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The Fpg/Nei Family of DNA Glycosylases: Substrates, Structures and Search for Damage

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Abstract

During the initial stages of the base excision DNA repair (BER) pathway, DNA glycosylases are responsible for locating and removing the majority of endogenous oxidative base lesions. The bifunctional formamidopyrimidine DNA glycosylase (Fpg) and endonuclease VIII (Nei) are members of the Fpg/Nei family, one of the two families of glycosylases that recognize oxidized DNA bases, the other being the HhH/GPD (or Nth) superfamily. Structural and biochemical developments over the past decades have led to novel insights into the mechanism of damage recognition by the Fpg/Nei family of enzymes. Despite the overall structural similarity among members of this family, these enzymes exhibit distinct features that make them unique. This review summarizes the current structural knowledge of the Fpg/Nei family members, emphasizes their substrate specificities, and describes how these enzymes search for lesions.

Keywords

Base excision repair; bifunctional-DNA glycosylase; lesion recognition, structure and substrates; substrate specificity

I. Introduction

Escherichia coli formidopyrimidine (Fapy) DNA glycosylase (Fpg) was originally discovered in Tomas Lindahl's laboratory as a DNA glycosylase that removes methylFapyG from alkylated DNA (1). The *E. coli* gene for Fpg was subsequently cloned (2) and the protein further characterized in a number of laboratories (3-6). *E. coli mutM* mutants were identified in Jeffrey Miller's laboratory as mutators that gave rise to G→T transversions (7). When the MutM protein was subsequently purified, it was found to be identical to Fpg (8). Following these initial findings there were a number of biochemical studies showing that 8-oxoguanine (8-oxoG) was also a substrate for Fpg and that Fpg preferred 8-oxoG over methylFapyG (9,10). Because of this substrate preference and because guanine is the most readily oxidized DNA base, the conclusion was drawn that 8-oxoguanine was the biologically relevant substrate for Fpg. These studies led to the formulation of the GO model for 8-oxoG repair (11) which proposed that when guanine is oxidized to 8-oxoguanine, it is

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removed by Fpg. If 8-oxoG is not removed prior to replication, A is often inserted opposite the 8-oxoG by DNA polymerases (12-15). If this occurs, the A can be removed by another glycosylase called MutY (16). The GO model also included MutT that removes 8-oxoguanine nucleoside triphosphates from the nucleotide pool by hydrolyzing them to 8-oxodGMP (17). Taken together these data supported the idea that 8-oxoguanine is a biologically important, potentially mutagenic oxidative DNA lesion. However recent studies have shown that unmethylated FapyG is also a good substrate for Fpg (18,19) and like 8-oxoG, A can also be incorporated opposite FapyG (20,21) and the incorporated A can be removed by MutY (22). FapyG, which is formed from the same adduct radical as 8-oxoG (23), appears to be responsible for a substantial number of mutations originally attributed to 8-oxoG and thus is also a biologically relevant substrate (24).

E. coli nei (endonuclease VIII) was originally discovered in the Wallace laboratory as an activity that recognizes oxidized pyrimidines (25,26). The gene was cloned and the protein sequence was shown to be very similar to that of Fpg (27). *nei* mutants had little or no phenotype, but, when coupled with an *nth* mutation, they were mutators leading to C→T transitions (27). The *nth* gene encodes endonuclease III which also recognizes oxidized pyrimidines with a substrate specificity that substantially overlaps that of Nei (for reviews see (28,29)).

It was not until the twenty-first century and the sequencing of the human genome that *in silico* analysis allowed the Wallace, Mitra, and Seeberg laboratories to identify, clone and characterize three Fpg/Nei homologs in mammalian cells, the so-called Neil1 (nei-like), Neil2 and Neil3 proteins (30-34). Mouse Neil1 and Neil3 were also found in mice nullizygous for *nth* (35). The substrate specificities of human NEIL1 and NEIL2 have been well-characterized (30-34,36-40). In addition, NEIL1 forms specific interactions with a number of replication proteins and is cell cycle regulated (41-44). Thus, it has been proposed that NEIL1 acts as a cow catcher ahead of the replication fork, eliminating potentially mutagenic lesions (42-44). NEIL2 prefers lesions in single-stranded DNA over duplex DNA and interacts with a number of transcription factors including RNA polymerase II and has been suggested to act in transcription-coupled repair (45). Although attempts had been made to determine the activity of NEIL3 (33,46,47), it has only been recently that NEIL3 has been purified and characterized (24,48) and its glycosylase activity shown to be similar to that of NEIL2 (24). In mice, Neil3 is present during embryonic development (49) and was found in brain stem cells (49,50). In humans, expression of NEIL3 has only been observed in thymus (51).

