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COMMENTARY

Base excision repair in a network of defence and tolerance

Hilde Nilsen[1] and Hans E.Krokan[2]

Institute of Cancer Research and Molecular Biology, Norwegian University of Science and Technology, N-7489 Trondheim, Norway

[1]Present address: Imperial Cancer Research Fund, Clare Hall Laboratories, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3LD, UK

[2]To whom correspondence should be addressed
Email: hans.krokan@medisin.ntnu.no

Introduction

Survival of a species depends on balanced generation of genetic variation but at the same time on the protection of the genome from changes that cause disease and fitness reduction. DNA repair pathways limit mutations but do not totally eliminate them. In fact, some DNA repair pathways are error prone. DNA repair thus has a central function not only in protecting the genome, but also in the generation of genetic diversity. Expression of DNA repair proteins is subject to a delicate balance where both too few and too many of a type may result in increased cytotoxicity and/or mutation (1–4). DNA repair is integrated with transcription, replication, cell cycle control and apoptosis in complex networks (5), a full discussion of which is beyond the scope of this article, and our abilities. While DNA repair is an ancient and conserved defence mechanism, various pathways, and additions to basic pathways, have evolved during different time periods (6). Furthermore, the pathways have overlapping specificities and function as back-up systems for each other. Thus, cytotoxic and mutagenic abasic sites (AP sites) may be dealt with by the relatively accurate mechanisms nucleotide excision repair (NER) (7,8), base excision repair (BER) and recombination repair, as well as by highly error-prone translesion DNA synthesis (TLS) (Figure 1; 9). In addition, different enzymes may substitute for each other in a specific pathway, or variants of a pathway. Conditions that govern the selection of a pathway in each case are not well understood. Furthermore, components of DNA repair systems, such as error-prone DNA polymerases, may also cause untargeted mutations at sites where no apparent damage is present. In this commentary we will address functional aspects of BER and different complementary functions in the light of recent discoveries with regard to mutations, cancer, evolution and ageing.

In summary, recent results from different DNA glycosylase-deficient mice have failed to demonstrate strongly increased mutation rates, increased cancer frequencies or other severely altered phenotypes. This may be due to overlap in functions between DNA glycosylases, as well as repair by alternative pathways. There is evidence that the BER pathway is essential, since deficiencies in the common steps downstream of the DNA glycosylase step are embryonic lethal (10). However, these proteins may have additional functions in development and the evidence is therefore not yet conclusive. The minor effects of deficiencies in DNA glycosylases may seem surprising, given the strong conservation of these enzymes. Possibly, deficiency of each of the enzymes may reduce fitness to an extent that, although small, results in negative selection such that glycosylase mutations are very infrequently observed in populations. We speculate that DNA glycosylases have as one major function to protect the long-term integrity of the genome over several generations.

Sources of damage to the genome

DNA is inherently unstable and decays even in the absence of exogenous challenge from DNA-reactive chemicals and radiation (11,12). By convention, DNA damage has been categorized as being of spontaneous origin or environmentally induced. However, in some cases this separation is not distinct. For example, polyunsaturated fatty acids, giving rise to DNA-

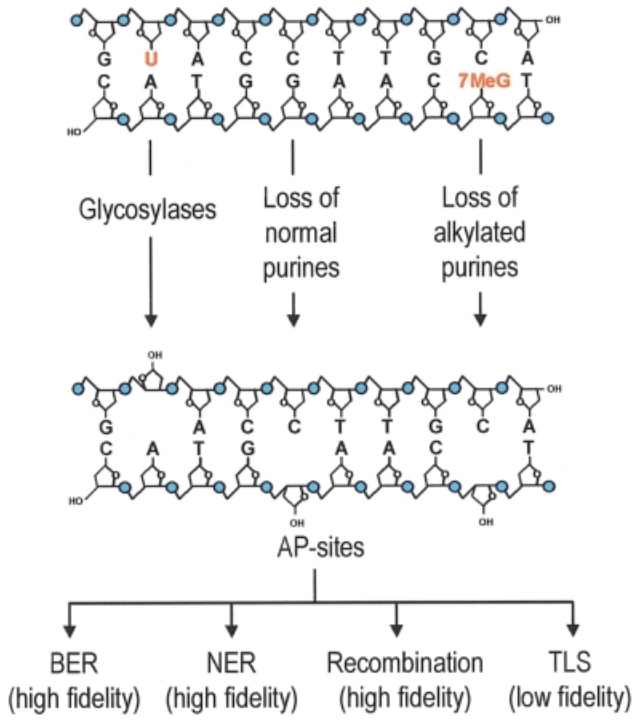

Fig. 1. AP sites are mainly generated by DNA glycosylases and depurination. Certain major alkylation products (e.g. 7-methylguanine and 3-methyladenine) increase rates of hydrolytic depurination by several orders of magnitude. Essentially error-free repair of AP sites is carried out by BER, NER and recombination repair, whereas error-prone DNA polymerases may synthesize DNA over the baseless site. This type of complexity in handling of damage may be more common than thought previously.

Abbreviations: AP sites, abasic sites; BER, base excision repair; dRP, deoxyribose 5-phosphate; HNPCC, hereditary non-polyposis colon cancer; MMR, mismatch repair; MPG, methylpurine-DNA glycosylase; NER, nucleotide excision repair; PCNA, proliferating cell nuclear antigen; Pol, DNA polymerase; RFC, replication factor C; TDG, thymine/uracil mismatch glycosylase; TLS, translesion DNA synthesis; UDG, uracil-DNA glycosylase; UNG, uracil-DNA glycosylase.

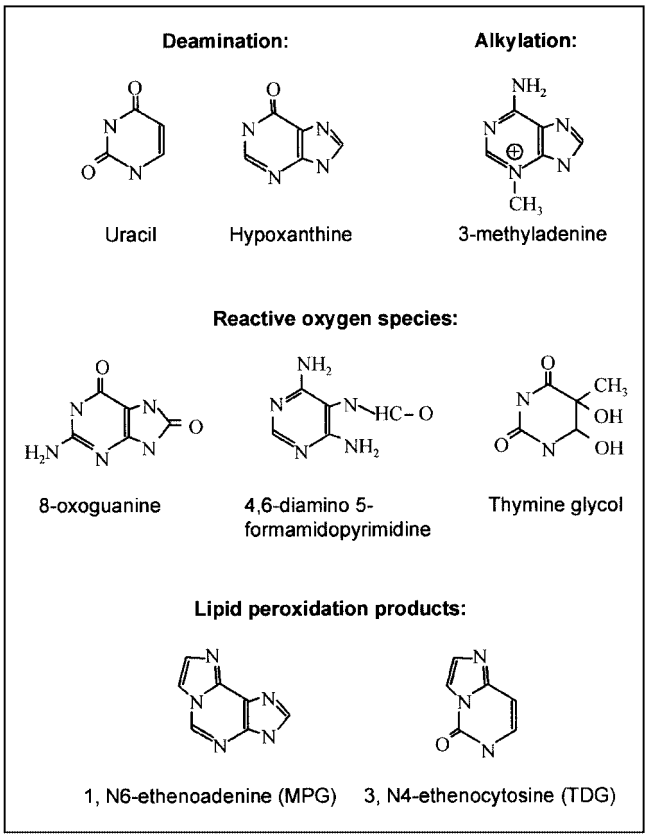

Fig. 2. Examples of DNA bases damaged by endogenous agents. In almost all cases exogenous agents may also produce the class of damage indicated.

reactive lipid peroxidation products, are natural components of cellular membranes, but their amount in the cell depends on the quantity ingested. In addition, the formation of lipid peroxidation products may be modulated by ingested antioxidants. BER is generally believed to constitute the primary defence against lesions formed by endogenous DNA-damaging agents. Figure 2 shows some examples of major base modifications repaired by BER. Water and reactive oxygen species are probably the main endogenous reactants damaging DNA in cells. Water attacks electrophilic centres to generate a number of hydrolysis products. The quantitatively most important arise from hydrolysis of the *N*-glycosidic bond of purines, resulting in loss of ~10 000 purines/human genome/day (13,14). Pyrimidines are lost at 5% of the rate of purines (11). The resulting AP sites are highly cytotoxic and mutagenic and, if left unrepaired, may rearrange to generate single-strand breaks. Hydrolytic deamination of exocyclic amino groups in cytosine and 5-methylcytosine results in generation of uracil and thymine, respectively, both mismatched to guanine. Similarly, adenine may deaminate to hypoxanthine and guanine to xanthine. The estimated rate of cytosine deamination ranges from 100 to 500 events/cell/day and will result in C→T transition mutations unless corrected by repair processes (15–17).

Oxidation of DNA by reactive oxygen species is the other major source of spontaneous DNA damage. Reactive oxygen species are formed as by-products from oxidative metabolism and from γ-irradiation. DNA-reactive fatty acid radicals, aldehydes and other compounds are formed during lipid peroxidation and cause various types of damage, including etheno adducts of pyrimidines and purines. Oxidation of bases occurs mainly at electrophilic carbon centres. Major products are the highly mutagenic 8-hydroxyguanine and ring-opened forms of purines (formamidopyrimidines) and pyrimidine glycols, all of which are cytotoxic and mutagenic (11,12,18–20). Oxidative attack on the DNA backbone may result in the generation of single-strand breaks, which are recombinogenic, cytotoxic and mutagenic. The rate of formation and endogenous levels of oxidative DNA damage in living cells are difficult to measure, but it is clear that oxidation reactions collectively constitute major sources of DNA damage (12,21). Several endogenous products of normal metabolism have been demonstrated or suspected to damage DNA. In addition to lipid peroxidation products and oxidants, these include alkylating agents, oestrogens, chlorinating agents, reactive nitrogen species, glycoxidation products, heme precursors and amino acids (22). The possible significance of all these agents, as well as of the long list of environmental agents (23), is beyond the scope of the present paper.

Base excision repair

The present review will concentrate on functional aspects of BER. For discussions of more comprehensive biochemical and structural aspects of DNA glycosylases, other recent reviews should be consulted (24–31). The mechanism of BER involves a damage-specific step performed by one of the DNA glycosylases (Table I) followed by damage-general steps involving the sequential action of different proteins which correct DNA by template-directed insertion of one or a few nucleotides, starting at the damaged site (Figure 3). Damage recognition and binding vary with different enzymes, but base flipping prior to excision appears to be a common trait. For monofunctional glycosylases hydrolysis of the glycosylic bond involves nucleophilic attack by an activated water molecule, in turn activated by the caboxyl side chain of an Asp residue. For bifunctional DNA glycosylases the nucleophile is an activated amino group of a Lys residue activated by an Asp residue. The amino group forms a Schiff base with C1' followed by β-elimination to cleave the DNA strand at the 3′-side of deoxyribose (lyase activity). The human monofunctional glycosylases include uracil-DNA glycosylase (UNG) (32–34), thymine/uracil mismatch glycosylase (TDG) (35), SMUG1 (36) and MBD4 (37,38), which have uracil as a common substrate but have generally narrow substrate specificities. In contrast, the monofunctional methylpurine-DNA glycosylase (MPG), also referred to as ANPG or AAG (39,40), removes a wide range of damaged purine bases and, surprisingly, may also remove undamaged purines at low rates (2). The bifunctional glycosylases include hOGG1 (41), which removes oxidized purines, and hNTH1 (42,43), which removes oxidized pyrimidines. The human MutY homologue, hMYH1 (44), removes adenine from 8-oxoguanine/A mismatches and harbours only weak, if any, lyase activity (45). The major AP endonuclease, HAP1 (also referred to as APE1 and Ref1), which is at the intersection of all the BER pathways, introduces a nick in the backbone 5′ of the AP site, thereby generating a 3′-OH end as a substrate for DNA polymerases (12). When BER is initiated by bifunctional glycosylases, the additional action of HAP1 results in a 1 nt gap flanked by 3′-OH and 5′-phosphate ends (26,46). Short-patch predominates when BER is initiated by the bifunctional glycosylases since the 1 nt gap is relatively refractory to displacement synthesis (47–49). If BER is initiated by monofunctional glycosylases, HAP1 leaves a nick with a 3′-OH and a 5′-deoxyribose 5-phosphate (dRP) end (26,50,51).

