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Oxidative DNA damage repair in mammalian cells:

A new perspective

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Abstract

Oxidatively induced DNA lesions have been implicated in the etiology of many diseases (including cancer) and in aging. Repair of oxidatively damaged bases in all organisms occurs primarily via the DNA base excision repair (BER) pathway, initiated with their excision by DNA glycosylases. Only two mammalian DNA glycosylases, OGG1 and NTH1 of *E. coli* Nth family, were previously characterized, which excise majority of the oxidatively damaged base lesions. We recently discovered and characterized two human orthologs of *E. coli* Nei, the prototype of the second family of oxidized base-specific glycosylases and named them NEIL (Nei-like)-1 and 2. NEILs are distinct from NTH1 and OGG1 in structural features and reaction mechanism but act on many of the same substrates. Nth-type DNA glycosylases after base excision, cleave the DNA strand at the resulting AP-site to produce a 3'-αβ unsaturated aldehyde whereas Nei-type enzymes produce 3'-phosphate terminus. *E. coli* APEs efficiently remove both types of termini in addition to cleaving AP sites to generate 3'-OH, the primer terminus for subsequent DNA repair synthesis. In contrast, the mammalian APE, APE1, which has an essential role in NTH1/OGG1-initiated BER, has negligible 3'-phosphatase activity and is dispensable for NEIL-initiated BER. Polynucleotide kinase (PNK), present in mammalian cells but not in *E. coli*, removes the 3' phosphate, and is involved in NEIL-initiated BER. NEILs show a unique preference for excising lesions from a DNA bubble, while most DNA glycosylases, including OGG1 and NTH1, are active only with duplex DNA. The dichotomy in the preference of NEILs and NTH1/OGG1 for bubble versus duplex DNA substrates suggests that NEILs function preferentially in repair of base lesions during replication and/or transcription and hence play a unique role in maintaining the functional integrity of mammalian genomes.

Keywords

Base excision repair; Reactive oxygen species; Oxidative DNA damage; PNK-dependent BER

1. Introduction

Reactive oxygen species (ROS) continuously generated as respiration by-products (1-5% of consumed O₂) in the mitochondria, are the most abundant, endogenous toxic agents in aerobic

organisms, including mammals [1,2]. Elevated cellular level of ROS is formed when cells are exposed to redox agents and ionizing radiation. ROS induces DNA damage, which includes a multitude of oxidized base lesions, abasic (AP) sites, single- and double-strand breaks containing 3' sugar fragments or phosphates and all of these are invariably cytotoxic and/or mutagenic [3]. Most sporadic cancers, as well as a variety of other pathophysiological states and the aging syndrome, are likely consequences of mutations, cell death or signaling alterations induced by oxidative DNA damage [4]. Nearly all oxidatively induced DNA lesions (except double-strand breaks), as well as single strand breaks, are repaired via the DNA base excision repair (BER) pathway in organisms ranging from *E. coli* to mammals [5-8]. Until recently BER was thought to be the simplest and most thoroughly defined of all repair processes. However, the BER pathway never received as much attention as the other repair pathways, i.e., nucleotide excision repair (NER) and DNA mismatch repair (MMR), presumably because deficiencies in these repair pathways have been linked to cancer and other diseases. Compared to other repair pathways, BER appears to be rather simple and requires five distinct enzymatic activities in the basic reaction steps. The critical step in BER pathway is the excision of damaged bases from the DNA by a class of enzymes called DNA glycosylases [8]; the repair is completed in several subsequent steps, beginning with cleavage of the DNA backbone by AP endonuclease (APE), or by the intrinsic AP lyase activity associated with oxidized base-specific DNA glycosylases [5]. The strand cleavage by APE generates 3'-OH and 5' deoxyribose phosphate (dRP) termini, while the AP lyases produce 3' blocking phosphoribose or phosphate termini together with 5' phosphates. In the subsequent step DNA polymerase β (Pol β), which has intrinsic dRP lyase activity, carries out both 5' end-cleaning and DNA repair synthesis with incorporation of the appropriate nucleotide at the site of the base damage [9,10]. Finally, the resulting nick is sealed by DNA ligase and thus the repair is completed [11]. Despite major advances in our understanding of the individual steps in BER that were made during the last decade, the physiological significance of the presence of multiple glycosylases with overlapping specificity for oxidized bases is still not clear. Here we provide a broad overview of the oxidized base-specific glycosylases, with special reference to the recently characterized family of mammalian DNA glycosylases and their potential roles in maintaining functional and structural integrity of mammalian genomes.

