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Neil3, the final frontier for the DNA glycosylases that recognize oxidative damage

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Abstract

DNA glycosylases are the enzymes that initiate the Base Excision Repair (BER) process that protects all organisms from the mutagenic and/or cytotoxic effects of DNA base lesions. Endonuclease VIII like proteins (Neil1, Neil2 and Neil3) are found in vertebrate genomes and are homologous to the well-characterized bacterial DNA glycosylases, Formamidopyrimidine DNA glycosylase (Fpg) and Endonuclease VIII (Nei). Since the initial discovery of the Neil proteins, much progress has been made on characterizing Neil1 and Neil2. It was not until recently, however, that Neil3 was shown to be a functional DNA glycosylase having a different substrate specificity and unusual structural features compared with other Fpg/Nei homologs. Although the biological functions of Neil3 still remain an enigma, this review highlights recent biochemical and structural data that may ultimately shed light on its biological role.

1. Introduction

The enzymes in the Base Excision Repair (BER) pathway are responsible for repairing the vast majority of endogenous lesions as well as various types of DNA damage produced during inflammation, by environmental chemicals and by ionizing radiation (for reviews see [1-4]). This pathway is highly conserved from bacteria to humans. If the lesion is a damaged base, repair is initiated by a DNA glycosylase that scans the DNA searching for the lesions it removes [5-7] (and for a review see [8]). Once a lesion is found, the glycosylase flips the damaged base into its active site pocket and cleaves the N-glycosyl bond releasing the damaged base (for a review see [9]). The glycosylases that recognize oxidized DNA bases fall into two structural families, the Helix-hairpin-Helix (HhH) superfamily and the Fpg/Nei family [9-11]. The founder of the HhH superfamily is endonuclease III (Nth) originally identified in *Escherichia coli* [12] but Nth orthologs are found across phyla including in humans [13-16]. The substrates for Nth are primarily oxidized pyrimidines. 8-Oxoguanine DNA glycosylase (Ogg), also an HhH superfamily member, removes oxidized purines and is primarily found in eukaryotes [17-21]. Another HhH superfamily member that excises oxidized bases is MutY. MutY is also highly conserved from bacteria to humans and specifically removes adenine misincorporated opposite 8-oxo-7,8-dihydroguanine (8-oxoG) and 2,6-diamino-4-hydroxyformamidopyrimidine (FapyG) [22-26].

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Formamidopyrimidine (Fapy) DNA glycosylase (Fpg) was originally identified in *E. coli* as a glycosylase that removes 2,6-diamino-4-hydroxy-5*N*-methylformamidopyrimidine (methylFapyG) from alkylated DNA [27, 28]. It was subsequently shown that its primary cellular role is to remove the products of free radical attack on purines such as 8-oxoG and FapyG [29-33]. Fpg is ubiquitous among the bacteria and is found in some fungi and plants. Endonuclease VIII (Nei) was also discovered in *E. coli* and recognizes oxidized pyrimidines with a substrate specificity that substantially overlaps that of Nth [34-36]. Nei proteins are sparsely distributed among the eubacteria and are primarily found in the *Actinobacteria*. Members of the Fpg/Nei family contain two conserved DNA binding motifs, an helix-two turns-helix (H2TH) motif and a zinc finger (for a review see [8]),

It wasn't until the 21st century that *in silico* analysis allowed the Wallace, Mitra, and Seeberg laboratories to identify three Fpg/Nei homologs in mammalian cells, namely endonuclease VIII like-1 (Neil1), Neil2 and Neil3 [37-41]. NEIL1 and NEIL2 were then successfully purified and characterized biochemically [37-42]. At about the same time, Takao and coworkers found residual activities in nuclear and mitochondrial extracts from the liver and lungs of *NTH1*$^{-/-}$ mice that were capable of removing thymine glycol (Tg) and urea from DNA. This work led to the characterization of the mouse ortholog of NEIL1 [43]. Because NEIL1 forms specific interactions with a number of replication proteins and its expression is cell cycle regulated, it has been suggested that it functions during replication to eliminate potentially mutagenic lesions [44-47]. In contrast, NEIL2 has a preference for lesions in single-stranded DNA and in bubble structures and interacts as well with a number of transcription factors including RNA polymerase II [39, 41, 42, 48]. Thus NEIL2 appears to act during transcription-coupled repair [48]. Neil3 has been the most elusive protein of the three Neil homologs. In the following sections we will highlight recent research that explores the final frontier for the DNA glycosylases that recognize oxidized DNA bases, that is, elucidation of the substrate specificity, structure and possible biological roles of Neil3.