Table I. Mammalian DNA glycosylases[a]

Name[c]	Size (amino acids)	Lyase activity	Cellular localization	Chromosome localization	Known substrates[b]
hUNG1	304	No	Mitochondria	12q24.1	ssU > U:G > U:A, 5-FU, (poor: 5-hydroxyU, isodialuric acid, alloxan)
hUNG2	313	No	Nuclei	12q24.1	ssU > U:G > U:A, 5-FU, (poor: 5-hydroxyU, isodialuric acid, alloxan)
mUNG1	295	No	Mitochondria	5	U, 5-FU
mUNG2	306	No	Nuclei	5	U, 5-FU
hSMUG1	270	No		12q13.1–q14	ssU>U:A, U:G
hTDG	410	No	Nuclei	12q24.1	U:G > εC:G > T:G
hMBD4	580	No		3q21	U or T in U/TpG:5-meCpG
hUDG2	327	No		5	U:A
hMPG	293	No		16p (tel)	3-mA, 7-mA, 3-mG,7-mG, 8-oxoG, hypoxanthine, εA, εG
mMPG		No			3-mA, 7-mA, 3-mG
hOGG1	345	Yes	Nuclei (1a)	3p25	Me-fapyG:C >> fapyG:C > 8-oxoG:C >> 8-oxoG:T
	424	Yes	Mitochondria (2a)		
mOGG1	345	Yes			8-oxoG:C >> 8-oxoG:T > 8-oxoG:G
hMYH	521	Yes?	Mitochondria	1p32.1–p34.3	A:G, A:8-oxoG >> C:A, 2-OH-A
	535	Yes?	Nuclei	1p32.1–p34.3	A:G, A:8-oxoG >> C:A, 2-OH-A
hNTH1	312	Yes	Nuclei (+ mitochondria?)	16p13.2–13.13	T/C-glycol, dihydrouracil, fapy
mNTH1	300	Yes		17A3	T-glycol, urea

[a]h, human; m, mouse.
[b]In mismatches the target base is on the left.
[c]Variant splice forms have also been identified for several DNA glycosylases and not all are included in the table.

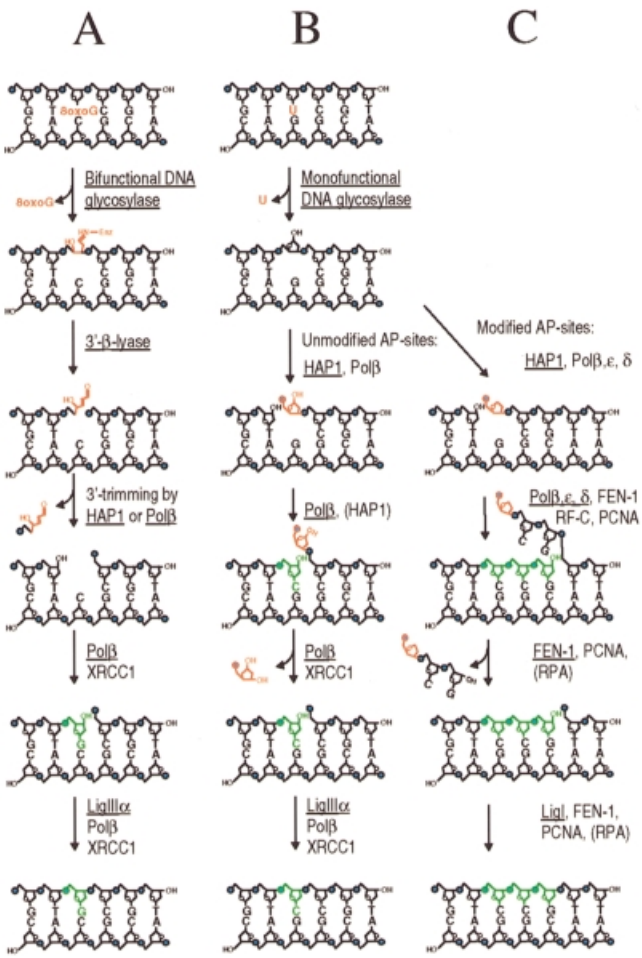

Fig. 3. The BER pathway is initiated by DNA glycosylases and may follow a short-patch (**A** and **B**) or a long-patch (**C**) route, in part depending on the type of initiating DNA glycosylase. The catalytic protein in each step is underlined.

When BER is initiated by monofunctional glycosylases it might proceed by the short-patch or the long-patch pathway (49,51,52). In the short-patch pathway DNA polymerase (Pol) β is recruited upon direct interaction with HAP1 to insert 1 nt (53,54). The dRP moiety is removed by the dRPase activity of Polβ, which involves Lys72 as the sole Schiff base nucleophile in the 8 kDa domain of this enzyme (55). Several lines of evidence suggest that the Polβ-dependent short-patch BER pathway is dominant, performing between 75 and 90% of all BER in human cells (53,56,57). It was recently shown that while Polδ or Polε could substitute for Polβ with regard to DNA synthesis there was no back-up for the dRPase activity and this might explain the neurotoxicity leading to embryonic lethality in Polβ-deficient mice (58,59). Resealing of the single-strand nick, presumably by DNA ligase III in complex with the scaffolding protein XRCC1 (60), completes the repair pathway (61,62).

Long-patch BER involves the synthesis of 2–8 nt stretches beginning at the damaged site. Insertion of only 2 nt is most frequent and the term 'long-patch' is therefore somewhat misleading (55,63–66). This pathway is particularly useful in cases where the dRP moiety is refractory to excision by Polβ (64). Strand displacement synthesis creates a flap structure from which the dRP moiety can be removed as part of an oligonucleotide. Strand displacement synthesis can be performed by Polβ (50,58), in addition to Polδ or Polε (65,66). Polβ-mediated long-patch BER does not absolutely require, but is stimulated by, proliferating cell nuclear antigen (PCNA) (50). In contrast, repair synthesis by Polδ or Polε is dependent on PCNA and replication factor C (RFC) (63). PCNA stimulates long-patch BER by appropriately positioning flap endonuclease I at the hinge and stimulating its nuclease activity to release the oligonucleotide containing the dRP moiety (50,64–66). RFC is required to load PCNA onto DNA (67). Since Polβ-deficient cells were found to be deficient in short-patch BER but proficient in PCNA-dependent long-patch BER, Polδ or Polε is believed to be the main polymerase in the long-patch pathway (54,56). RPA interacts with UNG2 (68,69) and is required for DNA synthesis by Pol or Polε in other repair

pathways and may stimulate long-patch BER, but its significance in BER remains unclear (63,70,71). Both DNA ligase I and III can seal the nick (50), but since PCNA stimulates the reaction it is supposed that DNA ligase I is responsible, since it interacts with PCNA (72).

A large fraction of nuclear UNG2 is localized to replication foci, where it has immediate access to replication factors that can be used for repair and interacts directly with PCNA and RPA (69). This suggests the possibility that long-patch BER might be mainly replication associated. It is also plausible that there is competition between the repair pathways for common factors. Some of the repair factors are induced when entering S phase, such as DNA glycosylases UNG2 (73,74) and hNTH1 (75). In contrast, Polβ is expressed at similar levels throughout the cell cycle (76). Polβ is apparently not present in replication foci (77) and it may therefore seem unlikely that it is involved in BER close to the replication fork. Moreover, post-translational modification, e.g. phosphorylation, may limit protein interaction to windows in the cell cycle, as has been shown for the interaction between PCNA and ligase I (78,79) and XRCC1 and ligase III (80). It is tempting to speculate that some factors may become limiting in certain situations, thus forcing repair into a different mode at these instances of time. As a consequence, identical lesions may be repaired by different pathways, depending on the status of the cell.

Knockout mice deficient in BER

Studies of BER deficiency using mouse knockout technology have at first sight been disappointingly inconclusive or, rather, have given results different from what was expected. Several known DNA glycosylases, as well as other BER proteins and proteins in other pathways, have been inactivated using this technology (see ref. 10 for comprehensive information). Based on genetic studies in *Escherichia coli* and yeast it was expected that mouse models would exhibit elevated mutation frequencies and, as a consequence, develop cancer at a relatively early age, as do most mismatch repair (MMR)-deficient mice (10). The first example of knockout mice deficient in a DNA glycosylase was mice deficient in the major activity for removal of 3-methyladenine (Mpg). Surprisingly, $Mpg^{-/-}$ mice developed normally, were fertile and did not demonstrate increased cancer incidence (81). Furthermore, Mpg-deficient mice show no increase in spontaneous mutation frequency and only a 3-fold increase in mutation frequency when exposed to methylating agents (82). Another example is Ung-deficient mice, which show less than 2-fold elevated mutation frequencies in the tissues analysed, but they do not seem to develop cancer at a significantly elevated rate nor do they show clear signs of any other disease (83). Ogg1-deficient mice show an up to 6-fold elevation of spontaneous mutation frequency in some tissues without developing tumours in these tissues, whereas other tissues remain at background mutation frequencies (21). Mouse knockouts of Myh1 (10) and Nth1 (J.Hoijmakers, van der Horst and Yasui, personal communication) exhibit a similar lack of altered phenotype. The spectrum of spontaneous mutations as measured in the *lacI* transgene in $ung^{-/-}$×BigBlue mice shows elevation in C→T transitions in spleen but not in thymus (83). Elevation of substitutions other than that expected is also seen in *Ogg1*-deficient mice (84), confirming lack of a simple and predictable relationship between genotype and mutation spectrum. Judged from the phenotype of mouse knockout models the importance of the individual DNA glycosylases in protection of genomic integrity appears to be reduced in mammalian cells compared with prokaryotes and lower eukaryotes. However, cell lines established from these mice appear to exhibit at least some of the biochemical characteristics expected with regard to sensitivity to DNA-damaging agents (82,85), accumulation of 8-oxoguanine (21) and accumulation of uracil (83).

