels of DNA damage (137). Moreover, extracts of Polβ null cells were impaired in their ability to complete the BER reaction initiated on uracil-containing DNA. When null cells were complemented by stable transfection with a full-length Polβ minigene, they exhibited normal resistance to MMS. Separate constructs with either the polymerase domain or the dRP lyase domain carrying an inactivating single-site mutation established that the ability to excise dRP from the incised DNA site was the function necessary for damage resistance, whereas other polymerases could substitute in the single-nucleotide gap-filling reaction (137).

Most investigations of BER in vitro have employed DNA molecules with one or several site-specifically altered base residues as substrates for particular DNA glycosylases. Recently, such in vitro studies have been extended to chromatin using reconstituted nucleosomes. The UNG and SMUG1 DNA glycosylases, APE1, and DNA ligases I and III have been investigated and they all function relatively efficiently with nucleosome substrates. Typically, a three- to tenfold decrease in rate of repair was observed, and damaged base residues facing either the histone octamer surface or the solvent were processed almost equally efficiently (10, 19,

104). Similar results were obtained with budding yeast in vivo, where all alkylated bases in nucleosomes were repaired but at a retarded rate compared with linker regions (84). In contrast, Polβ exhibited marked variation of activity at different positions in the nucleosome, and a small minority of sites appeared almost refractory to repair synthesis. One possibility is that Polβ needs to induce a 90° kink in DNA on binding both the 3′ primer terminus and the adjacent 5′ dRP moiety during BER, and this distortion would exact a high energetic cost at certain positions in the nucleosome. It seems unlikely that extensive chromatin remodeling by specific protein factors would be required to solve this problem. Instead, local resolution of the stalled repair intermediate might be promoted by an accessory BER factor. It has been proposed that poly (ADP-ribose) polymerase I (PARP1) could serve such a function (81); PARP1 binds to single-strand interruptions in DNA, which triggers rapid automodification of the enzyme and its subsequent release. However, in the reconstituted system, addition of PARP1 in the presence of its cofactor NAD failed to relieve the inhibition of Polβ activity by the nucleosome structure (104). Nevertheless, it seems possible that PARP1 could modulate one of the later reaction steps in BER, e.g., the switch between the short-patch versus long-patch pathway (Figure 3), or that it has a more relevant role in facilitating BER of higher-order chromatin structures. BER proceeds efficiently in extracts of *Parp-1* null cells, so there is no essential role for PARP-1 in this repair pathway, but the observation that PARP-1 activity appears to be required for repair of 8-oxoG lesions in Polβ null cells (83) suggests a role for PARP-1 in modulating the later stages of BER, possibly recruiting a component(s) of the minor pathway.

In the final steps of the short-patch BER pathway, the scaffold protein XRCC1 (16), which lacks catalytic activity, binds both Polβ and DNA ligase III (Lig3), and presumably brings them together during the repair reaction; Polβ and Lig3 do not interact directly. The XRCC1/Lig3 interaction employs BRCT motifs in both proteins, and Lig3 occurs as a heterodimer intracellularly. *Lig3* null cells do not seem to have been constructed, but since Lig3 is the only ligase found in mitochondria (79, 119), which contain circular DNA, deletion of the *Lig3* gene would most likely be a lethal event. *Xrcc1* null mice suffer early lethality at day 6.5 during embryogenesis, with increased DNA breakage and apoptosis (141). Chinese hamster ovary cell mutants defective in Xrcc1 are available; these cells exhibit tenfold increased susceptibility to MMS and similarly increased levels of spontaneous sister chromatid exchanges. Because Lig3 exists as a heterodimer with XRCC1, levels of this ligase are greatly decreased in the *Xrcc1* mutant cells, and the reduced level of Lig3 is the main reason for the inadequate BER in these cells (17).

CONCLUSION

In considering the genetic consequences and universality of endogenous DNA base damage, the evolutionary conservation of key enzymes and the marked mutator phenotypes of microbial repair-deficient mutants predicted that BER enzymes would serve as antimutators in mammalian cells, especially in regard to tumor

suppression (89). Knock-out mouse models have instead demonstrated rather subtle phenotypes and often unmasked previously unidentified factors; this elaboration is due not so much to gene duplication and functional redundancy, but rather to the specialization of multiple gene products that efficiently interface with replication, transcription, and DNA processing in the immune system. Thus, the consequences of DNA damage depend not only on the level and nature of the lesion but on its context in terms of cell type, cell cycle stage, and gene expression, and modulate differences in tumor spectrum and cancer susceptibility, even between mice and man (123). The genetic consequences of ablating a particular BER enzyme may be difficult to predict by extrapolation from microbial mutants, with recent evolutionary loss of the TDG and MYH DNA glycosylases making budding yeast a particularly wayward model. However, a genome-wide screen in *S. cerevisiae* for genes that suppress the accumulation of mutations identified oxidized guanine and uracil in DNA as major contributors to spontaneous mutagenesis (64), and if unrepaired, these endogenous DNA base lesions clearly contribute to neoplastic transformation of mammalian cells (4, 70, 97, 106, 127, 134, 154, 157). When actual levels of unrepaired spontaneous DNA base damage are carefully evaluated for their biological consequences, in compound mutant strains, even yeast exhibit phenotypic properties similar to those of cancer cells (39). So the base-excision repair field has in many ways come full circle and we might think again of BER in the context of tumorigenesis and potential cancer therapeutic targets (88), but now from our knowledge of mammalian rather than microbial systems. Polymorphisms in human BER genes that modulate susceptibility to endogenous DNA damage might be a promising area for future molecular epidemiological studies.

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