2. The human and mouse *Neil3* genes

In the most current version of the human genome sequence, the *NEIL3* gene is located on Chromosome 4q34.3 and encoded by the plus strand. Two genes encoded by the minus strand flank the *NEIL3* locus, the *Aspartylglucosaminidase* (*AGA*) gene on the telomeric end, and the *Vascular endothelial growth factor c* (*VEGFC*) gene on the centromeric side. The *NEIL3* gene spans about 53.25kb with 10 exons that vary in size resulting in a full-length NEIL3 protein of 605 amino acids, having a predicted molecular weight of 68 kDa. According to AceView (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi), there are two putative alternatively spliced variants that would give rise to proteins of 233 amino acids and 127 amino acids [49]. The mouse *Neil3* gene is located on Chromosome 8 B1.3, is encoded by the minus strand and is flanked by *Aga* and *Vegfc on* the centromeric side and the telomeric end, respectively. The full-length mouse Neil3 protein (MmuNeil3) consists of 606 amino acids. There are nine potential variants of MmuNeil3 predicted from alternative mRNA splicing [49].

The promoter region of the *NEIL3* gene exhibits characteristics of the cell cycle-regulated genes, which are GC-rich but TATA-less [50]. Several transcription factor binding sites, such as the E2F family transcription factors, Specificity Protein 1 (Sp1), cAMP response element-binding protein (CREB), Nuclear respiratory factor 1 (NRF-1), and Nuclear transcription factor Y (NF-Y) binding sites, as well as cell cycle dependent element (CDE)/ cell cycle gene homology region (CHR) cis-regulatory elements were predicted with several of the E2F, Sp1 and the CDE/CHR binding elements being in close proximity to the transcription initiation site [50]. The cell cycle-dependent expression pattern of *NEIL3* has

been experimentally confirmed, showing induction in early S phase with the highest levels in G2 phase [50-52]. *NEIL3* induction has been shown to be controlled by the Ras-dependent ERK-MAP kinase pathway, while the repression of *NEIL3* in G0 arrested cells appears to be mediated by E2F4 binding through the DREAM complex (DP1, RB p130, E2F4 and MuvB core complex) to one of several putative E2F sites or CDE/CHR sites [50, 53-55].

Overall, the full length NEIL3 and MmuNeil3 proteins share 74% sequence identity. The N-terminal half of the Neil3 proteins are homologous to the bacterial Fpg/Nei proteins, with the signature helix-two turns-helix (H2TH) motif and the zinc finger motif for DNA binding. In the conserved N-terminus, a valine replaces the catalytic proline found in most of the Fpg/Nei family members. The C-terminal extension of the Neil3 proteins contains additional zinc finger motifs, a RanBP-like zinc finger and two GRF zinc finger motifs. Figure 1 shows an alignment of the structural features of NEIL3 compared to those of NEIL1 and NEIL2, and bacterial Fpg and Nei.

3. Substrate specificity of Neil3

Analysis of the substrate specificity of the Neil3 proteins was significantly hampered by the difficulty in expressing and purifying active proteins. Seeberg and coworkers had found that lysates from insect cells overexpressing NEIL3 exhibited elevated excision activity on MeFapyG compared to uninfected cell lysates [40], providing the first evidence for the glycosylase activity of Neil3. However, subsequent studies failed to detect any glycosylase activity in Neil3 proteins using either purified or *in vitro* translated Neil3 proteins [43, 56-58]. The purified NEIL3 glycosylase domain did, however, exhibit a weak lyase activity on a single stranded DNA substrate [58]. Recently a protocol for expressing and purifying active mouse and human Neil3 proteins has been published [59]. Here it was shown that the N-terminal initiator methionine processing is critical for the activity of Neil3 proteins since the primary amine of the adjacent valine serves as a nucleophile required for catalysis [59]. This protocol led to the successful characterization of the glycosylase/lyase activities of MmuNeil3 [60].