2. Identification of oxidized base-specific mammalian DNA glycosylases

There are several distinct families of DNA glycosylases, which are likely to have evolved independently and are conserved in organisms ranging from *E. coli* to mammals [12]. Most DNA glycosylases have broad substrate specificities, but with preference for either pyrimidine or purine derivatives. Two oxidized base-specific DNA glycosylases were identified in mammals; NTH1 (endonuclease III homolog) and OGG1 (8-oxoguanine DNA glycosylase); both belonging to the endonuclease III (Nth) superfamily. The mouse and human OGG1 genes were cloned in 1997 by several groups, based on homology to yeast OGG1 [13-19]. Surprisingly, the cloning of the mammalian OGG1 cDNA was not preceded by identification of OGG activity in cell extracts. In an earlier study, an 8-oxoG-specific DNA endonuclease and a monofunctional DNA glycosylase were identified in HeLa cells [20]; however, neither activity appeared to be identical to that of the cloned hOGG1. We first identified an 8-oxoG DNA glycosylase activity in HeLa cells, partially purified it and showed that this 38 kDa OGG1 was identical to the cloned enzyme [21]. The mammalian ortholog of Nth, NTH1, was also identified in the same year and characterized by us and others [22-25]. NTH1 recognizes a wide range of oxidized pyrimidine derivatives, such as thymine glycol (Tg), 5-hydroxycytosine, dihydrouracil (DHU), urea, and at least six other oxidized pyrimidines in addition to AP-sites, and the ring-opened structure of 1, *N*⁶-ethenoadenine [8,26-29]. OGG1 is primarily responsible for the repair of oxidation products of guanine [8], such as 7,8-dihydro-8-oxoguanine (8-oxoG) and ring-opened fapy guanine (FapyG).

In addition to Nth, *E. coli* expresses two other oxidized base-specific DNA glycosylases, namely, MutM and Nei (Endonuclease VIII), which have significant sequence conservation and structural similarity. However, Nei like Nth, prefers oxidized pyrimidines, while MutM prefers oxidized purines and ring opened purines. Structural orthologs of Nei/MutM are absent in yeast and *Drosophila*, and were not identified in mammals until 2002. Because we had detected earlier MutM/Nei-like activity in HeLa extracts, we searched the human and mouse genomic databases and identified three orthologs of MutM/Nei, cloned, expressed and characterized these proteins. We originally named the proteins encoded by these genes, NEHs (Nei homolog) and since then these have been renamed NEILs (Nei-like) [30-33], all of which have distinct sizes (Table 1), and are located on different human and mouse chromosomes. Although we identified three such candidates (NEIL1, NEIL2 and NEIL3), we could not detect glycosylase activity of NEIL3. NEIL1 and NEIL2 are closer to Nei than to MutM in regards to amino acid sequence and substrate specificity. Both recombinant NEIL1 and NEIL2 prefer ROS-derived lesions of pyrimidines, although like *E. coli* Nei, NEIL1 does excise 8-oxoguanine from oligonucleotides [34]. NEIL1 shows high affinity for ring-opened purines-formamidopyrimidine (Fapy)-A and -G and is also active with thymine glycol, generated by oxidation of thymine. NEIL2 appears to prefer cytosine-derived lesions, particularly 5-hydroxyuracil and 5-hydroxycytosine [31]. It was also reported that the NEILs, unlike OGG1 and NTH1, are functional in recognition and removal of subsequent oxidation products of 8-oxoG, e.g., spiroiminodihydantoin (Sp) and guanidinohydantoin (Gu) [35]. The substrate preferences of the mammalian DNA glycosylases are summarized in Table 1.

