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REVIEW

Eukaryotic Endonuclease VIII-Like Proteins: New Components of the Base Excision DNA Repair System

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Abstract—Base excision DNA repair is necessary for removal of damaged nucleobases from the genome and their replacement with normal nucleobases. Base excision repair is initiated by DNA glycosylases, the enzymes that cleave the *N*-glycosidic bonds of damaged deoxynucleotides. Until recently, only eight DNA glycosylases with different substrate specificity were known in human cells. In 2002, three new human DNA glycosylases (NEIL1, NEIL2, and NEIL3) were discovered, all homologous to endonuclease VIII, a bacterial protein, which also participates in DNA repair. The role of these enzymes remains mostly unknown. In this review we discuss recent data on the substrate specificity of the NEIL enzymes, their catalytic mechanism, structure, interactions with other components of DNA repair system, and possible biological role in preventing diseases associated with DNA damage.

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Key words: oxidative stress, DNA repair, DNA glycosylases, NEIL proteins

DNA repair is one of the most important processes in cells. Chemical instability of nucleic acids, DNA polymerase errors during replication, and various exogenous and endogenous damaging agents constantly damage DNA in all living cells. The majority of DNA lesions, such as single-strand breaks, apurine-apyrimidine (AP) sites, and modified bases are repaired by the base excision repair (BER) system, a multistep process involving several enzymes acting successively (Scheme 1). In general, BER includes five stages (Scheme 1). The stage of 5′-end processing can follow the short-patch repair pathway with removal of the protruding 2′-deoxyribo-5′-phosphate (dRP-) fragment due to dRP-lyase activity of DNA polymerase β (Scheme 1, IV) or the long-patch pathway with synthesis of a DNA strand by DNA polymerase δ followed by displacement and degradation of the DNA strand on the 3′-side of the lesion.

The initiation stage of BER (Scheme 1, I) includes the hydrolysis of the *N*-glycosidic bond between the sugar-phosphate backbone and the damaged base catalyzed by specialized enzymes called DNA glycosylases [1-3]. About 20 DNA glycosylases with different substrate specificity to damaged nucleobases (oxidized, alkylated, etc.) are known. Mechanisms of their action may differ—there are monofunctional enzymes, which remove only the damaged base, and bifunctional ones, which remove the damaged base and make a break in the DNA strand by the β-elimination mechanism. In recent years a structural classification of DNA glycosylases has been developed based on the conservative sequences in their primary structure or tertiary structure motifs [2].

Oxidation of DNA significantly contributes to DNA damage [4]. DNA glycosylases participating in oxidized base repair belong to two main structural superfamilies. DNA glycosylases from the endonuclease III (Nth) superfamily are characterized by the presence of characteristic domains in their spatial structure—a barrel of six α-helices and a domain composed of four α-helices, a DNA binding helix-hairpin-helix motif, and a loop containing Gly, Pro, and a catalytic Asp residue [2, 5]. DNA

Abbreviations: AP, apurine-apyrimidine; BER, base excision repair; dRP, 2′-deoxyribo-5′-phosphate; FapyAde, 4,6-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-oxo-5-formamidopyrimidine; Fpg/Nei, formamidopyrimidine-DNA glycosylase/endonuclease VIII; Gh, guanidinohydantoin; Nth, endonuclease III; 5-OH-Ura, 5-hydroxyuracil; 8-oxoAde, 8-oxoadenine; 8-oxoGua, 8-oxoguanine; Sp, spiroiminodihydantoin; ThyGly, thymine glycol.

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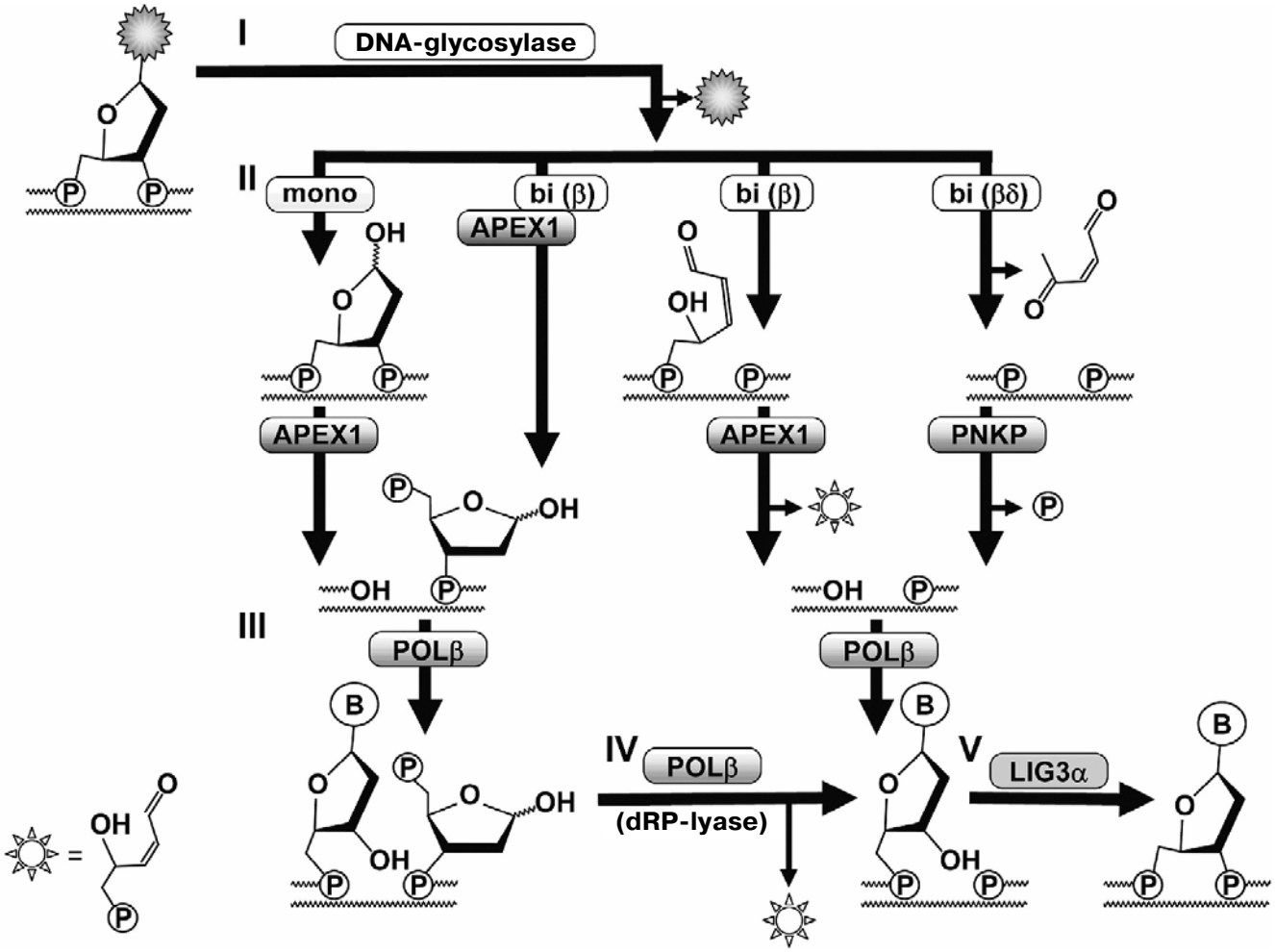