MmuNeil3 is a bifunctional DNA glycosylase that exhibits a broad substrate recognition spectrum and can excise both oxidized purines and pyrimidines but not 8-oxo-G. In oligodeoxyribonucleotides, the best substrates for MmuNeil3 are the further oxidation products of 8-oxoG, including spiroiminodihydantoin (Sp) and guanodinohydantoin (Gh) [60]. In ! -irradiated DNA, FapyA and FapyG are the best substrates but oxidized pyrimidines are also released [60]. Depending on the DNA sequence context, thymine glycol (Tg) can also be excised efficiently by MmuNeil3 (Zhou, et al, unpublished observation). In DNA containing single-stranded regions, in addition to Sp, Gh and Tg, the ring-saturated pyrimidines, dihydrothymine (DHT) and dihydrouracil (DHU), and the oxidized pyrimidines, 5-hydroxycytosine (5-OHC) and 5-hydroxyuracil (5-OHU) are all good substrates for MmuNeil3 [60]. Interestingly, MmuNeil3 exhibits a preference for lesions in single-stranded DNA as well as in bubble, fork and quadruplex structures ([60], Zhou et al, unpublished observation). Table 1 summarizes the substrate specificity of MmuNeil3 and for comparison, NEIL1 and NEIL2.

The glycosylase activity of human NEIL3 is similar to that of MmuNeil3 on both single-stranded and duplex DNA containing Tg and Sp [59]. NEIL3 and MmuNeil3 proteins exhibit very weak lyase activity which occurs primarily via β-elimination, leaving an α, β-unsaturated aldehyde at the 3'terminus [59, 60]. In contrast, most other Fpg/Nei family members exhibit robust lyase activity acting primarily via β,δ-elimination leaving cleavage products with 3′ phosphate termini (reviewed in [8, 41, 61]).

That Neil3 can function as a glycosylase *in vivo* has been demonstrated using *E. coli* cells deficient in combinations of various DNA glycosylases. Takao and coworkers observed that expression of the NEIL3 glycosylase domain in an *E. coli nth nei* mutant partially rescued the hydrogen peroxide sensitivity phenotype of these cells [58], suggesting an overlapping substrate specificity of NEIL3 with EcoNth and EcoNei proteins. It was further demonstrated that expression of the MmuNeil3 glycosylase domain in an *E. coli* triple mutant lacking Fpg, Nei, and MutY glycosylase activities greatly reduced both the spontaneous mutation frequency and the level of FapyG in the genomic DNA, suggesting that Neil3 plays an important role in repairing FapyG *in vivo* [60]. It is also possible that Neil3 proteins are responsible for repairing the further oxidation products of 8-oxoG *in vivo*, including Sp and Gh, since they are potentially mutagenic lesions [62, 63] and may contribute to the high mutation frequency observed in the triple mutant cells.

4. Structural features of Neil3

The crystal structure of unliganded MmuNeil3 has been solved (Liu, Imamura, Averill, Wallace and Doublié, manuscript under review). The catalytic core of MmuNeil3 exhibits a two-domain architecture similar to other Fpg/Nei family members. It contains a two-layered ! -sandwich flanked by ! -helices in the N-terminal domain, and a bundle of α-helices, two of which form the H2TH motif, followed by a zinc finger motif in the C-terminal domain. A strong positively-charged cleft lies between the N- and C-terminal domains of MmuNeil3 and is responsible for DNA binding. The active site of MmuNeil3 is located in this positively charged cleft between the two domains, where a valine serves the same function as the catalytic proline found in most other Fpg/Nei family members. In addition, one void-filling residue occupies the position of the everted lesion, in a manner similar to the analogous residue in other Fpg/Nei proteins [64-71]. Figure 2 shows a superposition of the catalytic domain of MmuNeil3 with that of human NEIL1.

The structure of MmuNeil3, however, presents two distinct features. First, the ! F-! 9-10 or “8-oxoG capping loop” which caps 8-oxoG in bacterial Fpg proteins [67, 69, 70, 72, 73], is truncated in Neil3. Therefore, this glycosylase cannot stabilize the flipped out 8-oxoG in the lesion-binding pocket. Secondly, Neil3 lacks two of the three canonical void-filling residues that stabilize the opposite strand after eversion of the base lesion [65, 68-71]. It also harbors acidic residues on either side of the damaged strand’s binding cleft, which create a negative charge and thus an electrostatic repulsion with the opposite strand. These distinct structural features of MmuNeil3 provide insight into the substrate specificity of Neil3 as well as its preference for DNA containing single-stranded regions.