3. Distinct structural features and reaction mechanisms

All DNA glycosylases identified so far that are involved in the repair of oxidative DNA damage possess intrinsic AP lyase activity. These glycosylases employ a common reaction mechanism involving several steps. The initial recognition of the damaged base involves bending of DNA at the damage site and subsequent flipping of the lesion into the active site pockets of these glycosylases [36,37]. The damaged base is displaced by a nucleophilic attack at C1' by an active site nucleophile, which is generally an internal lysine residue (hNTH1 and hOGG1) or an N-terminal proline (NEIL1 and NEIL2). There is some controversy as to whether the nucleophilic reaction will lead to an initial C1'-O4' bond cleavage before glycosidic bond cleavage or C1'-N9 cleavage before ring opening [36,38-40]. The direct displacement of the damaged base by the active site nucleophile will lead to the formation of an abasic Schiff base intermediate [40]. The transient Schiff base could be converted into a stable 'covalent complex' by reduction with sodium borohydride [41]. The stability of this Schiff base intermediate is crucial for the subsequent DNA lyase reaction, characterized by either a single lytic reaction at the 3' phosphodiester bond (β -elimination) or a successive double lytic reaction occurring at both the 3' and 5' phosphodiester bond (successive β and δ -elimination). The β -lyase reaction generates a one base gap containing 5'-phosphate and 3'-phospho α,β unsaturated aldehyde while the $\beta\delta$ -lyase produces a gap with 3' and 5' phosphates [40].

Tertiary structure of *E. coli* Nth, the first oxidized base-specific DNA glycosylase whose structure was solved by X-ray crystallography reveals the presence of two α -helical domains, a helix-hairpin-helix (HhH) motif and a [4Fe-4S] cluster loop [42-45]. The [4Fe-4S] cluster region, common in many mitochondrial OXPHOS complexes, is apparently not involved in redox chemistry in this case [44], but has been proposed to serve as an architectural element that positions a loop containing positively charged residues near the phosphodiester backbone of a target DNA molecule [45]. The HhH motif has been suggested to be the signature domain for DNA binding in Nth type DNA glycosylases [45]. Several eukaryotic homologs of *E. coli* Nth have been identified in yeast, plant, worm, mice and human [22,24,42,46-49]. The DNA-binding motifs, and the catalytic residues, such as Lys 212 and Asp 231 in human NTH1 (Fig. 1) corresponding to the catalytic Lys 120 and Asp138 of *E. coli* enzyme, are conserved

among these homologues, except that *S. cerevisiae* NTG1 (alternate name for NTH1) does not have an [4Fe-4S] cluster [45,46,50]. Although Lys 212 is essential for NTH1's activity, its substitution with Arg resulted in reduced DNA glycosylase/lyase activity and the mutant could still form a covalent complex with the substrate DNA after reduction with NaBH₄. Nonetheless, the substitution of K212 with Arg (but not Gln), uncoupled the glycosylase activity from the lyase activity. The uncoupled reaction could be a result of direct attack either by the nonionized form of the guanidino group of arginine that forms an unstable Schiff base that hydrolyzes before the β -elimination event or by the hydroxide ion to cleave the glycosidic bond. In either case, this reaction is followed by a secondary β -elimination event performed by random, "opportunistic" lysine residues primarily from the lysine-rich N-terminal tail region [51].

The other Nth ortholog, OGG1 share common structural features including the helix-hairpin-helix (HhH) motif with a closely located Lys (Lys 249 for hOGG1) and an Asp (Asp 268), 19 residues downstream of the HhH (Fig. 1). Lys 249 was shown to be involved in Schiff base formation following cleavage of the glycosidic bonds at 8-oxoG [15]. The highly conserved Asp 268 is the only amino acid residue in the active site that is capable of acid/base catalysis and was initially thought to be involved in the deprotonation of Lys 249 [36,52]. However, hOGG1 D268Q mutant still retains robust glycosylase/lyase activity and this argue against the role of Asp268 in acid/base catalysis [53], but rather could be important for electrostatically stabilizing the developing positive charge on O4' in the transition state of the base excision step [38,39,53].