II. Fpg/Nei Phylogeny

Sequence alignments of members of the Fpg/Nei family of glycosylases indicate that they share many structural and biochemical features (34). Some of the hallmark motifs of this family include conserved residues in the helix-two-turns-helix motif (H2TH), a zinc finger motif, and a common catalytic mechanism involving either an N-terminal proline (for example in NEIL1 and NEIL2) or a valine residue (as in human NEIL3 and the giant mimivirus Nei2 (MvNei2)) as the active site nucleophile. Despite these commonalities, each glycosylase prefers a different spectrum of oxidative lesions. Moreover, some of these

subfamilies have changed significantly in sequence from their common ancestor, making it difficult to infer the evolution of these enzymes.

Phylogenetic analysis and functional studies of the Fpg/Nei family indicate that in *Actinobacteria* alone, six gene clades occur, two within the Nei proteins and four within the Fpg clade (52). The plant and fungi clade is clearly part of the Fpg family while within metazoans, Neil2 and Neil3 form their own clade separate from Neil1. The Neil1 protein, like members of the plant and fungi Fpg/Nei proteins, does not have the canonical zinc finger, but possesses a "zincless-finger" motif, which lacks the four characteristic cysteine residues that coordinate a zinc ion. This motif superimposes well with the zinc finger domains of EcoNei and EcoFpg, despite the absence of sequence homology. In contrast to Neil1, both Neil2 and Neil3 possess a zinc finger domain: the former contains a C-H-C-C-type zinc finger whereas the latter has a RanBP-type zinc finger very similar to the one found in bacterial Fpg. Some shared conserved structural features suggest that the zincless fingers evolved independently of the zinc finger motifs. Recent evidence suggests that the Neil2 and Neil3 proteins evolved from a common ancestor while Neil1 evolved separately (Barrantes-Reynolds, unpublished data).

We speculate that horizontal gene transfer, a common occurrence in bacteria, seems to be a likely event in the initial evolution of EcoNei proteins from a common ancestor which contained at least one Fpg/Nei homolog and exhibited features similar to EcoFpg (53). Vertical evolution may have been responsible for the transfer of an early Fpg/Nei gene to early eukaryotes in which these Fpg/Nei homologs led to the diversification of the Fpg/Nei proteins in higher eukaryotes (34,52,53).

III. Fpg/Nei Structures

A. Introduction

Over the past decade, there has been a significant increase in the number of crystal structures of Fpg/Nei glycosylases (54-69). The advent of techniques such as reductive cross-linking using sodium borohydride has played an essential role in trapping stable protein-DNA complexes for the purposes of crystallization and to elucidate the mechanism and role of these intricate enzymes ((54-56,64-66,70,71) and for reviews see (70,71)). Other approaches successfully used to produce stable glycosylase/DNA complexes include the generation of site-directed mutants of active site residues to abolish catalysis and the use of non-cleavable substrates such as tetrahydrofuran (THF) that mimics an abasic (AP) site (60,72) and non-cleavable cyclopentane FapyG (cFapyG) (68). A summary of all the currently available crystal structures of the Fpg/Nei family of glycosylases and their substrate preferences is listed in Table 1. Crystal structures of Fpg proteins from various bacterial species like *Thermus thermophilus* (Tth) Fpg (without DNA)(54), *Escherichia coli* (EcoFpg) (55), *Geobacillus stearothermophilus* Fpg (BstFpg) (57,59,64-66) and *Lactococcus lactis* Fpg (LlaFpg) (58,60,63,68,69) complexed with DNA substrates have been determined. The Fpg/DNA complexes include Schiff base intermediates, non-covalent complexes with AP-site analogs, and recognition or end-product complexes. Although the structure of EcoNei as a Schiff base intermediate in a complex with DNA was solved (56), it wasn't until recently that the unliganded structure of EcoNei was determined which revealed

a unique and interesting interdomain global conformational change upon DNA binding (62). Furthermore, the structures of unliganded human NEIL1 (61), unliganded MvNei1 and MvNei1 in a complex with THF were subsequently obtained (67). The first crystal structures of an Nei bound to damaged bases were recently reported: MvNeil1 was captured in a complex with DNA containing either thymine glycol (Tg) or 5-hydroxyuracil (5-OHU). 104

Overall, the structures of the Fpg and Nei proteins are similar, with a distinct 2-domain architecture connected by a flexible hinge region (Figure 1A and B using EcoFpg and EcoNei as examples) (55,56,72). In general, the N-terminal region is predominantly β-sheet rich and is composed of a β-sandwich flanked by α-helices. The C-terminal domain comprises α-helices, two of which form a conserved H2TH motif, as well as two anti-parallel β-strands that fold into a zinc finger motif. These signature motifs are characteristic of both Fpg and Nei subfamilies. The zinc finger and H2TH motifs have been shown to be absolutely required for Fpg to bind to DNA (72-74). In addition to structural similarity, the members of this superfamily exhibit a similar multi-step catalytic mechanism that generally involves a nucleophilic attack at the C1′ position of the target nucleotide by an N-terminal proline residue (in the case of Fpg, Nei, and NEIL1) (75,76). A comparison of these structures is further discussed below.