5. Neil3 biology

The *in vitro* biochemistry studies have shown that the substrate specificity of the mouse and human Neil3 proteins largely overlap with those of Nth1, Neil1 and Neil2. *Neil3* orthologs are found only in the genome of vertebrates, organisms in which the brain and the adaptive immune system evolved. The question arises as to why vertebrates are equipped with Neil3 while other organisms can live without it. Since the discovery of Neil3, its biological role still remains an enigma; however, recent studies have provided insight into the expression patterns of *NEIL3* and *Neil3*, their subcellular localization and the phenotype of *Neil3* nullizygous mice thus providing hypotheses to be tested about the *in vivo* functions of the Neil3 proteins.

5.1 *Neil3* expression patterns

Using Northern blot analysis and semi-quantitative RT-PCR, *NEIL3* transcripts have only been found in thymus and testis [40, 57]. NEIL3 has also been shown to be highly expressed

in primary malignant melanomas associated with metastasis [74]. Moreover, tumor samples in general display a higher expression of NEIL3 than the corresponding normal tissues [75]. Mouse *Neil3* is also highly expressed in hematopoietic tissues such as spleen, bone marrow, thymus and in various mouse B cell lines [40, 57]. Using *in situ* hybridization, *Neil3* expression in the mouse brain has been localized to regions that harbor progenitor cells [76]. Apparently, expression of the mouse and human Neil3 proteins exhibits strong tissue specificity, which is unique for mammalian DNA glycosylases.

During embryonic development, MmuNeil3 shows a temporal expression pattern. For example, mouse *Neil3* transcripts were found to be highly expressed in the oocyte, unfertilized oocyte and zygote and this expression dramatically falls after the zygote stadium [75]. During embryogenesis, mouse *Neil3* transcripts were not detected until E8.5-11.5 which appeared to coincide with organogenesis [75]. In the mouse brain, *Neil3* is highly expressed in regions where neurogenesis occurs during embryogenesis and to a lesser extent in neonatal animals [75].

As mentioned in Section 2, human and mouse *Neil3* expression is also cell-cycle dependent. In mouse splenocytes, expression of *Neil3* is induced by mitogen stimulation [57]. Similarly, release from G0 by mitogen stimulation showed an induction of *NEIL3* expression in early S phase which was prolonged through G2/M phase in various cell lines, including human embryonic fibroblasts (HE), human fetal lung primary fibroblasts (MRC-5), human keratinocytes (HaCaT), and human epithelial breast cancer cells (MCF-7) [75]. In some genome-wide expression profiling screens, *NEIL3* was identified as being cell cycle-regulated with the highest expression in the G2 phase [51, 52].

5.2 Subcellular localization of the Neil3 proteins

The subcellular localization of NEIL3 was investigated by transient transfection of HeLa S3 cells with NEIL3 fused either by the C- or the N-terminus to the EGFP protein. In this study, recombinant NEIL3 was localized to the nucleus and in addition, it co-localized with Replication Protein A (RPA) [40]. Similarly, Torisu and coworkers, using polyclonal anti-MmuNeil3 antibodies, showed that recombinant MmuNeil3 is localized to HEK293T cell nuclei [57]. Also, in mouse thymocytes, Western blot analysis of nuclear and cytoplasmic fractions showed endogenous MmuNeil3 to be localized to the nucleus [57]. Thus far, Neil3 proteins have not been found in the mitochondria.

5.3 *Neil3* nullizygous mice

Torisu and coworkers generated a *Neil3* knockout mouse with a mixed genetic background, 129/Sv/Ev and C57BL/6, in which exon 1 of *Neil3* that contains the initiation codon and the coding sequence for the N-terminal catalytic residues was replaced by a *neo* cassette. These *Neil3* knockout mice were viable and apparently healthy for at least 24 weeks after birth and were able to reproduce, but they exhibited a tendency to have a reduced peripheral white blood cell count [57]. However, the mixed genetic background makes this result difficult to interpret. In addition, these mice were maintained in a pathogen-free environment, which might mask a potential phenotype in the immune system.