In contrast to the Nth superfamily, the Nei/MutM orthologs, NEIL1 and NEIL2 utilize N-terminal Pro as the active site nucleophile, and an essential conserved Lys (Lys 53 in NEIL1, Lys 49 in NEIL2) as proton donor. Both NEILs carry out $\beta\delta$ -elimination reaction to produce single strand breaks (SSBs) with phosphates at both 3' and 5' ends [6,30,31]. Like their bacterial counterparts, both these enzymes possess the helix-two turns-helix (H2TH) DNA-binding motif (Fig. 1). However, in spite of NEIL1 and NEIL2 having significant functional overlap and using the same reaction chemistry as MutM and Nei, only NEIL2 possesses a Zn finger motif (ZnF) near the C terminus [54]. It contains a unique sequence with three Cys and one His residues in an unusual ZnF configuration that is not homologous to the C4 type ZnF motifs of Nei/MutM. The Zn finger is essential for maintaining structural integrity of NEIL2, because mutations in the coordinating Cys or His not only abolish the DNA binding activity of the proteins, but also grossly alter its tertiary structure. We demonstrated how the β -hairpin zinc finger provides a necessary structural framework to position the conserved arginine (Arg-310 in NEIL2) and showed this Arg to be active in the enzyme's catalytic reaction by mutational studies [54]. NEIL1's structural motif also mimics the β -hairpin zinc finger found in members of the MutM/Nei class but lacks the loops harboring the canonical zinc-binding residues and therefore does not coordinate zinc. Interestingly, the critical Arg (Arg-277 in NEIL1) is placed in the loop connecting the two β -strands of the zinc-less finger and its mutation to Ala showed a strong reduction of glycosylase activity [55]. Thus the conserved Arg positioned on the edge of the β -hairpin loop may interact with the minor groove of DNA that may be critical for glycosylase activity in both zinc-containing (NEIL2) and zinc-less fingers (NEIL1).

4. Distinct APE-independent BER sub-pathways

In *E. coli*, the 3' blocked products of β - and $\beta\delta$ -elimination are both processed by the APEs (Xth and Nfo) [56]; to provide the 3'-OH terminus required for subsequent repair synthesis by a DNA polymerase. The major AP endonuclease in mammalian cells, APE1, primarily removes the 3' phospho α,β -unsaturated aldehyde generated by the β -lyase activity of OGG1 and NTH1. On the other hand, 3' phosphate generated by the $\beta\delta$ -lyase activity of NEILs, is a poor substrate of APE1 (unlike *E. coli* APEs) [57]. However, mammals, unlike bacteria, express high levels of polynucleotide kinase (PNK) with dual 5' kinase/3' phosphatase activities. The

role of mammalian PNK in repair of 3'-phosphate termini at DNA single-strand breaks, induced by ionizing radiation and ROS, have been implicated [58]. We thereby postulated a novel scenario for mammalian BER: a combination of NEILs and PNK could generate 3'-OH termini without involving APE1, an essential protein in mammals. We have shown that 5-hydroxyuracil-containing duplex oligo could be repaired using a reconstituted system containing NEIL1, PNK, DNA Polymerase β (Pol β), and DNA Ligase III α (Lig III α) without APE1 [57]. Very recently we provided similar evidence of PNK-mediated repair of damage-containing plasmid DNA initiated by NEIL2 [59]. APE1's weak DNA 3'-phosphatase activity was reported previously [60], and we have clearly shown that PNK is much more efficient in removing 3'-phosphate than APE1 [57]. The NEIL2 immunocomplex containing Pol β , PNK, Lig III α and XRCC1 (but not APE1), efficiently repairs 5-OHU [59], providing further support that APE1 is dispensable in NEIL-mediated BER. In mammals, another ortholog of *E. coli* Xth, named APE2, was recently identified [61]. While we could not detect any significant AP endonuclease or 3' phosphatase activity in recombinant hAPE2 [57], a recent report documented 3'-5' exonuclease and 3' phospho-diesterase activity in this protein. However, its 3' phosphatase activity was not examined [62]. The PNK-dependent repair thus represents a major pathway initiated by NEILs, providing a novel paradigm exclusively for mammals [57] (Fig. 2). 8-oxoG, a major ROS-induced lesion, is excised primarily by OGG1, which turns over poorly due to its binding to the product AP site. APE1 enhances OGG1 activity by competing for the AP site [63,64]. We then went on to show that NEIL1 acts like APE1 in enhancing OGG1 turnover [65] by competing for the AP site. Also our observation that NEIL1 can repair AP sites and 3' dRP, predict that the NEIL/PNK pathway could take over repair initiated by other DNA glycosylases. Taking together, we propose a comprehensive model for BER with three sub-pathways that are dictated by three types of DNA glycosylases present in mammalian cells (Fig. 2). Monofunctional DNA glycosylases (M) generate AP sites after base excision, and APE1 then cleaves the DNA strand 5' to the AP site (pathway II). For oxidized bases, DNA glycosylases/AP lyases that carry out either β elimination (pathway I) or $\beta\delta$ elimination (pathway III) determine the subsequent steps. APE1 is responsible for processing the β elimination product. However, when NEIL1 and NEIL2 carry out $\beta\delta$ elimination, PNK is required for generating 3'-OH termini. Furthermore, AP sites and 3' dRP generated by other DNA glycosylases can also be processed through a NEIL-PNK-dependent pathway. This alternative route of repair thus may provide important redundancy in mammalian BER, a critical safeguard against oxidative and spontaneous DNA damage.