In contrast to the glycosylases, deficiencies in the subsequent steps are embryonic lethal (10). One frequently stated explanation for this has been that there is an accumulation of repair intermediates generated by the glycosylases, in particular cytotoxic AP sites and dRP moieties. An implication would be that inactivation of the major glycosylases in these models should, at least in part, rescue the lethal phenotype at the expense of mutagenesis. To our knowledge these elaborate experiments have yet to be performed. Another possibility is that these models are lethal not because of BER deficiency, but rather due to the involvement of HAP1, Polβ and XRCC1 in developmental pathways. The N-terminal domain of HAP1 harbours a redox function (Ref1) that participates in activation of a number of proteins, among others p53, in response to oxidative stress (86,87). By using mutants of HAP1 that have lost endonuclease function but retained the redox activity, or vice versa, one should be able to determine whether redox function or AP endonuclease activity is required for survival during development. Polβ appears to be the major, possibly only, activity for removal of dRP moieties in mammalian cells (58,59) and this may be the cause of cyanosis and embryonic lethality of Polβ-deficient mice (88,89). However, there is also evidence that Polβ is involved in homologous recombination (90) and, therefore, one cannot presently conclude that the essentiality of this enzyme is due to its function in BER. DNA ligase III is stabilized through interaction with XRCC1 (60) and, accordingly, Xrcc1-deficient cells containing very little of this ligase activity are deficient in the ligation step in short-patch BER (62). Embryonic development in Xrcc1-deficient cells arrests around E6.5 (91). For comparison, DNA ligase I-deficiency is not lethal until E15.5–16.5 (92,93). It has been suggested that strand breaks might be generated through BER as part of a developmental programme and the inability to reseal these could be the cause of lethality of Xrcc1-deficient mice (91). The involvement of BER in development was earlier suggested based on absence of the conserved UDG and presence of a developmentally regulated dUTPase inhibitor in *Drosophila* (94,95). It was suggested that timed and tightly regulated incorporation of dUTP into DNA would prime DNA for degradation initiated by the action of a UDG activity in a developmental programme involving massive apoptosis (96). Interestingly, but maybe purely reflecting the reduced need for nucleotides in dying cells, dUTPase was recently shown to be degraded by caspase 3 during apoptosis in human cells (97). However, viable and developmentally normal Ung2-deficient mice may argue against this hypothesis, since the conserved Ung2 appears to predominantly remove misincorporated dUMP (83). But there may be redundancies even in this pathway and the involvement of uracil-initiated BER in a developmental programme remains an unproven and possibly weakened hypothesis.

Rearrangement of mitochondrial rDNA genes shows a correlation with age, suggesting a role for mitochondrial dysfunction in ageing (98). Accumulation of oxidative DNA damage in nuclear and mitochondrial DNA has similarly been proposed as one cause of cellular ageing (reviewed in ref. 99).

However, the evidence for accumulation of oxidative DNA damage, e.g. 8-oxoguanine, with age is still scarce. In fact, it was recently found that repair capacity (estimated as the level of 8-oxodeoxyguanosine glycosylase/AP lyase activity) increased with age, in contrast to capacity for uracil repair (100). Knockout mice deficient in glycosylases removing oxidative damage apparently do not show signs of increased cellular ageing (10). Furthermore, nutrient deprivation increases the lifespan in rodents and non-rodents (reviewed in ref. 101) but was also recently shown to increase oxidative stress and 8-oxoguanine levels in DNA in culture (102). As we see it, studies on the possible relationship between BER and ageing are still in their infancy and do not presently allow definite conclusions.

DNA glycosylases may have overlapping functions in BER and other DNA repair pathways also complement BER

It is clear that glycosylases are designed to remove mutagenic bases and thus limit mutagenesis. The biochemical data are strengthened by genetic data from bacteria and yeast. Thus, strains inactivated in each of the glycosylases exhibit increased spontaneous mutation frequencies. *Escherichia coli* *ung*$^-$ strains exhibit 3- to 5-fold increased global spontaneous mutation frequencies (103,104) and an up to 35-fold elevation of the spontaneous frequency of C→T transitions at individual sites (105–107). The simple relationship between altered genotype and types of mutation generated is challenged when mutation spectra are analysed more thoroughly, since unexpected types of mutations, specifically –1/–2 nt frameshifts, are similarly elevated in *ung*$^-$ strains (103). The loss of UDG activity was recently shown to increase transcription-associated –1 nt frameshift mutations in *Saccharomyces cerevisiae*, hence further complicating the relationship between genotype and classes of mutations generated (108). Other glycosylases have also been shown to limit mutagenesis in prokaryotes and lower eukaryotes. *Escherichia coli* endonuclease III (*nth* gene) and the yeast homologues Ntg1 and Ntg2 recognize pyrimidine glycols and several related compounds (109,110; reviewed in ref. 111). These enzymes have been shown to protect against spontaneous mutations and mutations induced by oxidative stress (112,113).

It was shown a long time ago that biochemically distinct enzymes could substitute for each other in bacterial BER, in that overexpression of Tag could complement AlkA deficiency in *E.coli* (1). Furthermore, repair of cyclobutane pyrimidine dimers is known to be carried out by at least three distinctly different pathways (direct repair by photolyase, NER and recombination) (reviewed in ref. 23). This line of research has recently been taken up again and an increasing overlap between repair pathways in the protection of genomic integrity has become apparent. Human NER can, in addition to well known helix-distorting lesions, also process AP sites, alkyl adducts and some mismatches (114), as well as oxidative base damage (115). Furthermore, BER of oxidized pyrimidines (e.g. thymine glycol) initiated by the human endonuclease III homologue hNTH1 requires XPG (47,116) and other repair proteins for optimal efficiency (117). At least 80% of thymine glycols are repaired by the short patch pathway (118). NER complements OGG1-initiated BER in the transcribed strand of active genes, but not in the non-transcribed strand (119). Repair of AP sites has turned out to be unexpectedly complex and involves BER, NER, recombination repair and TLS (7–9,114,120). Furthermore, genetic evidence has demonstrated overlapping specificities of the BER, NER repair, recombination and TLS pathways for oxidative base damage in *S.cerevisiae* (121). Repair of alkylation damage also involves more than one pathway. Thus, BER is complemented by recombination repair and NER for repair of alkylation damage (122,123). Surprisingly, it now appears that BER might have a modest protective effect compared with NER and recombination repair in response to alkylating agents in *Schizosaccharomyces pombe* (123).

One good example of intra-pathway complementation is removal of uracil from the genome. Several candidate enzymes that have uracil-DNA glycosylase activity have been identified in human cells, including TDG (35–37) and possibly UDG2 (124). Which one, if any, of these constitutes the major activity limiting mutagenesis from deamination of cytosine remains to be established. Among these glycosylases only TDG shows sequence similarity with the highly conserved UNG. The similarity is small and limited to one region of the protein. At the structural level, however, TDG and UNG are clearly related and the mechanism of catalysis is related, as recently reviewed (31). Human SMUG1 also shares two common sequence motifs with UNG and TDG, one involved in pyrimidine binding and one involved in glycosidic bond hydrolysis (31,36). In addition, MBD4 has a glycosylase domain and releases uracil, but is unrelated to UNG at the sequence level (37). Although the catalytic efficiency of these enzymes in uracil removal *in vitro* is low, they may complement UNG in BER *in vivo*. Therefore, the existence of several very distantly related, or unrelated, enzymes with overlapping, but not identical, specificities may explain why deficiencies in single activities generally have small effects.

UNG has a turnover number some 1000-fold higher than other glycosylases and as such represents a special case among glycosylases (125). The enzyme, particularly the nuclear form, is mainly expressed in late G_1 and S phase (73,74). These are properties compatible with the involvement of UNG2 in removal of misincorporated dUMP residues during replication (69,83). Bacterial and yeast Ung-deficient strains can tolerate high levels of dUMP stably incorporated into the genome, but when levels exceed 20% substitution they die, probably due to effects on RNA synthesis (126–128). Dietary folate deficiency (129,130) or treatment with drugs that interfere with dTTP synthesis, like methotrexate (131) and 5-fluorouracil (132), increases incorporation of uracil into DNA. The same treatments lead to accumulation of strand breaks and it has been suggested that these originate from attempted repair of U:A pairs initiated by UDG (133,134). Folate deficiency is also a risk factor for the development of a number of cancers (135,136) but appears to work as a tumour promoter rather than a mutagen (130). We are now in the position to test these hypotheses directly using *Ung*$^{-/-}$ mice.

In summary, different DNA glycosylases may substitute for each other in BER of base damage and biochemically distinct pathways may complement each other in repair of different lesions. The significance of each pathway in repair of a lesion almost certainly varies, but the rules that govern choice of pathway are generally not yet understood.

Are endogenous damage and cellular transactions major causes of mutations and cancer?

Mutation and promotion in cancer development

The evidence implying mutations as obligatory (but not necessarily sufficient) in carcinogenesis is strong. One major question

is whether mutations are mainly due to endogenous chemicals and processes or inflicted by exogenous agents. As outlined below, there is growing evidence indicating that endogenous factors are major contributors. The success of the Ames test in providing information on the mutagenic potential of numerous agents triggered extensive research on environmental mutagens as possible major contributors in carcinogenesis. However, with the exception of certain tissue-specific cancers clearly correlated with UV radiation, aflatoxin B_1 exposure and tobacco carcinogens, the contribution of exogenous mutagens to baseline levels of human cancers remains a question of debate (22,137,138). Still, epidemiological studies demonstrate wide variations in cancer incidences in populations in different geographical areas and even within distinct ethnic groups in different geographical locations, indicating that environmental factors and/or lifestyle contribute to a large fraction of cancers (139). However, the contribution of the environment to cancer is not necessarily mainly through mutagenesis. Moreover, the level of endogenously generated DNA damage may be sufficient to account for the mutations found in human cancer (18,138,140). Thus, the emphasis may shift towards mutations due to spontaneous DNA damage and promotion by environmental and endogenous factors as a more common general scheme for tumour development.

It seems likely that each of the many endogenous types of DNA damage individually contributes relatively little to the total DNA damage, but collectively this type of damage may still be dominant. It is implicit in this that each type of damage may be difficult to associate with a specific disease. A small fraction of this damage will escape repair and cause mutation. Some types of damage may be relatively infrequent, but close to 100% mutagenic upon replication prior to repair (such as uracil resulting from cytosine deamination and O^6-methylguanine). Other types of damage may be much more frequent but less mutagenic, e.g. uracil resulting from misincorporation of dUMP opposite A. However, even though DNA repair pathways have high fidelity, they are not infallible. A uracil base paired with A would become mutagenic upon repair essentially with the error frequency exhibited by the DNA synthesis step in BER. Repair of a uracil-containing substrate by BER in mammalian cell-free extracts was estimated to generate 1 error per 1900 nt inserted (141). The error rate of purified Polβ is apparently some 10-fold lower, but depends on several factors, such as patch size and sequence context (142; reviewed in ref. 143). Given that uracil resulting from cytosine deamination is mutagenic with close to 100% efficiency, a repair error frequency at this level would still limit mutagenesis dramatically. Incorporation of dUMP from dUTP, a normal intermediate, is limited by the enzyme dUTPase, which keeps dUTP concentrations low. It seems likely that misincorporation of dUMP is more common than deamination of cytosine. Thus, cells from Ung-deficient mice that have reduced capacity to remove misincorporated dUMP accumulate steady-state levels of some 2000 uracil residues/cell (83). If dUTPase activity were compromised or the ratio of dUTP to dTTP increased, e.g. by anti-folate drugs, an error rate of 1/1900 would seem unacceptable for repair of U:A base pairs, since misincorporation of dUMP would be greatly increased and therefore mutagenic. That increased incorporation of uracil into DNA during replication can be mutagenic, presumably through mechanisms in addition to erroneous BER, is illustrated by the increased spontaneous mutation frequency seen in *E.coli* dUTPase mutants (144). Inactivation of dUTPase in *ung*$^-$ strains has little effect on the spontaneous mutation frequency (104,106), suggesting that attempted repair of enhanced levels of U:A in an Ung-proficient background leads to generation of mutations, whereas misincorporated dUMP in an Ung-deficient background remains relatively innocuous in the short term. So, repair is apparently not always a good thing, and it has therefore been hypothesized that proof-reading activities must be associated with BER. A number of candidates that have $3'\rightarrow5'$ exonuclease activities compatible with such a function are known, including DNase III (145), WRN (146), p53 (147,148) and hMre11 (149). It remains to be shown, however, whether these proteins actually perform proof-reading *in vivo*. Interestingly, p53 has been demonstrated to directly stimulate BER *in vitro* by a so far unknown mechanism (150).