Another *Neil3* knockout mouse model in a pure C57BL/6 background has been reported by Sejersted and coworkers [77]. In this model, targeted disruption of the *Neil3* locus was achieved by replacing exons 3-5 that encode the fragment flanking the conserved H2TH motif with a *neo* cassette. Consistent with the previous result, these mice were viable, fertile and healthy into adulthood. However, a profound neuropathology after hypoxia-ischemia was observed, which was characterized by a reduced number of microglia and a loss of proliferating neuronal progenitors in the striatum [77]. In addition, aged *Neil3*$^{-/-}$ mice

exhibit learning and memory deficits and reduced anxiety-like behavior, which is associated with impaired neurogenesis and differentiation of the neural stem/progenitor cells [78]. In contrast to the phenotypes of mice deficient in other BER enzymes [79], including those deficient in glycosylases that recognize oxidative lesions [80-86] the phenotype of mice deficient in *Neil3* does not appear to be associated with genomic instability, but rather with impaired proliferative capacity of the neural stem/progenitor cells, highlighting the role of Neil3 in proliferating cells [77, 78].

6. What role does Neil3 play in the cell?

6.1 Neil3 might provide a "second line" of defense against oxidative DNA damage in proliferating cells

Neil3 is a bifunctional DNA glycosylase that reverses the phenotypes of *E. coli* cells deficient in the activities of several DNA glycosylases indicating that it is capable of repairing damaged DNA. Thus one potential role for Neil3 is to serve as a backup glycosylase to protect proliferating cells, the only cells where Neil3 is found, from the lethal and mutagenic effects of oxidative base lesions. Since Neil3 processes oxidative lesions in DNA with single-stranded regions, such as single-stranded DNA, fork, bubble and quadruplex structures ([59, 60] and (Zhou *et al.* unpublished observation)), it is possible that it might function during replication and/or in some special regions found in replicating genomes such as telomeres.

In fact there is some evidence suggesting that Neil3 might function during replication. Alignment of NEIL3 with known PCNA-binding proteins has identified a putative PCNA-binding motif (residues 410-416, Q1LDEEF) in NEIL3. Proliferating cell nuclear antigen (PCNA) is the sliding clamp responsible for DNA polymerase processivity [87]. As mentioned earlier, Morland *et al.* have shown that recombinant NEIL3 fused with EGFP protein co-localizes with RPA [40]. RPA is a single-stranded DNA binding protein which is essential for replication and other DNA transactions (reviewed in [88]). Studies have shown that the human MutY homologue (MYH) and uracil DNA glycosylase (UNG2) both localize to replication foci, interact with PCNA and RPA, and initiate post-replicative removal of lesions [89-91]. NEIL1 also physically interacts with PCNA and RPA and these proteins modulate its activity supporting the hypothesis that NEIL1 is involved in repairing the replicating genome [45, 47]. Since *Neil3* is expressed in proliferating cells and during S phase when replication takes place, it is possible that Neil3 proteins are also involved in replication-associated repair.

6.2 Neil3 might be involved in cell signaling

Like some DNA glycosylases that have functions beyond DNA repair, Neil3 proteins might not have evolved solely as a backup glycosylase to repair oxidative DNA damage. Instead, it might play a role outside of the canonical BER pathway. As mentioned Section 5.1, *Neil3* expression during pre-implantation exhibits the characteristics of a group of genes that have specific functions either in oogenesis, oocyte maturation, fertilization, and/or early phases of preimplantation development [75]. In addition, *Neil3* knockout mice do not exhibit the phenotypes associated with genomic instability, but rather, neural stem and progenitor cells were lost in these mice [77, 78], suggesting a function different from other oxidative DNA glycosylases. In a recent study, Reis and coworkers demonstrated that RNAi knockdown of Ogg1 and Neil3 decreased the ability of the embryonic neural stem cells (NSCs) to differentiate and resulted in decreased expression of both neuronal and astrocytic genes after mitogen withdrawal, as well as the stem cell maker Musashi-1 [92]. Furthermore, while cell survival remained unaffected, Neil3-deficient cells displayed decreased cellular proliferation rates along with an increase in HP1γ immunoreactivity, a sign of premature senescence

[92]. These results suggest that Neil3 might play a role in cell signaling pathways governing essential neural stem cell characteristics. Similarly, OGG1 has been shown to be involved in a cell signaling pathway that senses oxidative stress, contributes to the recruitment of transcription factors and the introduction of chromatin modifications, which results in the fine tuning of cellular processes that promote survival and successful cell specialization [93].