The importance of the NEIL/PNK pathway is further demonstrated *in vitro* when DNAs containing tandem dihydrouracil (DHU) are processed by hNTH1 and NEIL1 [66]. Tandem DNA lesions are induced not only by ionizing radiation, they are also generated by ROS and photosensitized reactions [67-69]. It was demonstrated that both hNTH1 and NEIL1 can remove only one of the two thus, leaving behind a gapped DNA containing a DHU residue at either 3' or 5' terminus. When the repair of tandem DHU is initiated by hNTH1, APE was shown to be essential for the removal of not only the 3' α,β -unsaturated sugar residue, but also the 3' DHU residue [66]. In contrast, when the repair of tandem DHU is initiated by NEIL1, the removal of 3' phosphate requires the 3' phosphatase activity of PNK. In this case, a complete repair of tandem DHU initiated by NEIL1 can proceed via two sub-pathways, a PNK dependent and a PNK/APE dependent pathway [66].

5. Modular protein domains and dynamic interactions

The sequence alignment of hNTH1 with NTHs from *E. coli*, archaea and other lower organisms shows that the human and other mammalian NTH1 has an N-terminal tail, absent in *E. coli* and archaea. This N-terminal tail (residues 1-95) contains putative sequences for both nuclear and mitochondrial translocation signals [42]. The structure and function of this tail was investigated. Controlled proteolysis cleaved hNTH1 into discrete fragments to generate a 25-

kDa core domain lacking the N-terminal 98 residues. Surprisingly, recombinant hNTH1 lacking up to 80 residues from the N-terminus had 4-5-fold higher activity than the full-length enzyme. Kinetic analysis revealed that release of the final product, an AP-site with 3'-nick, is the rate-limiting step in the multistep reaction catalyzed by hNTH1. The N-terminal tail regulates its overall catalytic turnover by reducing the rate of product release by 5-7-fold without affecting glycosylase or AP-lyase activities or the steady state equilibrium concentration of the Schiff base intermediate [25]. The inhibitory role of the N-terminal tail in catalytic turnover could explain the low activity of human NTH1 compared to the *E. coli* Nth [22,45]. Further studies demonstrated that the full-length hNTH1 positively cooperates in product formation as a function of enzyme concentration. At the protein concentration when cooperativity in turnover is observed, the enzyme forms a dimer independent of DNA-binding. We have shown that this dimerization independent of substrate binding, regulates the turnover of the enzyme in an allosteric manner at the product release step [70]. Intriguingly, the dimerization-dependant activation is distinct from stimulation of NTH1 by other protein factors (e.g., XPG, YB-1, APE1 [71-74]). The latter regulates hNTH1 activity via distinct mechanisms by affecting certain kinetic steps of the enzyme reaction. Thus the N-terminal tail of hNTH1 both inhibits the product release and is also required for its dimerization [25]. The high intranuclear concentrations of hNTH1 and other studies such as, protein cross-linking[8,70] and mammalian 2-hybrid analysis (S. Choudhury and R. Roy, unpublished results) strongly support the possibility of its dimerization *in vivo*, whose significance remains to be elucidated. Our data further revealed that 22-55 residues in the N-terminal tail of hNTH1 is indispensable for dimerization [70] and physical interactions with XPG. However, the activation of hNTH1's activity by Y-box binding protein (YB1) is achieved via direct protein-protein interaction [73].