B. Substrate Preference

Bacterial Fpg proteins characteristically excise oxidized purines, whereas bacterial Nei and the Neil proteins excise oxidized pyrimidines (for reviews see (28,29,34,71,72)). Interestingly, the Fpg proteins share similar substrate specificity with Ogg whereas the substrate specificity of the Nei family members overlaps with that of the Nth family. However, all Fpg/Nei family members share sequence homology. Glycosylases vary in their discrimination of the bases opposite the lesion as well as their preference for the nature of the DNA, *i.e.* single-stranded DNA, double-stranded DNA, or bubble-containing substrates. Fpg primarily exhibits a substrate preference for purines such as 8-oxoG and FapyG, albeit oxidized pyrimidines are also removed (77,78) whereas bacterial Nei and the rest of the eukaryotic family members such as NEIL1 and NEIL2 recognize a wider array of substrates.

Recently, it was determined that EcoFpg is more efficient at removing spiroiminodihydantoin (Sp), a further oxidation product of 8-oxoG, from double-stranded DNA substrates than 8-oxoG itself (79,80). EcoNei, like endonuclease III, recognizes Tg, dihydrothymine (DHT), β-ureidoisobutyric acid and urea residues (for reviews see (28,29,34,72,76). EcoNei can also recognize 5-hydroxycytosine (5-OHC), 5-hydroxyuracil (5-OHU) and uracil glycol (81). MvNei1 and NEIL1 share substrate preferences for oxidized pyrimidines in duplex DNA and also recognize and process lesions from single-stranded DNA (82,83). Although 8-oxoG is not a preferred substrate for NEIL1, its further oxidation products guanidinohydantoin (Gh) and Sp are both excellent substrates for these enzymes when paired opposite C rather than A (39,40,83). The NEIL1 protein also excises Tg, 5,6-dihydrouracil (DHU), FapyA and FapyG, as well as 5-OHU, 5-OHC, and oxanine (30,31,84-86). Bacterial Fpg (87,88) and all the eukaryotic members of the Fpg/Nei family recognize lesions in single-stranded DNA (31,36,83). NEIL2 and MmuNeil3 prefer to excise

lesions present in single-stranded, bubble or forked DNA structures over duplex DNA (24,36,39,42-44,48).

C. Comparison of Structures of the Fpg/Nei Family

As mentioned above, there are currently crystal structures of Fpg proteins from four bacterial species, namely TthFpg, EcoFpg, BstFpg, and LlaFpg (54,55,57-60,63-66,68,69). All four proteins share the same domain structure and considerable sequence homology. Structures of intermediates covalently linked to duplex DNA indicate that the DNA binds to the enzyme in a positively charged groove that runs roughly orthogonal to the DNA axis (55,59). Bacterial Fpg binds DNA in the minor groove and the damaged base is extruded through the major groove. The DNA appears to be severely kinked at the lesion point (~66º roll angle in the case of EcoFpg (55)) upon enzyme binding thereby allowing the extruded base to be positioned in the active site for catalysis. The minor groove is widened considerably at the lesion site, however, the rest of the DNA duplex surrounding the lesion retains canonical B-form (55,59). Upon nucleotide eversion, three highly conserved residues in the bacterial Fpg proteins namely Met74, Arg109 and Phe111 (in EcoFpg) fill the void that is created and stabilize the opposite base (Figure 2A) (55). Met74 is part of the β4/5 loop and occupies the position of the extruded base by entering through the minor groove while Arg109 and Phe111 are part of a loop connecting strands β7 and β8. Phe111 is wedged between the base opposite the lesion (a cytosine) and the neighboring base, and causes unstacking of these bases leading to the severe kinking of the DNA. Additionally, Arg109 forms H-bonds with the opposite base leading to discrimination against A as the opposite base (55,89).

The analogous residues in MvNei1 (Leu84, Arg114, and Phe116) (61), human NEIL1 (Met81, Arg118, and Phe120) (67) and *Arabidopsis thaliana* Fpg (AthFpg) (Met78, Arg126 and Phe128) (Stephanie Duclos, Pierre Aller, Pawel Jaruga, Miral Didzaroglu, Susan S. Wallace and Sylvie Doubie, manuscript submitted to DNA Repair) are similar to those seen in the bacterial Fpg proteins which discriminate against A as a base opposite the lesion (89). In contrast, EcoNei inserts three consecutive residues Gln69, Leu70 and Tyr71 into the void created upon base extrusion (56). The three residues are located on a loop connecting β4 and β5. Tyr71 is wedged between the orphaned base and its 3′ neighbor and stabilizes the severely kinked DNA. The void-filling residues are lacking in MmuNeil3, which appears to be related to the preference of this enzyme for single-stranded DNA (Minmin Liu, Kayo Imamujra, Sylvie Dolublie and Susan S. Wallace, manuscript in preparation).