6.3 Neil3 and the immune system

A putative role in lymphocytes and/or other immune cells has been proposed for Neil3 since it is highly expressed in lymphatic cells and tissues and the *Neil3* knockout mice showed a slightly reduced number of white blood cells [57]. However, because of the mixed genetic background, the phenotypes of these mice are hard to interpret and the function of Neil3 in lymphocytes remains unknown. There are much data that indicate that DNA glycosylases in general are involved in adaptive immunity. For example, the cooperation of cytidine deaminase and uracil DNA glycosylase activities are central to the genetic transactions associated with antibody diversification in the adaptive immune system such as somatic hypermutation (SHM) and class switch recombination (CSR) [94, 95]. Neil1 has also been shown to be important for the rapid expansion of germinal center (GC) B cells as the Neil1 knockout mice exhibit reduced expansion of GC B cells, a decreased frequency of Ig gene hypermutation, as well as a lower production of antibody against a T-dependent antigen during both primary and secondary immune responses [96].

6.4 NEIL3 and HIV replication

In a recent genome-scale RNAi screen for host factors required for HIV replication, NEIL3 was identified as one of the novel host factors. Knockdown of NEIL3 expression by siRNA reduced HIV infection and this effect was rescued by expression of the cDNA in a non-targeted form [97]. However, the mechanism behind the possible involvement of NEIL3 in HIV replication is still unknown and warrants further investigation.

6.5 NEIL3 and cancer

NEIL3 is highly expressed in various human cancer cells and tissues and in primary malignant melanomas associated with metastasis, suggesting that NEIL3 is required for the maintenance of cancer cell growth or progression of malignancy [74, 75]. This observation is in keeping with the data showing that Neil3 proteins are only found in dividing cells. Also, using a high-throughput SNP array, *NEIL3* was shown to exhibit a high frequency of loss of heterozygosity in hepatocellular carcinomas suggesting that *NEIL3* might be a potential tumor suppressor gene supporting its potential role in DNA repair [98]. The function of NEIL3 in different types of cancer cells will be of interest for future studies.

7. The next steps: What does Neil3 interact with and how is it modified?

As with many other DNA glycosylases, the activity and functions of Neil3 proteins may depend on their interactions with other proteins. So far, there is little information about the interacting partners of Neil3 proteins. Besides PCNA and RPA, other downstream proteins involved in the cellular pathways initiated by Neil3 are still unknown. Neil3 harbors a unique insertion in its N-terminal domain and a long C-terminal extension, which are predicted to be disordered. These regions are likely to govern protein-protein interactions. In addition, the observation that either over expression or knock down of Neil3 in cells induces cell death suggests that Neil3 functions are tightly regulated [56, 92]. Thus identifying the interaction partners of Neil3 should help to elucidate the cellular pathways in which Neil3 proteins play a role.

The activity of Neil3 might also be modulated by post-translational modifications. Several sites of phosphorylation, acetylation, and ubiquitination have been predicted in human and mouse Neil3 proteins (PhosphoSitePlus; http://www.phosphosite.org). Further experiments are needed to verify these modifications and their potential roles in modulating the activity or functions of the Neil3 proteins.

8. Summary

Since Neil3 proteins were identified in vertebrate genomes about a decade ago, information has accumulated regarding their substrate specificity, structure, and expression profiles. Unlike most other DNA glycosylases that recognize oxidative DNA base lesions, NEIL3 and MmuNeil3 exhibit a marked preference for lesions in DNA with single-stranded regions [59, 60]. The crystal structure has revealed features of Neil3 that could contribute to its preference for single-stranded substrates: Neil3 lacks two void-filling residues that stabilize the DNA strand complimentary to the lesion-containing strand after eversion of the base. Neil3 also harbors negatively-charged residues which would hinder binding of the complimentary strand. Although the *in vivo* function of Neil3 remains unknown, recent data suggest that Neil3 activities are required in proliferating cells/tissues [50, 58, 60, 75-78, 92]. In addition, because Neil3 proteins have evolved in organisms with a developed brain and an adaptive immune system, it is plausible that, in addition to functions in DNA repair, Neil3 proteins might have acquired functions important for the development of brain and the immune system. Indeed, a recent study of *Neil3*$^{-/-}$ mice has established a link between Neil3 function and adult neurogenesis [78]. Future work is necessary to define the detailed molecular pathways involving the functions of Neil3 proteins.