In spite of a few conserved structural motifs (including the N-terminal peptide PE(G/L)P(E/L)), an essential Lys (Lys 53 in NEIL1, Lys 49 in NEIL2) and a helix-2T-helix motif, a hallmark of Nei-type proteins, there is very limited sequence identity between NEIL1 and NEIL2 (Fig. 1). The X-ray crystallographic structure of an enzymatically active deletion construct of NEIL1 (lacking 56 C-terminal residues) indicated the presence of a Zn-less finger, which is internal [55], unlike the C-terminal Zn finger present in other Nei orthologs. We have previously shown that the interacting region of NEIL1 lies within its C-terminal domain (between residues 289 and 350) [57]. NEIL1 interacts via this flexible domain with downstream BER proteins, including Pol β and Lig III α . The tertiary structure of NEIL2 has not yet been elucidated. However, limited proteolytic digestion suggests that there are two major domains in NEIL2, the N-terminal domain (NTD; spanning residues 1-198) and the C-terminal domain (CTD; containing residues 199-331). Our data further show that in the case of NEIL2, the 133 C-terminal residues, including the ZnF motif that is necessary for DNA binding and catalysis are dispensable for interactions with Pol β , Lig III α and XRCC1 polypeptides [59]. It is remarkable that the same regions of Pol β (N-terminal 140 aa residues) and Lig III α (C-terminal 175 residues) are involved in interaction with both NEILs. That both NEIL1 and NEIL2 interact with similar domains of the interacting partners suggest a unique structural similarity between the NEILs in spite of the significant difference in their primary sequences. The physiological significance of NEIL's binding to the same region of Pol β and Lig III α warrants further investigation. The interacting domain of NEIL1 is localized in its C-terminal domain, which is dispensable for its *in vitro* glycosylase/lyase activity [57]. In contrast, not only is the C-terminal domain of NEIL2 essential, but the interacting domain, which is at the N-terminus, is also indispensable for activity. That both the NEIL enzymes show direct interaction with the downstream BER proteins including Lig III α , the last enzyme in BER, implies that the glycosylases control the pathway and so suggests their vital role in repair coordination. Furthermore, a FLAG-NEIL2 immunocomplex was capable of completing repair of a 5-OHU-containing plasmid DNA [59]. This multiprotein complex is likely to be dynamic, because the same NEIL2 molecule could not simultaneously interact with multiple partners via its common

interacting domain. A possible explanation for such a complex is that it allows coordinated hand-off among repair proteins, which we also believe to be the case for NEIL1. Taken together, the association of NEILs with downstream BER proteins supports a model of repair coordination in which NEILs recruit these proteins to form a larger complex at the site of DNA damage.

6. Unique activity of NEILs on bubble DNA substrates: possible role in RAR/TCR

Both NTH1 and OGG1 excise their substrate lesions only from duplex DNA, which is perhaps expected because the undamaged strand provides the template for repair synthesis of the damaged strand. In our studies with the NEILs we unexpectedly discovered that these enzymes have high activity in excising base lesions from single-stranded DNA or unpaired sequences in bubble DNA [75]. NEIL2 has higher activity with bubble DNA relative to ds or ss DNA, while NEIL1 shows similar activity with ss versus ds DNA. These results have significant implications, because bubble and ss DNA represent transient intermediates in transcription and replication. It was reported previously that preferential repair of the oxidative lesion, 8-oxoG located in the transcribed sequences does not involve OGG1 [76]. We have hence proposed that NEIL 1 and NEIL2, unlike OGG1 and NTH1 are preferentially involved in BER linked to transcription and/or genome replication [75] (Fig. 3). Although the observed activity of NEIL1 for 8-oxoG and oxidized purines is rather low [30], this result may not reflect the *in vivo* situation where the activity could be significantly enhanced in the presence of other proteins. Again NEIL1 with its S-phase specific regulation [30] may be involved with the replication machinery and recognize and excise the lesion from the single-stranded region, generated by DNA helicase. The resulting strand break will interrupt the coordination of daughter strand synthesis, which will then stall the DNA polymerase. DNA polymerase may regress which will allow reannealing of separated strands and repair of strand break. Although there is no evidence so far for preferential repair of oxidized bases in the replicative genome, it is tempting to speculate pre-replicative repair of template and post-replicative repair of misincorporated lesions in the nascent strands in association with MMR proteins (Fig. 3). The latter gains support from an *in vitro* observation which indicates that NEIL1 can actively excise 8-oxoG or 5-OHU in the primer strand recessed from the 3' terminus by 1-3 nucleotides, while both OGG1 or NTH1 are inactive or only weakly active on such substrates [77]. In contrast NEIL2, which is cell cycle-independent, may be involved in transcription-coupled base excision repair (TC-BER) of oxidized bases (Fig. 3). Transcription coupled repair (TCR) of bulky adducts (e.g., UV photoproducts), which prevent RNA polymerase elongation, was identified as a distinct sub-pathway of NER [78,79]. The blockage of RNA Polymerase II at the damage site triggers repair of the transcribed strand, which is required for survival and maintenance of genomic integrity. We should point out that repair of oxidized bases via TC-BER sub-pathway may not be analogous with the repair of bulky adducts via NER. Because, oxidized bases do not block transcription (at least *in vitro*); how TC-BER is activated in the *in vivo* repair of these lesions is not clear. We propose that NEIL2-initiated strand incision in the transient bubble, generated ahead of the growing RNA chain, will prevent forward movement of the transcription complex. This strand break will then block transcription followed by regression of the transcription complex and collapse of the bubble structure. The single base gap is then repaired from the duplex DNA, followed by resumption of transcription. Thus unlike bulky adducts which directly block transcription and initiate the TCR, the oxidized bases trigger TCR after the strand break by AP lyase activity of the DNA glycosylase.