In addition to the void-filling residues, the Fpg/Nei proteins contain an absolutely conserved Lys residue (Lys57 in EcoFpg) and a conserved Asn (Asn169 in EcoFpg), which is part of the H2TH motif (55). Lys 57 forms salt-bridges with P^{-1} and P^{-2} while Asn169 forms bonds through backbone and side-chain amides to P^{-1} and P^{0} of the DNA (Figure 2B and C). Another highly conserved residue in the Fpg/Nei family of proteins is Arg259 (in EcoFpg), which is part of the zinc finger motif and is involved in the formation of salt bridges with the phosphodiester backbone (55) (Figure 2B). In the bacterial Fpg and eukaryotic Fpg/Nei proteins, there is no gross conformational change in the overall domain structure upon DNA binding (55,57,59,64,72). On the other hand, the side-chains of the

void-filling residues and conserved residues in the hallmark motifs, show small changes. The development of disulfide-crosslinking techniques used to study the structure of BstFpg bound to lesion-containing DNA and undamaged DNA indicate that the enzyme possesses intrahelical recognition of the damage and can detect the subtle differences between the damaged base and its undamaged counterpart even at an initial encounter ((64,65) and see below). Comparing 8-oxoG with guanine in DNA suggests that the enzyme induces a local conformational change in the DNA backbone in which the sugar pucker (C2′-endo) adopts a different conformation (C4′-exo) to prevent a steric clash between the 8-oxo group of 8-oxoG and the C2′ of the sugar (64).

A loop region (called the αF-β9/10 loop) in the α-helical C-terminal domain of the Fpg proteins is presumed to be involved in lesion recognition. In the unliganded structure of TthFpg, this lesion recognition loop is ordered (54), but in structures of BstFpg bound to DNA containing an AP site, the density for this loop disappears suggesting that this region is disordered (57). In the presence of lesion-containing DNA in complex with catalytically inactive enzyme, the density for this loop resurfaces indicating conformational mobility upon catalysis (59,64,65). This loop plays a key role in the recognition of 8-oxoG: a projection from the loop wraps around the damaged base forming an extensive network of hydrogen bonds (59). This same loop was shown to wrap around FapyG in the LlaFpg structure (60). The major difference between the binding of Fpg to 8-oxoG and FapyG lies in the fact that binding in the extrahelical base-binding pocket of Fpg for the former lesion occurs in the *syn* conformation whereas FapyG is in the *anti* conformation. However, despite the difference in base conformations, a similar type of interaction exists between the main-chain carbonyl carbons of conserved residues S218 (in LlaFpg) and S221 (in BstFpg), which bind to the protonated N1 and N7 of FapyG and 8-oxoG, respectively. Similarly, the conserved I220 (in LlaFpg) and the analogous V223 (in BstFpg) use their main-chain group for hydrogen bonding with the carbonyl moiety at position 6 of both lesions (60).

The αF-β9/10 loops of bacterial Fpgs are functionally similar and are of comparable length (~27 residues) and conformation. In contrast, in the eukaryotic members of the family, which do not recognize 8-oxoG, this putative lesion-recognition loop is generally shorter or even missing as in the case of NEIL1 (61), AthFpg (Stephanie Duclos, Pierre Aller, Pawel Jaruga, Miral Didzaroglu, Susan S. Wallace and Sylvie Doublie, Manuscript submitted to DNA Repair) and MmuNeil3 (Liu et. al., manuscript in preparation). A superposition of BstFpg bound to DNA (containing 8-oxoG:C, (59)) with the unliganded human NEIL1 (61) and the MvNei1 enzymes illustrates that this loop wraps around the lesion only in the case of BstFpg (Figure 3). In contrast, in the case of NEIL1, the loop is replaced by an α-helix; the loop is shorter in MvNei1, and in both cases this segment is unable to wrap around the lesion (Figure 3). These data are consistent with the fact that 8-oxoG is not a good substrate for NEIL1 or any of the eukaryotic and mimivirus enzymes that are missing this loop. In fact, deletion of the αF-β9/10 loop in EcoFpg yielded a variant that retains catalytic ability on oxidized pyrimidines and FapyG, but not 8-oxoG, implying that this loop is important for stabilizing 8-oxoG and not the other lesions (Stephanie Duclos, Pierre Aller, Pawel Jaruga, Miral Didzaroglu, Susan S. Wallace and Sylvie Doublie, Manuscript submitted to DNA Repair).