The appreciation of DNA repair as a two-edged sword has recently been substantiated by the finding that overexpression of repair enzymes may lead to an increase in spontaneous mutagenesis. A mutator phenotype after overexpression of Polβ may be explained by substitution of the high fidelity polymerases Polδ/ε by the lower fidelity polymerase Polβ (4). In the case of alkylguanine glycosylases the mutator phenotype may be explained by accumulation of mutagenic repair intermediates such as AP sites (1,3), due to the ability of this broad substrate range glycosylase to remove normal bases (2). Transcription arrest due to DNA damage recruits DNA repair components required for transcription-coupled repair, a subpathway in NER (151). Perhaps one should consider the possibility that pausing of transcription at certain sequences might recruit repair proteins and trigger aberrant DNA repair in undamaged DNA ('illusory defects'), as hypothesized in the case of 'mismatch' repair (152). At least for BER, the probability of aberrant repair reactions may be limited by orchestration of the process: DNA repair appears to be highly coordinated through protein–protein interactions resulting in timely recruitment of the correct protein for individual reaction steps (26,51,53,93,153,154).

Error-prone DNA polymerases in generation of endogenous mutations

Mutation generated in the absence of DNA damage due to slippage during replication, predominantly in mononucleotide runs or in other repeat regions, is well established. Replicative polymerases generate these errors with a frequency of 10^{-7}, but the error rate is lowered to 10^{-10} by the action of MMR pathways (23). The recent discovery of human low fidelity DNA polymerases (reviewed in refs 155,156) have also forced us to expand our thinking on how mutations are generated in that a significant fraction may not be caused by DNA damage. Thus, replication of undamaged DNA by Polη was recently found to result in one misincorporation per 32 nt inserted (157). Polι is apparently even more sloppy, but fidelity varies widely depending on the template base, with the highest error frequency (3:1) for misincorporation at template thymidines (158). It is not known, however, whether Polη, or other sloppy polymerases such as Polι/μ/λ/Φ (155), participate in replication of normal DNA *in vivo*. Another important aspect is whether Polμ or Polλ, both related to Polβ, can substitute for Polβ in BER. To our knowledge it has not been demonstrated whether the Polβ homologues have dRPase activity. Inactivating mutations in Polη lead to cancer development, as seen in XP-V patients (159), probably due to substitution by a polymerase with lower fidelity or an altered substrate spectrum, for example Polι (158) or Polζ (159). Along these lines, it

was recently suggested that *E.coli* replicative DNA polymerase is not responsible for a considerable fraction of mistakes made during replication, as a *dinB* mutation reduced replication-associated mutation 5-fold in a *mutS* background (B.S.Strauss, personal communication). Similarly, overexpression of PolΦ (Polθ or hDINB) in mammalian cells increased mutagenesis (160).

Germline mutation rates per 'effective genome' (expressed part) appear to be conserved between species (161). It has been estimated that the genome of each human zygote contains at least 20 *de novo* gene mutations (162), of which one is expected to give a slightly altered phenotype (163,164). Local mutation rates are highly variable within the genome and also between the same loci in different cell types (165). Still, the somatic mutation rate appears to be fairly consistent within a given locus between different species, between individuals and over time. Largely due to methodological limitations, however, it is still not clear whether global mutation rates have evolved to a favourable level (163,166). Changes in mutation frequencies, rather than their exact magnitude, would be expected to be of great importance for the human population, if not for individuals (163).

Does transcription across non-blocking DNA lesions contribute to mutations?

Base damage in DNA repair genes and other genes involved in DNA metabolism may also be implicated in mutagenesis, even if the gene is correctly repaired. Transcription over miscoding non-blocking lesions in the time window between damage and repair may result in altered transcripts that encode inactive, dominant negative or even directly mutagenic proteins. The concept of 'mutated' transcripts encoding proteins with altered functions has been demonstrated with uracil in the coding strand (167), adding to the complexity of the possible origin of spontaneous mutations. Mutagenic DNA glycosylases that excise normal bases and create AP sites *in vivo* have been generated by site-directed mutagenesis involving single nucleotides in the *UNG* gene (9,168), demonstrating the principle that mutagenic proteins may arise from simple base damage. Such proteins might persist in the cell for hours or days and disturb normal physiology or even directly or indirectly cause mutations. When the original damage causing the miscoding is eventually repaired no trace of the original damage is left at the DNA level, but secondary mutations remain. It would seem difficult to verify such a mechanism of mutagenesis, but given the large number of replicating cells and the significant burden of non-blocking DNA damage, it can be calculated that such damage is almost certainly bound to happen relatively frequently in multicellular organisms.

It is not clear which cellular processes are dominant in maintaining the global mutation rate and to what extent their establishment relies on the presence of endogenous or exogenous DNA damage. Somewhat surprisingly, many error-prone DNA polymerases appear to be highly expressed in the testes (169,170). This provides a possible alternative explanation for the observed male bias in *de novo* generated base substitution mutations in germ cells believed to originate from errors arising during normal conservative replication (163).

Is a major role of BER to protect the long-term integrity of the genome?

A puzzle in the light of the knockout phenotypes is what function we can envisage that would establish a selective pressure to conserve the genes for DNA glycosylases such as the highly conserved *UNG* gene through billions of years of evolution. The catalytic domain of human UNG shares 55% identity with the *E.coli* and 91% identity with the mouse homologues (33,34). In a search of more than 60 cell lines and paraffin-embedded tissues we have not identified mutations or polymorphisms in exons altering the activity of products of the human *UNG* gene (171). Thus, it appears that this gene shows less than expected sequence variation (164), suggesting that there is selective pressure to preserve the sequence in a species. In spite of the lack of an apparent phenotype of $Ung^{-/-}$ mice, the most likely explanation is probably that most mutations in coding sequences specifying UNG proteins give a small but sufficient reduction in fitness to select against them. A small reduction in fitness would be hard to detect under most laboratory settings, but may have profound effects on long-term fitness (163). Thus, one possibility is that UNG and other DNA glycosylases are important for the conservation of long-term integrity of the genome. They may serve the species to a greater extent than the individual. An Ung-deficient cell in a knockout animal does not need to compete with a wild-type version of itself, thus avoiding selection for fitness under laboratory conditions. An important question is how elevated the global mutation rate must be to have deleterious consequences on population fitness or to affect cancer incidence. Hereditary non-polyposis colon cancer (HNPCC) patients inherit one mutated allele in the MMR genes, most often in the *MSH2* or *MLH1* genes (reviewed in ref. 172). Cell lines derived from HNPCC patients show microsatellite instability and often more than a 100-fold increased mutation frequency (173,174). This is a good example of a mutator phenotype as a basis for cancer development, as suggested (175). However, it is becoming appreciated that the relationship between mutation frequency and cancer development in HNPCC is complex (reviewed in ref. 176). An increased mutation frequency is not necessarily a prerequisite for cancer development nor does an increase in mutations necessarily cause cancer. For instance, rare cases of HNPCC have been reported where affected individuals inherited dominant negative mutant alleles. They had deficient MMR and an increased mutation frequency in normal tissue, but they did not develop more tumours and more widely located tumours than other HNPCC patients (176,177). Furthermore, HNPCC patients do not develop more tumours per individual than MMR-proficient patients with sporadic colon cancer, although HNPCC patients are certainly cancer prone. This is in contrast to familial adenomatous polyposis cancer, where large numbers of colon tumours, often several thousands, develop. The mutated gene involved, *APC*, encodes a relatively colon-specific promoter protein that is also mutated early in sporadic cancer, indicating the relative importance of promotion over mutation as the rate limiting factor (reviewed in ref. 140). These findings indicate that elevated mutation frequency is insufficient to cause cancer in all tissues and underline the importance of tissue-specific promotion factors in overcoming growth control. This is supported by mathematical/statistical studies indicating that the 'normal' somatic mutation rate is indeed sufficiently high to explain the background incidence of cancer in the human population (140,178,179). An increased mutation rate would almost certainly speed up the process, but the rate limiting steps in tumour development would in any case be the ability to overcome the cell proliferation control mechanism (140). Clonal expansion of cells carrying

a mutation in growth regulatory genes (in the form of a benign tumour/hyperplasia) enhances the chances of secondary mutations proportional to the number of cells. Secondary mutations in MMR genes or genes controlling genomic stability might speed up diversification of expanding clones. Furthermore, MMR-deficient cells might be able to avoid elimination by the immune system due to mutations in β-globulin genes, but, due to other mutations, they may also present new antigens that stimulate removal of the cells by the immune system (180). Thus, HNPCC has proven very instructive, and is a relatively common genetic disease, but it may not be a widely valid model for carcinogenesis.

Future perspectives

We have discussed what the function of BER is. Perhaps this is the wrong question, because we know the function: it is simply to repair DNA. We need a better understanding of the *mechanisms* of DNA repair and the *consequences* of defects in DNA repair. Scientifically, prevention of cancer is not a function of DNA repair, but cancer may be a consequence of inadequate repair. In fact, all repair systems were well established long before cancer entered the arena in multicellular organisms. In addition to understanding the consequences, we certainly need to know more about the mechanisms of repair and regulation of each mechanism, as well as regulation of the interacting network.

BER is more versatile than other DNA repair mechanisms since it is initiated by at least eight different DNA glycosylases and follows at least two different paths downstream. In contrast, NER is initiated by an invariant protein complex and MMR is initiated by only two different complexes, depending on the type of damage (reviewed in ref. 181). It might well be that repair of spontaneously arising DNA damage is so important that a highly versatile network of overlapping mechanisms is required to maintain sufficient genomic stability. An important task will be to examine the relative importance of the different enzymes with overlapping specificities, as well as the relative importance of alternative pathways in repair of a given lesion. We also need to know the level of damage induction, as well as the efficiency, fidelity, cell cycle regulation and speed of repair. We may also need better methods to identify minor phenotypic changes, as well as a better understanding of genotype–phenotype relationships. Cancer is an obvious phenotype to look for when one expects an increase in mutation rates, perhaps too obvious at the expense of other changes, like ageing and metabolic disease. For Ung deficiency, myeloblastic anemia (182) and spina bifida (183) might be two candidate conditions to look at, since both are correlated with folate deficiency, which may increase the ratio between dUTP and dTTP and therefore also uracil incorporation. The complexity of DNA repair mechanisms that may employ several independent pathways for one specific damage, as well as one specific enzyme or pathway for several different types of damage, is beginning to be understood. Regulation of the complex network of interactions in DNA repair, as well as interactions of DNA repair processes with other cellular processes, are even less well understood. The concept of genetically unrelated pathways that complement each other in the defence against mutations certainly seems to represent a general cellular strategy rather than an exception (184).