7. Redundancy of BER enzymes: distinctive biological roles

It is an interesting and important question why mammals provide so many enzymes for oxidative purine/pyrimidine damage [80]. The presence of redundant BER enzymes may in a

way signify their importance in the cells. The down-regulation of NEIL1 expression in mouse embryonic stem cells sensitizes these cells to γ -irradiation [81], establishing its protective role *in vivo*. Furthermore we demonstrated that reactive oxygen species (ROS) up-regulate hNEIL1 expression through activation of the transcription factors, CREB/c-Jun [82]. Recently the *NEIL1*^{-/-} mice have been reported to manifest a combination of diseases associated with the metabolic syndrome [83], the pathogenesis of this syndrome being attributed to ROS exposure [84]. Neither OGG1 nor NTH1 null mice shows any phenotype [85,86], although there was some accumulation of oxidative lesions in OGG1^{-/-} mice and slower repair kinetics of thymine glycol in NTH1^{-/-} mice. Recently evidence was collected for association of polymorphism in these DNA glycosylases with cancer and other diseases. For example, polymorphism in NEIL1 have been linked to gastric cancer [87], while those in OGG1 and subsequent less activity have been related to higher risk for prostate cancer and smoking-related lung cancer [88-90]. In other studies, the activity and expression of NTH1 and OGG1 were found to be significantly altered during the acute (16-18 weeks) and early chronic (24 weeks) phases of hepatitis in the Long Evans Cinnamon (LEC) rat, an animal model for Wilson's disease (WD) which is said to cause a "genetically induced" oxidative condition and higher risk for liver cancer [89]. Several studies indicated that SMUG1 and UNG1 are also capable of excising oxidized derivatives of pyrimidines and siRNA-mediated downregulation of these genes in mouse embryo fibroblasts significantly increased radio sensitivity [91,92]. In view of the presence of multiple DNA glycosylases further studies are required before we could evaluate their contribution to normal cellular functions.

8. Future perspectives

In this review we have attempted to provide a glimpse of the complexities of repair processes for oxidative DNA damage. A few other aspects of *in vivo* repair that we have not previously discussed are important areas of ongoing research in our own and other laboratories.