The bacterial Nei proteins are composed of members that share several characteristics with Fpg but also provide some unique variations. The crystal structure of EcoNei reveals a similar structural fold and conservation of motifs present in the Fpg proteins (56,62). A striking difference between the unliganded and DNA-bound EcoNei structures is a large conformational change of about 50 degrees between the N- and C-terminal domains. This is the only DNA glycosylase to date that has been reported to display a DNA-induced global conformational change, in which the glycosylase transforms from an elongated “open” form to a “closed” DNA-bound form (56,62). This conformational change was not observed for the MvNei1 protein upon DNA binding (67).

The structure of human NEIL1 reveals the presence of a structural motif composed of two antiparallel β-strands that mimic the zinc finger fold. This motif superimposes well with the zinc finger of EcoNei and the bacterial Fpg proteins (59,61) (Figure 3). However, the canonical Cys residues, and the loops connecting the β-strands of the zinc finger are missing in NEIL1, which prevents the coordination of a zinc atom (Figure 3). This motif termed “zincless-finger” contains a highly conserved Arg277 residue which, when mutated, significantly diminishes glycosylase activity (56,61). This zincless motif is also harbored by MvNei1 (61,67), and the plant and fungal Fpg glycosylases (Stephanie Duclos, Pierre Aller, Pawel Jaruga, Miral Didzaroglu, Susan S. Wallace and Sylvie Doublie, Manuscript submitted to DNA Repair).

In summary, members of the Fpg/Nei family are structurally similar, but display significant variations in conserved domains/motifs involved in DNA interactions. One of the main differences between EcoNei and the bacterial Fpg proteins is the composition of the void-filling, intercalation triad. In EcoNei, all three residues are located on the same β4/β5 loop and are consecutive, i.e. Gln69, Leu70 and Tyr71. In bacterial Fpg proteins, NEIL1, MvNei1 and AthFpg as mentioned above, the residues that constitute the triad reside in two different loops. Another difference between EcoFpg and the eukaryotic family members lies in the lesion-recognition loop located in the C-terminal domain of both proteins. In bacterial Fpg, the damaged base is everted from the DNA helix and is enveloped in a deep pocket, which is capped by the lesion recognition loop at one end (Figure 3). This loop is missing in EcoNei and the eukaryotic members for which a structure exists, including NEIL1, AthFpg and Neil3 and appears to be required for excising 8-oxoG. The vast repertoire of substrates of the Fpg/Nei family members and their different preferences for opposite bases and DNA substrates (single-stranded, double-stranded, or bubble DNA substrates) warrants further structural and biochemical scrutiny.

IV. Glycosylases Search for Lesions

It has long been a question in the field as to how DNA glycosylases locate the lesions they recognize in a sea of undamaged bases. This issue is complicated by the fact that a glycosylase flips out the damage from the DNA helix into its active site pocket in order to perform its enzymatic function. Furthermore, glycosylases do not use biochemical energy and rely on thermal energy so that lesions are found through random collisions between the glycosylase and the DNA molecule. Because of this, three-dimensional diffusion is considered to be too inefficient to account for the number of lesions the glycosylase must

excise. Glycosylases are thought to bind to a non-specific site on the DNA molecule and slide along the DNA by one-dimensional diffusion until the enzyme finds the lesion or disassociates from DNA. There have been a number of hypotheses proposed for the lesion search itself. One model suggests that the glycosylase binds to an extruded DNA lesion and then moves along the DNA testing every single base (90). This appears to be unlikely since both kinetics (91) and single-molecule studies (92,93) have shown that glycosylases scan DNA close to diffusion limits making it thermodynamically impossible to sequentially extrude and examine every base. In the second model, the DNA glycosylase traps a randomly extruded damaged base. This extrusion is more likely with lesions since hydrogen bonding and stacking interactions would be altered compared to the normal bases. This appears to be the mechanism used by uracil DNA glycosylase (94). In the third model, glycosylases slide along the DNA molecule and are able to recognize their particular substrate by specific interactions between the glycosylase and the DNA molecule. This model has been suggested by structural studies (64,65) and by a recent single-molecule study (95).

Two groups have attempted to address the question of how glycosylases search for a lesion using single-molecule approaches (92,93,95). In the first study (92), human OGG1 (oxoguanine DNA glycosylase, a member of the HhH superfamily) labeled with Cy3, was observed to undergo one-dimensional sliding along DNA that was stretched by shear flow. A similar diffusive motion was observed with BstFpg. These same authors showed that the one-dimensional diffusion constants measured were consistent with the glycosylases diffusing along the DNA helix in a rotational manner (93). In a recent study from our laboratory (95), quantum dot-labeled *E. coli* Fpg, Nei, and Nth were imaged in the absence of flow. In this study the glycosylases were shown to diffuse along the DNA with a broad distribution of rates that ranged over two orders of magnitude. This broad distribution was common to all three glycosylases suggesting that both the Fpg/Nei family and HhH superfamily scan using a similar mechanism. When the diffusive behavior was analyzed further, the three glycosylases were shown to exhibit a continuum of motion that was in keeping with rotational diffusion along the DNA molecule and that ranged from a slow, subdiffusive to a faster, unrestricted diffusive behavior.