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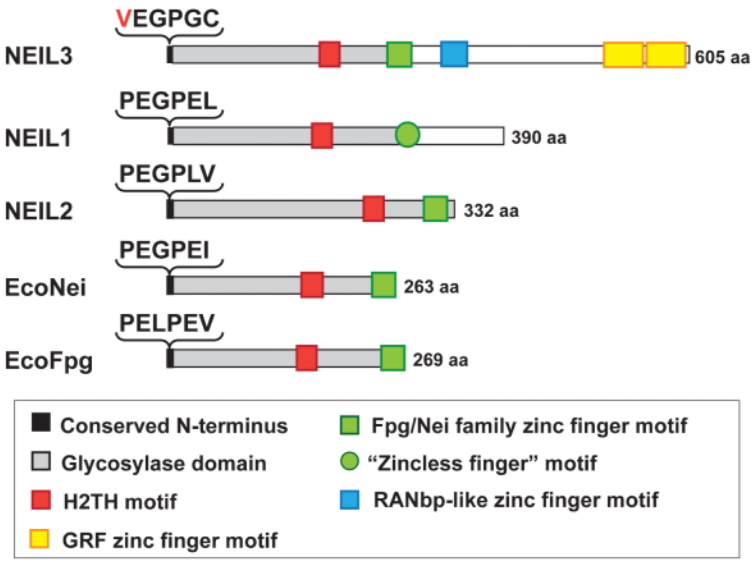

Figure 1.

Alignment of the structural features of NEIL3 with representative members of the Fpg/Nei family. Domains and motifs in the scheme are described in the box below. Sequences of the conserved N-terminus are shown in the bracket. NEIL1: human NEIL1; NEIL2: human NEIL2; EcoNei: *Escherichia coli* Nei; EcoFpg: *Escherichia coli* Fpg.