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References

1. Kaasen,I., Evensen,G. and Seeberg,E. (1986) Amplified expression of the tag^+ and $alkA^+$ genes in *Escherichia coli*: identification of gene products and effects on alkylation resistance. *J. Bacteriol.*, **168**, 642–647.
2. Berdal,K.G., Johansen,R.F. and Seeberg,E. (1998) Release of normal bases from intact DNA by a native DNA repair enzyme. *EMBO J.*, **17**, 363–367.
3. Glassner,B.J., Rasmussen,L.J., Najarian,M.T., Posnick,L.M. and Samson,L.D. (1998) Generation of a strong mutator phenotype in yeast by imbalanced base excision repair. *Proc. Natl Acad. Sci. USA*, **95**, 9997–10002.
4. Canitrot,Y., Cazaux,C., Fréchet,M., Bouayadi,K., Lesca,C., Salles,B. and Hoffmann,J.-S. (1998) Overexpression of DNA polymerase β in cell results in a mutator phenotype and decreased sensitivity to anticancer drugs. *Proc. Natl Acad. Sci. USA*, **95**, 12586–12590.
5. Kohn,K.W. (1999) Molecular interaction map of the mammalian cell cycle control and DNA repair systems. *Mol. Biol. Cell*, **10**, 2703–2734.
6. Eisen,J.A. and Hanawalt,P.C. (1999) A phylogenomic study of DNA repair genes, proteins and processes. *Mutat. Res.*, **435**, 171–213.
7. Snowden,A., Kow,Y.W. and Van Houten,B. (1990) Damage repertoire of the *Escherichia coli* UvrABC nuclease complex includes abasic sites, base-damage analogues and lesions containing adjacent 5′ or 3′ nicks. *Biochemistry*, **29**, 7251–7259.
8. Torres-Ramos,C.A., Johnson,R.E., Prakash,L. and Prakash,S. (2000) Evidence for the involvement of nucleotide excision repair in the removal of abasic sites in yeast. *Mol. Cell. Biol.*, **20**, 3522–3528.
9. Otterlei,M., Kavli,B., Standal,R., Skjelbred,C., Bharati,S. and Krokan,H.E. (2000) Repair of chromosomal abasic sites *in vivo* involves at least three different repair pathways. *EMBO J.*, in press.
10. Friedberg,E.C. and Meira,L.B. (2000) Database of mouse strains carrying targeted mutations in genes affecting cellular responses to DNA damage: version 4. *Mutat. Res.*, **459**, 243–274.
11. Lindahl,T. (1993) Instability and decay of the primary structure of DNA. *Nature*, **362**, 709–715.
12. Lindahl,T. and Wood,R.D. (1999) Quality control by DNA repair. *Science*, **286**, 1897–1905.
13. Lindahl,T. and Nyberg,B. (1972) Rate of depurination of native DNA. *Biochemistry*, **11**, 3610–3618.
14. Nakamura,J., Walker,V.E., Upton,P.B., Chiang,S.Y., Kow,Y.W. and Swenberg,J.A. (1998) Highly sensitive apurinic/apyrimidinic site assay can detect spontaneous and chemically induced depurination under physiological conditions. *Cancer Res.*, **58**, 222–225.
15. Lindahl,T. and Nyberg,B. (1974) Heat-induced deamination of cytosine residues in deoxyribonucleic acid. *Biochemistry*, **13**, 3405–3410.
16. Frederico,L.A., Kunkel,T.A. and Shaw,B.R. (1990) A sensitive genetic assay for the detection of cytosine deamination: determination of rate constants and activation energy. *Biochemistry*, **29**, 2532–2537.
17. Shen,J.C., Rideout,W.M. and Jones,P.A. (1994) The rate of hydrolytic deamination of 5-methyl cytosine in double-stranded DNA. *Nucleic Acids Res.*, **22**, 972–976.
18. Marnett,L.J. (2000) Oxyradicals and DNA damage. *Carcinogenesis*, **21**, 361–370.
19. Ide,H., Kow,Y.W. and Wallace,S.S. (1985) Thymine glycols and urea residues in M13 DNA constitute replicative blocks *in vitro*. *Nucleic Acids Res.*, **13**, 8035–8052.
20. Rouet,P. and Essigmann,J.M. (1985) Possible role for thymine glycol in the selective inhibition of DNA synthesis on oxidized DNA templates. *Cancer Res.*, **45**, 6113–6118.
21. Klungland,A., Roswell,I., Hollenbach,S., Larsen,E., Daly,G., Epe,B., Seeberg,E., Lindahl,T. and Barnes,D.E. (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc. Natl Acad. Sci. USA*, **96**, 13300–13305.
22. Burcham,P.C. (1999) Internal hazards: baseline DNA damage by endogenous products of normal metabolism. *Mutat. Res.*, **443**, 11–36.
23. Friedberg,E.C., Walker,G.C. and Siede,W. (1995) *DNA Repair and Mutagenesis.* ASM Press, Washington, DC.
24. Seeberg,E., Eide,L. and Bjoras,M. (1995) The base excision repair pathway. *Trends Biochem. Sci.*, **20**, 391–397.
25. Krokan,H.E., Standal,R. and Slupphaug,G. (1997) DNA glycosylases in the base excision repair of DNA. *Biochem. J.*, **325**, 1–16.

26. Parikh,S.S., Mol,C.D., Hosfield,D.J. and Tainer,J.A. (1999) Envisioning the molecular choreography of DNA base excision repair. *Curr. Opin. Struct., Biol.*, **9**, 37–47.
27. McCullough,A.K., Dodson,M.L. and Lloyd,R. (1999) Initiation of base excision repair: glycosylase mechanisms and structures. *Annu. Rev. Biochem.*, **68**, 55–85.
28. Krokan,H.E., Nilsen,H., Skorpen,F., Otterlei,M. and Slupphaug,G. (2000) Base excision repair of DNA in mammalian cells. *FEBS Lett.*, **476**, 73–77.
29. Wilson,S.H. and Kunkel,T.A. (2000) Passing the baton in base excision repair. *Nature Struct. Biol.*, **7**, 176–178.
30. Mol,C.D., Parikh,S.S., Putnam,C.D., Lo,T.P and Tainer,J.A. (1999) DNA repair mechanisms for the recognition and removal of damaged DNA bases. *Annu. Rev. Biophys. Biomol. Struct.*, **28**, 101–128.
31. Schärer,O.D. and Jiricny,J. (2000) Recent progress in the biology, chemistry and structural biology of DNA glycosylases. *Bioessays*, in press.
32. Lindahl,T., Ljungquist,S., Siegert,W., Nyberg,B. and Sperens,B. (1977) DNA N-glycosidases: properties of uracil-DNA glycosidase from *Escherichia coli*. *J. Biol. Chem.*, **252**, 286–294.
33. Olsen,L.C., Aasland,R., Wittwer,C.U., Krokan,H.E. and Helland,D.E. (1989) Molecular cloning of human uracil-DNA glycosylase, a highly conserved DNA repair enzyme. *EMBO J.*, **8**, 3121–3125.
34. Nilsen,H., Otterlei,M., Haug,T., Solum,K., Nagelhus,T.A., Skorpen,F. and Krokan,H.E. (1997) Nuclear and mitochondrial uracil-DNA glycosylases are generated by alternative splicing and transcription from different positions in the *UNG* gene. *Nucleic Acids Res.*, **25**, 750–755.
35. Neddermann,P., Gallinari,P., Lettieri,T., Schmid,D., Truong,O., Hsuan,J.J., Wiebauer,K. and Jiricny,J. (1996) Cloning and expression of human G/T mismatch-specific thymine-DNA glycosylase. *J. Biol. Chem.*, **271**, 12767–74.
36. Haushalter,K.A., Stukenberg,P.T., Kirschner,M.W. and Verdine,G.L. (1999) Identification of a new uracil-DNA glycosylase family by expression cloning using synthetic inhibitors. *Curr. Biol.*, **9**, 174–185.
37. Hendrich,B., Hardeland,U., Ng,H.-H., Jiricny,J. and Bird,A. (1999) The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. *Nature*, **401**, 301–304.
38. Petronzelli,F., Riccio,A., Markham,G.D., Seeholzer,S.H., Stoerker,J., Genuardi,M., Yeung,A.T., Matsumoto,Y. and Bellacosa,A. (2000) Biphasic kinetics of the human DNA repair protein MED1 (MBD4), a mismatch-specific DNA N-glycosylase. *J. Biol. Chem.*, in press.
39. O'Connor,T.R. and Laval,F. (1990) Isolation and structure of a cDNA expressing a mammalian 3-methyladenine-DNA glycosylase. *EMBO J.*, **9**, 3337–3342.
40. Vickers,M., Vyas,P., Harris,P., Simmons,D. and Higgs,D. (1993) Structure of the human 3-methyladenine DNA glycosylase gene and localization close to the 16p telomere. *Proc. Natl Acad. Sci. USA*, **90**, 3437–3441.
41. Radicella,J.P., Dherin,C., Desmaze,C., Fox,M.S. and Boiteux,S. (1997) Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **94**, 8010–8015.
42. Aspinwall,R., Rothwell,D.G., Roldan-Arjona,T., Anselmino,C., Ward, C.J., Cheadle,J.P., Sampson,J.R., Lindahl,T., Harris,P. and Hickson,I.D. (1997) Cloning and characterization of a functional human homolog of *Escherichia coli* endonuclease III. *Proc. Natl Acad. Sci. USA*, **94**, 109–114.
43. Ikeda,S., Biswas,T., Roy,R., Izumi,T., Boldogh,I., Kurosky,A., Sarker,A.H., Seki,S. and Mitra,S. (1998) Purification and characterization of human NTH1, a homolog of *Escherichia coli* endonuclease III. *J. Biol. Chem.*, **273**, 21585–21593.
44. Slupska,M.M., Baikalov,C., Luther,W.M., Chiang,J.H., Wei,Y.F. and Miller,J.H. (1996) Cloning and sequencing a human homolog (hMYH) of the *Escherichia coli* mutY gene whose function is required for the repair of oxidative DNA damage. *J. Bacteriol.*, **178**, 3885–3892.
45. Slupska,M.M., Luther,W.M., Chiang,J.H., Yang,H. and Miller,J.H. (1999) Functional expression of hMYH, a human homolog of the *Escherichia coli* MutY protein. *J. Bacteriol.*, **181**, 6210–6213.
46. Hosfield,D.J., Guan,Y., Haas,B.J., Cunningham,R.P. and Tainer,J.A. (1999) Structure of the DNA repair enzyme endonuclease IV and its DNA complex: double-nucleotide flipping at abasic sites and three-metal-ion catalysis. *Cell*, **98**, 397–408.
47. Klungland,A., Höss,M., Gunz,D., Constantinou,A., Clarkson,S.G., Doetsch,P.W., Bolton,P.H., Wood,R.D. and Lindahl,T. (1999) Base excision repair of oxidative DNA damage activated by XPG protein. *Mol. Cell*, **3**, 33–42.
48. Dianov,G.L., Bischoff,C., Piotrowski,J. and Bohr,V.A. (1998) Repair pathways for processing of 8-oxoguanine in DNA by mammalian cell extracts. *J. Biol. Chem.*, **273**, 33811–33816.
49. Fortini,P., Parlanti,E., Sidorkina,O.M., Lavla,J. and Dogliotti,E. (1999) The type of DNA glycosylase determines the base excision repair pathway in mammalian cells. *J. Biol. Chem.*, **274**, 15230–15236.
50. Klungland,A. and Lindahl,T. (1997) Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *EMBO J.*, **16**, 3341–3348.
51. Kubota,Y., Nash,R.A., Klungland,A., Schär,P., Barnes,D.E. and Lindahl,T. (1996) Reconsitution of DNA base excision-repair with purified human proteins: interaction between polymerase β and the XRCC1 protein. *EMBO J.*, **15**, 6662–6670.
52. Wang,Z., Wu,X. and Friedberg,E.C. (1997) Molecular mechanism of base excision repair of uracil-containing DNA in yeast cell-free extracts. *J. Biol. Chem.*, **272**, 24064–24071.
53. Bennett,R.O., Wilson,D.M.III, Wong,D. and Demple,B. (1997) Interaction of human apurinic endonuclease and DNA polymerase β in the base excision repair pathway. *Proc. Natl Acad. Sci. USA*, **94**, 7166–7169.
54. Fortini,P., Pascucci,B., Parlanti,E., Sobol,R.W., Wilson,S.H. and Dogliotti,E. (1998) Different DNA polymerases are involved in the short- and long-patch base excision repair in mammalian cells. *Biochemistry*, **37**, 3575–3580.
55. Deterding,L.J., Prasad,R., Mullen,G.P., Wilson,S.H. and Tomer,K.B. (2000) Mapping of the 5′-2-deoxyribose-5-phosphate lyase active site in DNA polymerase β by mass spectrometry. *J. Biol. Chem.*, **275**, 10463–10471.
56. Sobol,R.W., Horton,J.K., Kühn,R., Gu,H., Singhal,R.K., Prasad,R., Rajewsky,K. and Wilson,S.H. (1996) Requirement of mammalian DNA polymerase-β in base-excision repair. *Nature*, **379**, 183–186.
57. Nealon,K., Nicholl,I.D. and Kenny,M.K. (1996) Characterization of the DNA polymerase requirement of human base excision repair. *Nucleic Acids Res.*, **24**, 3763–3770.
58. Dianov,G.L., Prasad,R., Wilson,S.H. and Bohr,V.A. (1999) Role of DNA repair polymerase β in the excision step of long patch mammalian base excision repair. *J. Biol. Chem.*, **274**, 13741–13743.
59. Sobol,R.W., Prasad,R., Evenski,A., Baker,A., Yang,X.-P., Horton,J.K. and Wilson,S.H. (2000) The lyase activity of the DNA repair protein β-polymerase protects from DNA-damage-induced cytotoxicity. *Nature*, **405**, 807–810.
60. Nash,R.A., Caldecott,K., Barnes,D.E. and Lindhal,T. (1997) XRCC1 protein interacts with one of two distinct forms of DNA ligase III. *Biochemistry*, **36**, 5207–5211.
61. Tomkinson,A.E. and Mackey,Z.B. (1998) Structure and function of mammalian DNA ligases. *Mutat. Res.*, **407**, 1–9
62. Cappelli,E., Taylor,R., Cevasco,M., Abbondandolo,A., Caldecott,K. and Frosina,G. (1997) Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. *J. Biol. Chem.*, **272**, 23970–23975.
63. Stucki,M., Pascucci,B., Parlanti,E., Fortini,P., Wilson,S.H., Hübscher,U. and Dogliotti,E. (1998) Mammalian base excision repair by DNA polymerases δ and ε. *Oncogene*, **17**, 835–843.
64. Gary,R., Kim,K., Cornelius,H.L., Park,M.S. and Matsumoto,Y. (1999) Proliferating cell nuclear antigen facilitates excision in long-patch base excision repair. *J. Biol. Chem.*, **274**, 4354–4363.
65. Matsumoto,Y., Kim,K., Hurwitz. J., Gary,R., Levin,D.S., Tomkinson,A.E. and Park,M.S. (1999) Reconsitution of proliferating cell nuclear antigen-dependent repair of apurinic/apyrimidinic sites with purified human proteins. *J. Biol. Chem.*, **274**, 33703–33708.
66. Pascucci,B., Stucki,M., J′nsson,Z.O., Dogliotti,E. and Hübscher,U. (1999) Long patch base excision repair with purified human proteins. *J. Biol. Chem.*, **274**, 33696–33702.
67. Kelman,Z. and Hurwitz,J. (1998) Protein-PCNA interactions: a DNA-scanning mechanisms? *Trends Biochem. Sci.*, **23**, 236–238.
68. Nagelhus,T.A., Haug,T., Singh,K.K., Keshav,K.F., Skorpen,F., Otterlei,M., Bharati,S., Lindmo,T., Benichou,S., Benarous,R. and Krokan,H.E. (1997) A sequence in the N-terminal region of human uracil-DNA glycosylase with homology to XPA interacts with the C-terminal part of the 34-kDa subunit of replication protein A. *J. Biol. Chem.*, **272**, 6561–6566.
69. Otterlei,M., Warbrick,E., Nagelhus,T.A., Haug,T., Slupphaug,G., Akbari,M., Aas,P.A., Steinsbekk,K., Bakke,O. and Krokan,H.E. (1999) Post-replication base excision repair in replication foci. *EMBO J.*, **18**, 3834–3844.
70. DeMott,M.S., Zigman,S. and Bambara,R.A. (1998) Replication protein A stimulates long patch DNA base excision repair. *J. Biol. Chem.*, **273**, 27492–27498.
71. Dianov,G.L., Jensen,B.R., Kenny,M.K. and Bohr,V.A. (1999) Replication protein A stimulates proliferating cell nuclear antigen-dependent repair of abasic sites in DNA by human cell extracts. *Biochemistry*, **38**, 11021–11025.