As described earlier, members of the Fpg/Nei family of DNA glycosylases have three void-filling residues that are inserted into the DNA helix and aid in flipping out the damaged base, and as well stabilize the DNA helix (55,56,59,64-67). The HhH superfamily uses a similar mechanism (96,97). Interestingly, a crystal structure of BstFpg crosslinked to undamaged DNA revealed that one of these void-filling residues, a phenylalanine, was found to be wedged into the helix occupying a position analogous to its position in the Fpg complex bound to damage-containing DNA (64) (Figure 2A). These data, together with kinetics data (98,99), suggest that the phenylalanine may be acting as a wedge that scans for deformability of the base pair such as in the sugar pucker. Interestingly, when the corresponding *E. coli* wedge residue, Phe111, was mutated to an alanine, there was a significant increase in the mean diffusion constant compared to the wild-type protein (95). Moreover, the diffusive properties characteristic of wild-type were altered, that is, the slow, subdiffusive population of glycosylases was selectively lost. Similar results were observed

when the analogous residues in Nei and Nth were mutated (Dunn *et al.* unpublished observations) suggesting that the slow subdiffusive glycosylases are those interrogating the DNA for damages. Taken together, the data support the idea that the Fpg/Nei family of DNA glycosylases diffuse one-dimensionally along the DNA molecule with diffusion constants that are consistent with rotation around the DNA molecule, presumably in the minor groove where they bind. It also appears that at least part of the glycosylase search mechanism may be accomplished by insertion of a particular wedge residue that senses the topography of the minor groove and pauses either to check for damage at random locations or in response to subtle deformations of the DNA helix.

V. Concluding Remarks

Advances in the structural biology and biochemistry of glycosylases have led to a better understanding of how these complex enzymes recognize and excise damaged bases. Based on current *in vitro* studies, we can speculate on the mechanisms of specific lesion recognition. However, despite the vast knowledge gained, several unanswered questions still remain. For instance, we know that the Fpg/Nei family members recognize a broad range of substrates but it is not clear how these enzymes discriminate among each of these lesions and how they distinguish these from undamaged bases. Moreover, as some glycosylases are active at different times in the cell cycle and interact with a number of protein partners, how are these enzymes involved in processes such as DNA replication or transcription? Additionally, it remains difficult to classify certain members of the Fpg/Nei family under a specific subfamily. For example, even though members of the Fpg/Nei family of proteins are structurally similar, some elements such as the intercalation triad present in NEIL1 and MvNei1 suggest that these members could be classified under the Fpg sub-family contrary to the Nei sub-family after which they were originally named. Many aspects of phylogenetic characterization, lesion recognition, substrate specificities and the biological functions of this glycosylase family still remain to be elucidated.

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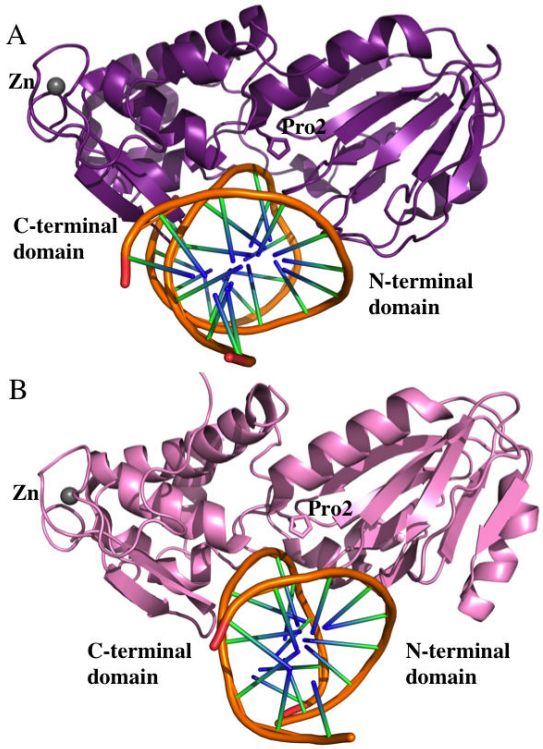

Figure 1. Overall structural comparison between EcoFpg and EcoNei

A. EcoFpg bound to DNA. Both and N- and C-terminal domains are colored in purple (PDB ID 1K82) (55). B. EcoNei bound to DNA. N- and C-terminal domains are shown in pale pink. (IK3W from the PDB) (56). Zinc atoms are shown as gray spheres in both cases and the DNA is displayed as a ribbon. PyMol was used to generate the Figures (DeLano Scientific, The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.).