- i. *In vivo regulation of BER sub-pathways*: *In vitro* repair studies using mammalian and *Xenopus* cell-free extracts and also purified proteins, show that both short patch and long patch BER take place. Thus the cellular choice and *in vivo* regulatory mechanisms of short and long patch BER sub-pathways governed by these damage-specific BER enzymes still remain intriguing.
- ii. *Posttranslational modifications of BER proteins*: There are many reports of modifications of BER proteins, including phosphorylation, acetylation, ubiquitination, etc., which dramatically affect organelle targeting, repair activity, as well as interactions [93,94]. We believe posttranslational modifications are fundamental means to regulate BER.
- iii. *Impact of chromatin structure on BER*: Little is known about how the BER proteins are recruited to the damaged site and perform their job in the highly condensed chromatin. Most *in vitro* repair studies have been carried out with naked DNA. It was shown earlier that repair activities are severely affected in the presence of histones [95]. Thus the DNA lesions in highly condensed chromatin of eukaryotic cells pose a challenge for the BER proteins to recognize, initiate and complete the repair process. Indeed, this may be one more reason for the evolution of repair- and transcription-associated BER (also, see below).
- iv. *Repair of DNA damage in active versus inactive regions*: The majority of the mammalian genomes (90-95%) is not transcribed in any given cell type; these inactive regions could thus tolerate significant variation and/or DNA base modification. However, the repair of active sequences, especially in terminally differentiated cells is critical for cell survival and homeostasis. Whether such repair of oxidized bases via BER or some other processes takes place remains to be elucidated. Future

challenge and excitement lies in unraveling those mysteries of mammalian DNA repair, particularly for repair of oxidative damage.

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Fig. 1. Sequence alignment of critical domains of OGG1 with NTH1, and NEIL1/NEIL2 with *E. coli* Nei/Fpg using T-coffee (<http://www.ch.embnet.org/software/Tcoffee.html>), the homology scores being indicated in color (blue to red). Positions from N-termini are numerically shown in the primary sequences. Critical side chains and boxes are indicated with triangles (catalytic Lys/Asp for OGG1/NTH1; catalytic Pro 1 and Lys for the NEILs; FeS clusters for NTH1 and CHCC type ZnF for NEIL2) or a bar (HhH or H2TH) along with the alignments. The consensus rows (Cons) depict identical match (*), chemically similar residues (“:” and “.”).

Fig. 2.

A model for APE and PNK-dependent BER pathways in mammalian cells. Three BER sub-pathways (I, II, and III) defined by the type and reaction mechanism of DNA glycosylases are shown. Monofunctional glycosylases (M) generate AP sites which are cleaved by APE1 to leave a 5' deoxyribosephosphate terminus. It is removed by Pol β producing a single-nucleotide gap necessary for nucleotide addition (pathway II). When NTH1 and OGG1 carry out β elimination, APE1 removal of the resulting 3' dRP generates a single nucleotide gap with a 3'-OH (pathway I). With NEILs as the initial glycosylase, a 3' phosphate terminus is generated which is then removed by PNK (pathway III). Reprinted from Ref. [57, Fig 7]. Copyright 2004 by Cell Press, with permission from Elsevier.

Fig. 3. Schematic representation of TCR and RAR of the nuclear genome: potential involvement of distinct DNA glycosylases. Transcription requires chromatin unfolding as shown by distinct appearance of nucleosomes in transcribed vs. nontranscribed regions of chromatin. ‘X’ indicates oxidative DNA damage, which may be present endogenously or is incorporated in the nascent strand. Transcription and replication complexes are shown. The requirement of chromatin unfolding in Global genome repair (GGR) is not indicated, and the naked form of DNA during RAR as depicted in this cartoon may not be accurate. Adapted from Ref. [6, Fig. 3], with permission from Elsevier.

Table 1
Comparative properties of human DNA glycosylases

	OGG1	NTH1	NEIL1	NEIL2
Size (kDa)	38	36	43	36
Preferred substrates	8-oxoG, Fapy G	Tg, 5OHU, DHU	Fapy A, Fapy G, Tg, 5OHU	Hydantoins (Sp, Gu), 5 OHU
Structural type	Nth	Nth	MutM/Nei	MutM/Nei
Conserved motif	HhH	HhH	H2TH	H2TH
Zn finger motif	Absent	Absent	Absent	Present
[4Fe-4S] cluster loop	Absent	Present	Absent	Absent
Catalytic residue	Lys 249	Lys 212	Pro 1	Pro 1
AP lyase product	3' dRP	3' dRP	3' P	3' P
Dispensable sequences	C-terminal 20 and N-terminal 10 aa	N-terminal 80 aa	C-terminal 101 aa	C-terminal 11 aa

*Sp, spiroiminodihydantoin; Gu, guanidinohydantoin.