72. Levin,D.S., Bai,W., Yao,N., O'Donnell,M. and Tomkinson,A.E. (1997) An interaction between DNA ligase I and proliferating cell nuclear antigen: implications for Okazaki fragment synthesis and joining. *Proc. Natl Acad. Sci. USA*, **94**, 12863–12868.
73. Slupphaug,G., Olsen,L.C., Helland,D., Aasland,R. and Krokan,H.E. (1991) Cell cycle regulation and *in vitro* hybrid arrest analysis of the major human uracil-DNA glycosylase. *Nucleic Acids Res.*, **19**, 5131–5137.
74. Haug,T., Skorpen,F., Aas,P.A., Malm,V., Skjebred,C. and Krokan,H.E. (1998) Regulation of expression of nuclear and mitochondrial forms of human uracil-DNA glycosylase. *Nucleic Acids Res.*, **26**, 1449–1457.
75. Luna,L., Bjørås, Hoff,E., Rognes,T. and Seeberg,E. (2000) Cell-cycle regulation, intracellular sorting and induced overexpression of the human NTH1 DNA glycosylase involved in removal of formamidopyrimidine residues from DNA. *Mutat. Res.*, **460**, 95–104.
76. Zmudzka,B.Z., Fornace,A., Collins,J. and Wilson,S.H. (1988) Characterization of DNA polymerase β mRNA: cell-cycle and growth response in cultured human cells. *Nucleic Acids Res.*, **16**, 9587–9596.
77. Li,C., Cao,L.G., Wang,Y.L. and Baril,E.F. (1993) Further purification and characterization of a multienzyme complex for DNA synthesis in human cells. *J. Cell. Biochem.*, **53**, 405–419.
78. Montecucco,A., Rossi,R., Levin,D.S., Gary,R., Park,M.S., Montycka,T.A., Ciarrocchi,G., Villa,A., Biamonti,G. and Tomkinson,A.E. (1998) DNA ligase I is recruited to sites of DNA replication by an interaction with proliferating cell nuclear antigen. Identification of a common targeting mechanism for the assembly of replication factories. *EMBO J.*, **17**, 3786–3795.
79. Rossi,R., Villa,A., Negri,C., Scovassi,I., Ciarrocchi,G., Biamonti,G. and Montecucco,A. (1999) The replication factory targeting sequence/PCNA-binding site is required in G_1 to control the phosphorylation status of DNA ligase I. *EMBO J.*, **18**, 5745–5754.
80. Taylor,R.M., Moore,D.J., Whitehouse,J., Johnson,P. and Caldecott,K.W. (2000) A cell cycle-specific requirement for the XRCC1 BRCT II domain during mammalian DNA strand break repair. *Mol. Cell. Biol.*, **20**, 735–740.
81. Engelward,B.P., Weeda,G., Wyatt,M.D., Broekhof,J.L., de Wit,J., Donker,I., Allan,J.M., Gold,B., Hoeijmakers,J.H. and Samson,L.D. (1997) Base excision repair deficient mice lacking the Aag alkyladenine DNA glycosylase. *Proc. Natl Acad. Sci. USA*, **94**, 13087–13092.
82. Elder,R.H., Jansen,J.G., Weeks,R.J., Willington,M.A., Deans,B., Watson,A.J., Mynett,K.J., Bailey,J.A., Cooper,D.P., Rafferty,J.A., Heeran,M.C., Wijnhoven,S.W.P., van Zeeland,A.A. and Margison,G.P. (1998) Alkylpurine-DNA-*N*-glycosylase knockout mice show increased susceptibility to induction of mutations by methyl methanesulfonate. *Mol. Cell. Biol.*, **18**, 5828–5837.
83. Nilsen,H., Rosewell,I., Robins,P., Skjelbred,C.F., Andersen,S., Slupphaug,G., Daly,G., Krokan,H.E., Lindahl,T. and Krokan,H.E. (2000) Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication. *Mol. Cell*, **5**, 1059–1065.
84. Minowa,O., Arai,T., Hirano,M., Monden,Y., Nakai,S., Fukuda,M., Itoh,M., Takano,H., Hippou,Y., Aburatani,H., Masumura,K., Nishimura,S. and Noda,T. (2000) Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. *Proc. Natl Acad. Sci. USA*, **97**, 4156–4161.
85. Engelward,B.P., Dreslin,A., Christensen,J., Huszar,D., Kurahara,C. and Samson,L. (1996) Repair-deficient 3-methyladenine DNA glycosylase homozygous mutant mouse cells have increased sensitivity to alkylation-induced chromosome damage and cell killing. *EMBO J.*, **15**, 945–952.
86. Xanthoudakis,S. and Curran,T. (1992) Identification and characterization of Ref-1, a nuclear protein that facilitate AP-1 DNA binding activity. *EMBO J.*, **11**, 653–665.
87. Prieto-Alamo,M.J. and Laval,F. (1999) Overexpression of the human HAP1 protein sensitizes cells to the lethal effect of bioreductive drugs. *Carcinogenesis*, **20**, 415–419.
88. Gu,H., Marth,J.D., Orban,P.C. Mossmann,H. and Rajewsky,K. (1994) Deletion of a DNA polymerase β gene segment in T-cells using cell type-specific gene targeting. *Science*, **265**, 103–106.
89. Esposito,G., Texido,G., Betz,U.A., Gu,H., Muller,W., Klein,U. and Rajewsky,K. (2000) Mice reconstituted with DNA polymerase β-deficient fetal liver cells are able to mount a T cell-dependent immune response and mutate their Ig genes normally. *Proc. Natl Acad. Sci. USA*, **97**, 1166–1171.
90. Plug,A.W., Clairmont,C.A., Sapi,E., Ashley,T. and Sweasy,J.B. (1997) Evidence for a role for DNA polymerase β in mammalian meiosis. *Proc. Natl Acad. Sci. USA*, **94**, 1327–1331.
91. Tebbs,R.S., Flannery,M.L., Meneses,J.J., Hartmann,A., Tucker,J.D., Thompson,L.H., Cleaver,J.E. and Pedersen,R.A. (1999) Requirement for the *Xrcc1* DNA base excision repair gene during early mouse development. *Dev. Biol.*, **208**, 513–529.
92. Bentley,D.J., Selfridge,J., Millar,J.K., Hole,N., Ansell,J.D. and Melton,D.W. (1996) DNA ligase I is required for fetal liver erythropoiesis but is not essential for mammalian cell viability. *Nature Genet.*, **13**, 489–491.
93. Prasad,R., Singhal,R.K., Srivastava,D.K., Molina,J.T., Tomkinson,A.E. and Wilson,S.H. (1996) Specific interaction of DNA polymerase β and DNA ligase I in a multiprotein base excision repair complex from bovine testis. *J. Biol. Chem.*, **271**, 16000–16007.
94. Nation,M.D., Guzder,S.N., Giroir,L.E. and Deutsch,W.A. (1989) Control of *Drosophila* deoxyuridine triphosphatase. Existence of a developmentally expressed protein inhibitor. *Biochem. J.*, **259**, 593–596.
95. Dudley,B., Hammond,A. and Deutsch,W.A. (1992) The presence of uracil–DNA glycosylase in insects is dependent on developmental complexity. *J. Biol. Chem.*, **267**, 11964–11967.
96. Deutsch,W.A. (1995) Why do pupating insects lack an activity for the repair of uracil-containing DNA? One explanation involves apoptosis. *Insect Mol. Biol.*, **4**, 1–5.
97. Brockstedt,E., Rickers,A., Kostka,S., Laubersheimier,A., Dorken,B., Wittmann-Liebold,B., Bommert,K. and Otto,A. (1998) Identification of apoptosis-associated proteins in a human Burkitt lymphoma cell line. Cleavage of heterogeneous nuclear ribonucleoprotein A1 by caspase 3. *J. Biol. Chem.*, **273**, 28057–28064.
98. Melov,S., Schneider,J.A., Coskun,P.E., Bennett,D.A. and Wallace,D.C. (1999) Mitochondrial DNA rearrangements in aging human brain and *in situ* PCR of mtDNA. *Neurobiol. Aging*, **20**, 565–571.
99. Johnson,F.B., Sinclair,D.A. and Guarente,L. (1999) Molecular biology of ageing. *Cell*, **96**, 291–302.
100. Souza-Pinto,N.C., Croteau,D.L., Hudson,E.K., Hansford,R.G. and Bohr,V.A. (1999) Age-associated increase in 8-oxo-deoxyguanosine glycosylase/AP lyase activity in rat mitochondria. *Nucleic Acids Res.*, **27**, 1935–1942.
101. Nicolas,A.S., Lanzmann-Petithory,D. and Vellas,B. (1999) Caloric restriction and aging. *J. Nutr. Health Aging*, **3**, 77–83.
102. Conlon,K.A., Grollman,A.P. and Berrios,M. (2000) Immunolocalization of 8-oxoguanine in nutrient-deprived mammalian tissue culture cells. *J. Histotechnol.*, **23**, 37–44.
103. Fix,D.F. and Glickman,B.W. (1987) Asymmetric cytosine deamination revealed by spontaneous mutational specificity in an Ung^- strain of *Escherichia coli. Mol. Gen. Genet.*, **209**, 78–82.
104. Duncan,B.K. and Weiss,B. (1978) In Hanawalt,P.C., Friedberg,E.C. and Fox,C.F. (eds), *DNA Repair Mechanisms.* Academic Press, New York, NY, pp. 183–186.
105. Duncan,B.K. and Miller,J.H. (1980) Mutagenic deamination of cytosine residues in DNA. *Nature*, **287**, 560–561.
106. Duncan,B.K. and Weiss,B. (1982) Specific mutator effects of *ung* (uracil-DNA glycosylase) mutations in *Escherichia coli*. *J. Bacteriol.*, **151**, 750–755.
107. Fix,D.F. and Glickman,B.W. (1986) Differential enhancement of spontaneous transition mutations in the *lacI* gene of an Ung^- strain of *Escherichia coli. Mutat. Res.*, **175**, 41–45.
108. Morey,N.J., Greene,C.N. and Jinks-Robertson,S. (2000) Genetic analysis of transcription-associated mutation in *Saccharomyces cerevisiae*. *Genetics*, **154**, 109–120.
109. Dizdaroglu,M., Bauche,C., Rodriguez,H. and Laval,J. (2000) Novel substrates of *Escherichia coli* nth protein and its kinetics for excision of modified bases from DNA damaged by free radicals. *Biochemistry*, **39**, 5586–5592.
110. You,H.J., Swanson,R.L., Harrington,C., Corbett,A.H., Jinks-Robertson,S., Senturker,S., Wallace,S.S., Boiteux,S., Dizdaroglu,M. and Doetsch,P.W. (1999) *Saccharomyces cerevisiae* Ntg1p and Ntg2p: broad specificity N-glycosylases for the repair of oxidative DNA damage in the nucleus and mitochondria. *Biochemistry*, **38**, 11298–11306.
111. Cadet,J., Bourdat,A.G., D'Ham,C., Duarte,V., Gasparutto,D., Romieu,A. and Ravanat,J.L. (2000) Oxidative base damage to DNA: specificity of base excision repair enzymes. *Mutat. Res.*, **462**, 121–128.
112. Najrana,T., Saito,Y., Uraki,F., Kubo,K. and Yamamoto,K. (2000) Spontaneous and osmium tetroxide-induced mutagenesis in an *Escherichia coli* strain deficient in both endonuclease III and endonuclease VIII. *Mutagenesis*, **15**, 121–125.
113. Alseth,I., Eide,L., Pirovano,M., Rognes,T., Seeberg,E. and Bjoras,M. (1999) The *Saccharomyces cerevisiae* homologues of endonuclease III from *Escherichia coli*, Ntg1 and Ntg2, are both required for efficient repair of spontaneous and induced oxidative DNA damage in yeast. *Mol. Cell. Biol.*, **19**, 3779–3787.
114. Huang,J.C., Hsu,D.S., Kazantsev,A. and Sancar,A. (1994) Substrate spectrum of human excinuclease: repair of abasic sites, methylated bases, mismatches and bulky adducts. *Proc. Natl Acad. Sci. USA*, **91**, 12213–12217.