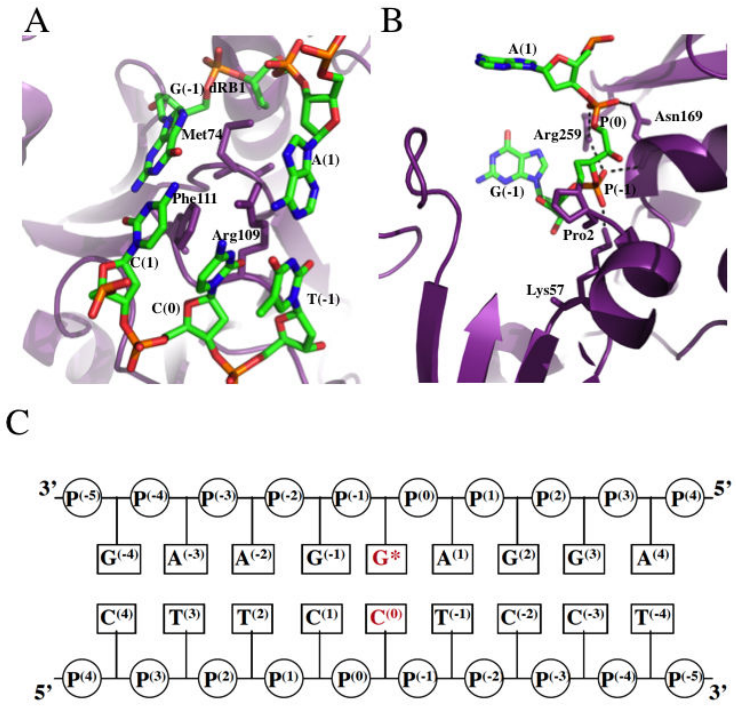

Figure 2. Specific interactions between EcoFpg and DNA

A. Triad of void-filling residues Met74, Phe111 and Arg109 that intercalate into the DNA causing severe kinking at the site of the damage. B. Interaction of conserved residues Lys57, Asn169 and Arg259 with DNA phosphates surrounding the ring-opened deoxyribitol moiety (dRb1) (PDB ID code 1K82 (55)). C. DNA sequence context present in the crystal structure of EcoFpg bound to DNA indicating the typical nomenclature used to describe the phosphates and the bases surrounding the lesion. The lesion is indicated by G* while $C^{(0)}$ is the opposite base, both of which are indicated in red lettering.

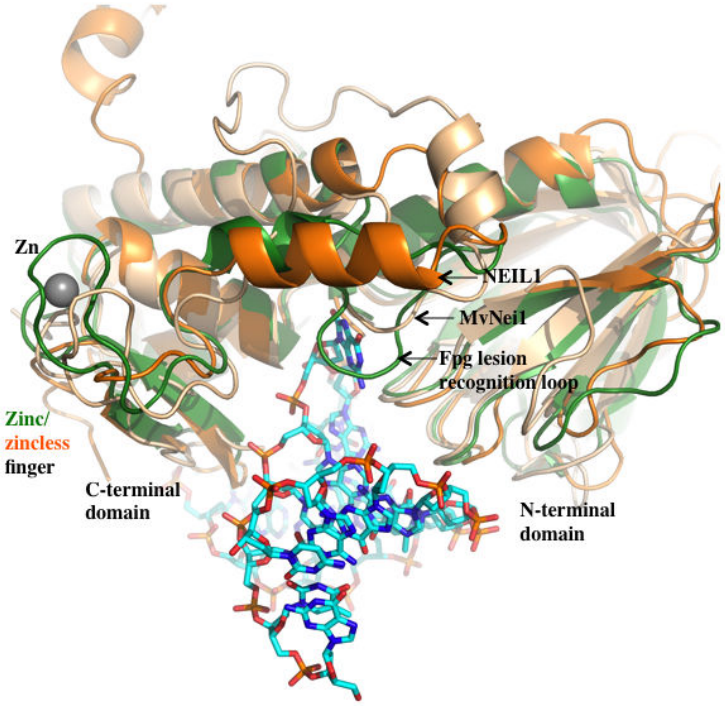

Figure 3. Superposition of BstFpg (E3Q mutant, green) bound to DNA containing 8-oxoG with human NEIL1 (orange) and MvNei1 (beige)

Overall the three proteins superimpose well with an RMSD of 1.1 Å – 1.3 Å upon aligning C-α of analogous residues from the NEIL1 and MvNei1 with BstFpg (performed using COOT (100) and Superimpose (M. Rould, personal communication)). Key differences among the three enzymes are the zinc/zinc-less finger in Fpg vs. NEIL1 and MvNei1, and the presence of the lesion-recognition loop in Fpg. The 8-oxoG containing DNA is displayed as a ball and stick model. (PDB ID codes for the BstFpg protein, NEIL1 and MvNei1 are 1R2Y, 1TDH and 3A42 respectively).