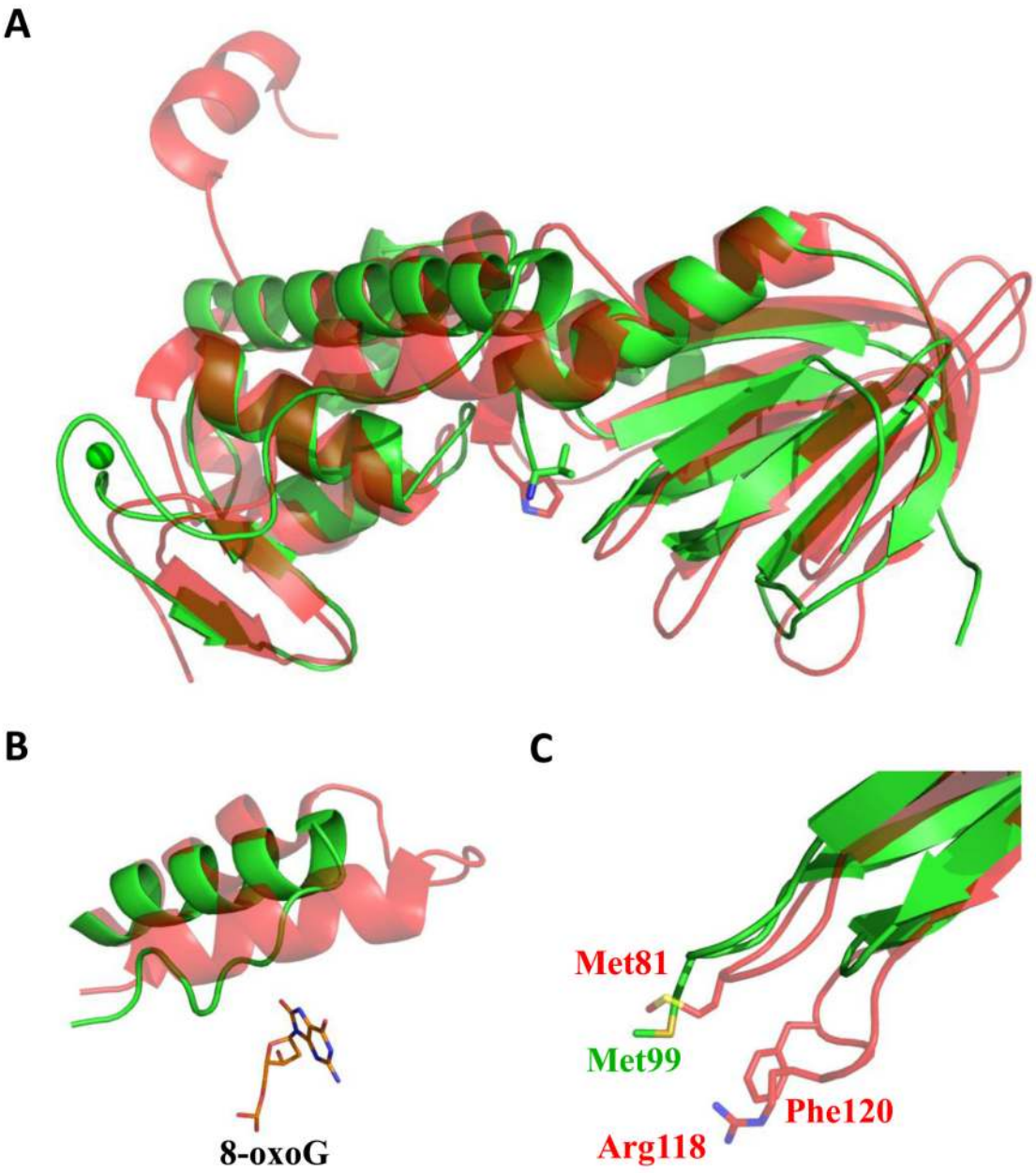

Figure 2.
Structural comparison of MmuNeil3 (colored in green) with NEIL1 (colored in red). (A) Superposition of the glycosylase domain of MmuNeil3 with that of NEIL1 (PDB ID code 1TDZ [99]). The N-terminal proline of NEIL1 and N-terminal valine of MmuNeil3 are represented as a stick model, and the zinc metal ion in MmuNeil3 is shown as a green sphere. (B) Close-up view of the ! F-! 9 loop. Superposition of the ! F-! 9 loop from MmuNeil3! 324 (residues 224-248) with that of NEIL1 (residues 228-261). The flipped-out 8-oxoG from the *Bacillus stearothermophilus* Fpg-DNA complex (PDB ID code: 1R2Y; [100]) is shown in orange. (C) Close-up view of the "void-filling residues" (Met81, Arg118 and Phe120 for NEIL1, and Met99 for MmuNeil3! 324).

Table 1

Comparison of the substrate specificity of Neil3 to that of Neil1 and Neil2.

Protein	Preferred lesions	Other lesions recognized	Preferred DNA structures	References
NEIL1	Sp, Gh, AP, FapyG, FapyA	DHU, DHT, Tg, 5-OHMH, 5-OHC, 8-oxoG, 8-oxoA, MeFapyG	Duplex DNA>> Bubble, Bulge, and Fork DNA> Single-stranded DNA	[1-11]
NEIL2	Sp, Gh, 5-OHC, AP	5-OHU, DHT, DHU, Tg, 8-oxoG,	Bubble, Fork, and Single-stranded DNA> Duplex DNA	[5, 8, 12-14]
MmuNeil3	Sp, Gh, Tg, FapyG, FapyA	DHU, DHT, 5OHMH, 5-OHC, 5-OHU, 8-oxoA, AP	Large bubble and Single-stranded DNA> Small bubble, Fork DNA>> Duplex DNA	[6, 9, 15, 116]

8-oxoG, 8-oxo-7,8-dihydroguanine;

8-oxoA, 7,8-dihydro-8-oxoadenine;

FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine;

FapyA, 4,6-diamino-5-formamidopyrimidine;

MeFapyG, 2,6-diamino-4-hydroxy-5N-methylformamidopyrimidine;

Sp, Spiroiminodihydantoin;

Gh, Guanidinohydantoin;

Tg, thymine glycol;

5-OHC, 5-hydroxycytosine;

5-OHU, 5-hydroxyruacil;

5OHMH, 5-hydroxy-5-methylhydantion;

DHT, 5,6-dihydrothymine;

DHU, 5,6-dihydrouracil;

AP, apurinic or apyrimidinic site;