115. Reardon,J.T., Bessho,T., Kung,H.C., Bolton,P.H. and Sancar,A. (1997) *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in xeroderma pigmentosum patients. *Proc. Natl Acad. Sci. USA*, **94**, 9463–9468.
116. Cooper,P.K., Nouspikel,T., Clarkson,S.G. and Leadon,S.A. (1997) Defective transcription-coupled repair of oxidative base damage in Cockaynes syndrome from XP group G. *Science*, **275**, 990–993.
117. LePage,F., Kwoh,E.E., Avrutskaya,A., Gentil,A., Leadon,S.A., Sarasin,A. and Cooper,P.K. (2000) Transcription-coupled repair of 8-oxoGuanine: requirements for XPG, TFIIH and CSB and implications for Cockayne syndrome. *Cell*, **101**, 159–171.
118. Dianov,G.L., Thybo,T., Dianova,I.I., Lipinski,L.J. and Bohr,V.A. (2000) Single nucleotide patch base excision repair is the major pathway for removal of thymine glycol from DNA in human cell extracts. *J. Biol. Chem.*, **275**, 11809–11813
119. Le Page,F., Klungland,A., Barnes,D.E., Sarasin,A. and Boiteux,S. (2000) Transcription coupled repair of 8-oxoguanine in murine cells: the ogg1 protein is required for repair in nontranscribed sequences but not in transcribed sequences. *Proc. Natl Acad. Sci. USA*, **97**, 8397–8402.
120. Lin,J.J. and Sancar,A. (1989) A new mechanism for repairing oxidative damage to DNA: (A)BC excinuclease removes AP sites and thymine glycols from DNA. *Biochemistry*, **28**, 7979–7984.
121. Swanson,R.L., Morey,N.J., Doetsch,P.W. and Jinks-Robertson,S. (1999) Overlapping specificities of base excision repair, nucleotide excision repair, recombination and translesion synthesis pathways for DNA base damage in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **19**, 2929–2935.
122. Xiao,W. and Chow,B.L. (1998) Synergism between yeast nucleotide and base excision repair pathways in the protection against methylation damage. *Curr. Genet.*, **33**, 92–99.
123. Memisoglu,A. and Samson,L. (2000) Contribution of base excision repair, nucleotide excision repair and DNA recombination to alkylation resistance of the fission yeast *Saccharomyces pombe*. *J. Bacteriol.*, **182**, 2104–2112.
124. Muller,S.J. and Caradonna,S. (1991) Isolation and characterization of a human cDNA encoding uracil-DNA glycosylase. *Biochim. Biophys. Acta*, **1088**, 197–207.
125. Slupphaug,G., Eftedal,I., Kavli,B., Bharati,S., Helle,N.M., Haug,T., Levine,D.W. and Krokan,H.E. (1995) Properties of a recombinant human uracil-DNA glycosylase from the *UNG* gene and evidence that *UNG* encodes the major uracil-DNA glycosylase. *Biochemistry*, **34**, 128–138.
126. Tye,B.-K., Chien,J., Lehman,I.R., Duncan,B.K. and Warner,H.R. (1978) Uracil incorporation: a source of pulse-labelled DNA fragments in the replication of the *Escerichia coli* chromosome. *Proc. Natl Acad. Sci. USA*, **75**, 233–237.
127. Warner,H.R., Duncan,B.K., Garrett,C. and Neuhard,J. (1981) Synthesis and metabolism of uracil-containing deoxyribonucleic acid in *Escherichia coli*. *J. Bacteriol.*, **145**, 687–695.
128. El-Hajj,H.H., Wang,L. and Weiss,B. (1992) Multiple mutant of *Escherichia coli* synthesising virtually thymineless DNA during limited growth. *J. Bacteriol.*, **174**, 4450–4456.
129. Blount,B.C., Mack,M.M., Ewhr,C.M., MacGregor,J.T., Hiatt,R.A., Wang,G., Wickramasinghe,S.N., Everson,R.B. and Ames,B.N. (1997) Folate deficiency causes uracil misincorporation into human DNA and chromosomal breakage: implications for cancer and neuronal damage. *Proc. Natl Acad. Sci. USA*, **94**, 3290–3295.
130. Melnyk,S., Pogribna,M., Miller,B.J., Basnakian,A.G., Pogribny,I.P. and James,S.J. (1999) Uracil misincorporation, DNA strand-breaks and gene amplification are associated with tumourigenic cell transformation in folate deficient/repleted Chinese hamster ovary cells. *Cancer Lett.*, **146**, 35–44.
131. Goulian,M., Bleile,B. and Tseng,B.Y. (1980) Methotrexate-induced misincorporation of uracil into DNA. *Proc. Natl Acad. Sci. USA*, **77**, 1956–1960.
132. Kubo,K., Shiomi,A., Asaeda,A., Ohashi,F., Matsuyama,S., Ide,H. and Takamori,Y. (1998) Induction of fragile sites by fluorodeoxyuridine and caffeine accompanies with misincorporation of endogenous uridine nucleotide into DNA of feline fibroblasts. *J. Vet. Med. Sci.*, **60**, 1293–1297.
133. Canman,C.E., Radany,E.H., Parsels,L.A., Davis,M.A., Lawrence,T.S. and Maybaum,J. (1994) Induction of resistance to fluorodeoxyuridine cytotoxicity and DNA damage in human tumor cells by expression of *Escherichia coli* deoxyuridinetriphosphatase. *Cancer Res.*, **54**, 2296–2298.
134. Parsels,L.A., Parsels,J.D., Wagner,L.M., Loney,T.L., Radany,E.H. and Maybaum,J. (1998) Mechanism and pharmacological specificity of dUTPase-mediated protection from DNA damage and cytotoxicity in human tumor cells. *Cancer Chemother. Pharmacol.*, **42**, 357–362.
135. Skibola,C.F., Smith,M.T., Kane,E., Roman,E., Rollinson,S., Cartwright,R.A. and Morgan,G. (1999) Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc. Natl Acad. Sci. USA*, **96**, 12810–12815.
136. Ames,B.N. (1999) Cancer prevention and diet: help from single nucleotide polymorphisms. *Proc. Natl Acad. Sci. USA*, **96**, 12216–12218.
137. Morley,A.A. and Turner,D.R. (1999) The contribution of exogenous and endogenous mutagens to *in vivo* mutations. *Mutat. Res.*, **428**, 11–15.
138. Robertson,K.D. and Jones,P.A. (2000) DNA methylation: past, present and future directions. *Carcinogenesis*, **21**, 461–467.
139. World Cancer Fund/American Institute for Cancer Research (1997) Patterns of cancer. In *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. American Institute for Cancer Research, Washington, DC, Ch. 1.2, pp. 35–52.
140. Tomlinson,I. and Bodmer,W. (1999) Selection, the mutation rate and cancer: ensuring that the tail does not wag the dog. *Nature Med.*, **5**, 11–12.
141. Sanderson,R.J. and Mosbaugh,D.W. (1998) Fidelity and mutational specificity of uracil-initiated base excision DNA repair synthesis in human glioblastoma cell extracts. *J. Biol. Chem.*, **273**, 24822–24831.
142. Osheroff,W.P., Jung,H.K., Beard,W.A., Wilson,S.H. and Kunkel,T.A. (1999) The fidelity of DNA polymerase β during distributive and processive DNA synthesis. *J. Biol. Chem.*, **274**, 3642–3650.
143. Holmquist,G.P. (1998). Endogenous lesions, S-phase-independent spontaneous mutations and evolutionary strategies for base excision repair. *Mutat. Res.*, **400**, 59–68.
144. Sedgewick,W.D., Brown,O.E. and Glickman,B.W. (1986) Deoxyuridine misincorporation causes site-specific mutational lesion in the *lacI* gene of *Escherichia coli*. *Mutat. Res.*, **162**, 7–20.
145. Höss,M., Robins,P., Naven,T.J.P., Pappin,D.J.C., Sgouros,J. and Lindahl,T. (1999) A human DNA editing enzyme homologous to the *Escherichia coli* DnaQ/MutD protein. *EMBO J.*, **18**, 3868–3875.
146. Kamath-Loeb,A.S., Shen,J.C., Loeb,L.A. and Fry,M. (1998) Werner syndrome protein. II. Characterization of the integral 3′→5′ DNA exonuclease. *J. Biol. Chem.*, **273**, 34145–34150.
147. Mummenbrauer,T., Janus,F., Muller,B., Wiesmuller,L., Deppert,W. and Grosse,F. (1996) p53 protein exhibits 3′ to 5′ exonuclease activity. *Cell*, **85**, 1089–1099.
148. Janus,F., Albrechtsen,N., Dornreiter,I., Wiesmuller,L., Grosse,F. and Deppert,W. (1999) Different regulation of the p53 core domain activities 3′- to- 5′ exonuclease and sequence-specific DNA binding. *Mol. Cell. Biol.*, **19**, 2155–2168.
149. Paull,T. and Gellert,M. (1998) The 3′ to 5′ exonuclease activity of Mre11 facilitates repair of double-strand breaks. *Mol. Cell*, **1**, 969–979.
150. Offer,H., Wolkowicz,R., Matas,D., Blumenstein,S., Livneh,Z. and Rotter,V. (1999) Direct involvement of p53 in the base excision repair pathway of the DNA repair machinery. *FEBS Lett.*, **450**, 197–204.
151. Hanawalt,P.C. and Spivak,G. (1999) Transcription-coupled repair. Which lesions? Which diseases? In Dizdaroglu,M. and Karakaya,A. (eds), *Advances in DNA Damage and Repair*. Kluwer Academic/Plenum Press, New York, NY, pp. 169–179.
152. Ninio J. (2000) Illusory defects and mismatches: why must DNA repair always be (slightly) error prone? *Bioessays*, **22**, 396–401.
153. Parikh,S.S., Mol,C.D., Slupphaug,G., Bharati,S., Krokan,H.E. and Tainer,J.A. (1998) Base excision repair initiation revealed by crystal structures and binding kinetics of human uracil-DNA glycosylase with DNA. *EMBO J.*, **17**, 5214–5226.
154. Marintchev,A., Mullen,M.A., Maciejewski,M.W., Pan,B., Gryk,M.R. and Mullen,G.P. (1999) Solution structure of the single-strand break repair protein XRCC1 N-terminal domain. *Nature Struct. Biol.*, **6**, 884–893.
155. Hübscer,U., Nasheuer,H.P. and Syväoja,J.E. (2000) Eukaryotic DNA polymerases, a growing family. *Trends Biochem. Sci.*, **25**, 143–147.
156. Friedberg,E.C., Feaver,W.J. and Gerlach,V.L. (2000) The many faces of DNA polymerases: strategies for mutagenesis and for mutational avoidance. *Proc. Natl Acad. Sci. USA*, **97**, 5681–5683.
157. Matsuda,T., Bebenek,K., Masutani,C., Hanaoka,F. and Kunkel,T.A. (2000) Low fidelity DNA synthesis by human polymerase η. *Nature*, **404**, 1011–1013.
158. Tissier,A., McDonald,J.P., Frank,E.G. and Woodgate,R. (2000) Poliota, a remarkably error-prone human DNA polymerase. *Genes Dev.*, **14**, 1642–1650.
159. Masutani,C., Kusumoto,R., Yamada,A., Dohmae,N., Yokoi,M., Yuasa,M., Araki,M., Iwai,S., Takio,K. and Hanaoka,F. (1999) The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase η. *Nature*, **399**, 700–704.
160. Ogi,T., Kato,T. and Ohmori,H. (1999) Mutation enhancement by DINB1, a mammalian homologue of the *Escherichia coli* mutagenesis protein DinB. *Genes Cells*, **4**, 607–618.
161. Drake,J.W., Charlesworth,B., Charlesworth,D. and Crow,J.F. (1998) Rates of spontaneous mutation. *Genetics*, **148**, 1667–1686.