Table 1

Summary of current crystal structures of the Fpg/Nei glycosylases

Protein	Mutation	Complex	Substrate:Opposite base	Resolution (Å)	PDB ID/ References	Substrate Specificity
TthFpg	-		-	1.90	1EE8 (54)	8-oxoG, FapyG, Me-FapyG, FapyA (54)
EcoFpg	-	SBI	-	2.10	1K82 (55)	Sp = 8-oxoG, FapyG, Me-FapyG, FapyA, and Gh (6,18,72,79,89,101-103)
BstFpg	-	SBI	-	1.70	1L1Z (57)	8-oxoG, FapyG, Me-FapyG, FapyA (57,59,64-66)
	-	SBI	-	2.40	1L2B	
	-	RC	rAb:C	1.80	1L1T	
	-	RC	rAb:T	2.20	1L2C	
	-	RC	rAb:G	2.00	1L2D	
	E3Q	LRC	8-oxoG:C	2.34	1R2Y (59)	
	E3Q	LRC	DHU:C	1.63	1R2Z (64)	
	E3Q-DXL	CC1	8-oxoG:C	2.35	2F5S	
	E3Q-DXL	CC2	8-oxoG:C	2.35	2F5Q	
	E3Q-DXL	IC1	A:T	2.00	2F5N	
	E3Q-DXL	IC2	A:T	2.00	2F5P	
	E3Q-DXL	IC3	G:C	2.05	2F5O	
	E3Q-DXL	LRC3	8-oxoG:C	1.85	3GPY (65)	
	Δ220-235-DXL	EC3	8-oxoG:C	1.89	3GO8	
	V222P-DXL	EC3	8-oxoG:C	2.05	3GP1	
	T224P-DXL	EC3	8-oxoG:C	2.15	3GPP	
	Δ220-235-DXL	EC4	8-oxoG:C	1.62	3GPU	
	Δ220-235-DXL	IC4	G:C	1.78	3GPX	
	E3Q-DXL	LRC5	8-oxoG:C	1.70	3GQ4	
	Δ220-235-DXL	EC5	8-oxoG:C	1.83	3GQ3	
	V222P-DXL	IC5	G:C	1.90	3GQ5	
	N174C-DXL	XGC	G:C	2.60	3JR4 (66)	

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Protein	Mutation	Complex	Substrate:Opposite base	Resolution (Å)	PDB ID/ References	Substrate Specificity
	N174C-DXL	LRC	8-oxoG:C	1.70	3JR5	
LlaFpg	P1G	RC	Pr:C	2.55	1KFV (58)	8-oxoG, FapyG, Me-FapyG, FapyA, Hyd (58,60,63,68,69)
	ΔP1	LRC	cFapydG:C	1.80	1TDZ (60)	
	P1G	RC	Pr:C	1.90	1NNJ (63)	
	P1G	RC	THF:C	1.90	1PJJ	
	-	RC	Pr:C	1.90	1PJI	
	-	RC	THF:C	1.95	1PM5	
	-	RC	cFapydG:C	1.95	1XC8 (68)	
	-	RC	N7-Benzyl-FapyG:C	1.90	3C58	
	-	LRC	cHyd:C	1.80	2XZF (69)	
	-	DPC	cHyd:C	1.80	2XZU	
EcoNei		SBI	-	1.42	1K3W (56)	Tg, 5-OHU, 5-OHC, DHT, DHU, Sp, Gh (34,72,76,81)
		SBI	-	1.25	1K3X	
	-	-	-	2.80	1Q39 (62)	
	E2A	-	-	2.30	1Q3C	
	R252A	-	-	2.05	1Q3B	
NEIL1	ΔC-terminal56	-	-	2.10	1TDH (61)	Sp=Gh>Tg>DHU>5-OHU>5-OHC>DHT>FapyG=FapyA>>8-oxoG (30,36,39,44,61,83,86)
MvNei1	-	RC	THF:C	2.20	3A46 (67)	Sp=Gh>Tg>5-OHU>5-OHC>DHT=DHU>>8-oxoG (39,67,82)
	-		-	2.60	3A42	
	-		-	2.30	3A45	

Schiff-base intermediate: SBI

Disulfide cross-linking: DXL

Recognition complex: RC

Lesion Recognition Complex: LRC

DNA-protein covalent: DPC

Interrogation Complex: IC

Control Complex: CC

Encounter Complex: EC

Extrahelical G complex: XGC

5-hydroxy-5-methylhydantoin: Hyd or cHyd, where c refers to a carbanucleoside