162. Sommer,S.S. and Ketterling,R.P. (1994) How precisely can data from transgenic mouse mutation-detection systems be extrapolated to humans? : lessons from the human factor IX gene. *Mutat. Res.*, **307**, 517–531.
163. Crow,J.F. (1997) The high spontaneous mutation rate: is it a health risk? *Proc. Natl Acad. Sci. USA*, **94**, 8380–8386.
164. Simpson,A.J.G. and Camargo,A.A. (1998) Evolution and the inevitability of human cancer. *Semin. Cancer Biol.*, **8**, 439–445.
165. Turker,M.S. (1998) Estimation of mutation frequencies in normal mammalian cells and the development of cancer. *Semin. Cancer Biol.*, **8**, 407–419.
166. Metzgar,D. and Wills,C. (2000) Evidence for the adaptive evolution of mutation rates. *Cell*, **101**, 581–584.
167. Viswanathan,A., You,H.J. and Doetsch,P.W. (1999) Phenotypic change caused by transcriptional bypass of uracil in nondividing cells. *Science*, **284**, 159–162.
168. Kavli,B., Slupphaug,G., Mol,C.D., Arvai,A.S., Peterson,S.B., Tainer,J.A. and Krokan,H.E. (1996) Excision of cytosine and thymine from DNA by mutants of human uracil-DNA glycosylase. *EMBO J.*, **15**, 3442–3447.
169. Ohashi,E., Ogi,T., Kusumoto,R., Iwai,S., Masutani,C., Hanaoka,F. and Ohmori,H. (2000) Error-prone bypass of certain lesions by the human DNA polymerase Φ. *Genes Dev.*, **14**, 1589–1594.
170. Gerlach,V.L., Aravind,L., Gotway,G., Schultz,R.A., Koonin,E.V. and Friedberg,E.C. (1999) Human and mouse homologs of *Escherichia coli* DinB (DNA polymerase IV), members of the UmuC/DinB superfamily. *Proc. Natl Acad. Sci. USA*, **96**, 11922–11927.
171. Kvaløy,K., Nilsen,H., Steinbekk,K.S., Nedal,A., Monterotti,B., Akbari,M. and Krokan,H.E. (2001) Sequence variation in the human uracil-DNA glycosylase (UNG) gene. *Mutat. Res*., **461**, 325–338.
172. Lynch,H.T. and de la Chapelle,A. (1999) Genetic susceptibility to non-polyposis colorectal cancer. *J. Med. Genet.*, **36**, 801–818.
173. Glaab,W.E. and Tindall,K.R. (1997) Mutation rate at the *hprt* locus in human cancer cell lines with specific mismatch repair-gene defects. *Carcinogenesis*, **18**, 1–8.
174. Richards,B., Zhang,H., Phear,G. and Meuth,M. (1997) Conditional mutator phenotypes in hMSH2-deficient tumour cell lines. *Science*, **277**, 1523–1526.
175. Loeb,L.A. (1998) Cancer cells exhibit a mutator phenotype. *Adv. Cancer Res.*, **72**, 25–56.
176. Kinzler,K.W. and Vogelstein,B. (1996) Lessons from hereditary colorectal cancer. *Cell*, **87**, 159–170.
177. Parsons,R., Li,G.M., Longley,M., Modrich,P., Liu,B., Berk,T., Hamilton,S.R., Kinzler,K.W. and Vogelstein,B. (1995) Mismatch repair deficiency in phenotypically normal human cells. *Science*, **268**, 738–740.
178. Tomlinson,I.P.M., Novelli,M.R. and Bodmer,W.F. (1996) The mutation rate and cancer. *Proc. Natl Acad. Sci. USA*, **93**, 14800–14803.
179. Herrero-Jimenez,P., Thilly,G., Southam,P.J., Tomita-Mitchell,A., Morgenthaler,S., Furth,E.E. and Thilly,W.G. (1998) Mutation, cell kinetics and subpopulations at risk for colon cancer in the United States. *Mutat. Res.*, **400**, 553–578.
180. Bicknell,D.C., Kaklamanis,L., Hampson,R., Bodmer,W.F. and Karran,P. (1996) Selection for β-2-microglobulin mutation in mismatch repair-defective colorectal carcinomas. *Curr. Biol.*, **6**, 1695–1697.
181. Lindahl,T., Karran,P. and Wood,R.D. (1997) DNA excision repair pathways. *Curr. Opin. Genet. Dev.*, **7**, 158–169.
182. Koury,M.J., Horne,D.W., Brown,Z.A., Pietenpol,J.A., Blount,B.C., Ames,B.N., Hard,R. and Koury,S.T. (1997) Apoptosis of late-stage erythroblasts in megaloblastic anemia: association with DNA damage and macrocyte production. *Blood*, **89**, 4617–4623.
183. Molloy,A.M., Mills,J.L., Kirke,P.N., Weir,D.G. and Scott,J.M. (1999) Folate status and neural tube defects. *Biofactors*, **10**, 291–294.
184. Wagner,A. (2000) Robustness against mutations in genetic networks of yeast. *Nature Genet.*, **24**, 355–361

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