Stages of short-patch BER in higher eukaryotes. I, damaged bases can be removed by monofunctional DNA glycosylase (mono), bifunctional DNA glycosylase catalyzing β-elimination (bi (β)), or bifunctional DNA glycosylase catalyzing βδ-elimination (bi (βδ)); II, processing of 3′-end; III, replacement of damaged nucleotide; IV, processing of 5′-end; V, ligation

Scheme 1

glycosylases from the formamidopyrimidine-DNA glycosylase/endonuclease VIII superfamily (Fpg/Nei) are characterized based on their typical structure that includes N-terminal and C-terminal domains connected by a flexible linker. The base of the N-terminal domain is a two-sheet β-sandwich consisting of eight antiparallel strands. The PE helix, which is an α-helix carrying catalytic Pro1 and Glu2 residues on its N terminus (an initiating Met residue is removed from all proteins from the Fpg/Nei superfamily during polypeptide maturation, and the numbering of amino acids starts from the next Pro residue) is adjacent to the side of this β-sandwich. The C-terminal domain contains a DNA-binding helix-two turn-helix motif characteristic of the entire superfamily, and in many cases a Cys4-type zinc finger consisting of two antiparallel β-strands.

Despite the complete lack of similarity of primary and tertiary structures of the enzymes from the Fpg/Nei and Nth superfamilies, their substrate specificity may overlap. For example, the human OGG1 (oxoguanine-DNA glycosylase 1) enzyme from the Nth superfamily and the Fpg enzyme of *Escherichia coli* from the Fpg/Nei superfamily remove the same set of oxidized purine bases from DNA, and the substrates for the Nth and Nei enzymes are practically the same oxidized pyrimidine bases [2, 5, 6].

The three-dimensional structures of damaged DNA complexes with DNA glycosylases show that all DNA glycosylases share a common mechanism of accessing a damaged base—kinking of the DNA and intercalation of a hydrophobic amino acid residue into the double helix, which pushes the damaged base into the active site of the enzyme where nucleophilic attack on the C1′ atom is exerted by a water molecule or an amino group of the enzyme. However, the patterns of bonds formed by the same damaged base in the active site may considerably differ for different enzymes.

GENERAL PROPERTIES OF NEIL PROTEINS

It was long thought that repair of oxidized bases in eukaryotic DNA is catalyzed by two enzymes from the Nth superfamily: oxidized pyrimidines serve as substrates for DNA glycosylase NTHL1 (endonuclease three-like protein 1) and oxidized purines are processed by DNA glycosylase OGG1 [7]. However, residual activities removing thymine glycol (ThyGly) and urea residues from DNA were recently found in nuclear and mitochondrial extracts from liver and lungs of *NTHL1*$^{-/-}$ mice [8]. Based on the sequences of bacterial Fpg and Nei proteins, several groups of researchers independently discovered three genes in the mouse and human genomes encoding DNA glycosylases belonging to the Fpg/Nei superfamily and having primary structure more similar to that of the Nei protein [9-14]. These proteins were named NEIL1, NEIL2, and NEIL3 (see table).

Although the total sequence homology between eukaryotic NEIL proteins and bacterial proteins from the Fpg/Nei superfamily is not very high, all three NEIL proteins contain five motifs characteristic to this superfamily in both domains (Scheme 2). The first motif in NEIL1 and NEIL2 contains the N-terminal Pro1 residue, the α-amino group of which acts as a nucleophile in the DNA glycosylation reaction and forms a Schiff base with the C1′ atom [15, 16]. NEIL3 has a Val residue in place of Pro1, the primary amine of which performs the same catalytic function [17]. The Glu2 residue, which is necessary for the DNA glycosylation activity [18, 19], is also preserved in all three NEIL protein sequences. Site-directed mutagenesis of Pro1 and Glu2 inactivates the NEIL1 and NEIL2 enzymes, confirming the importance of these residues for catalysis [12].

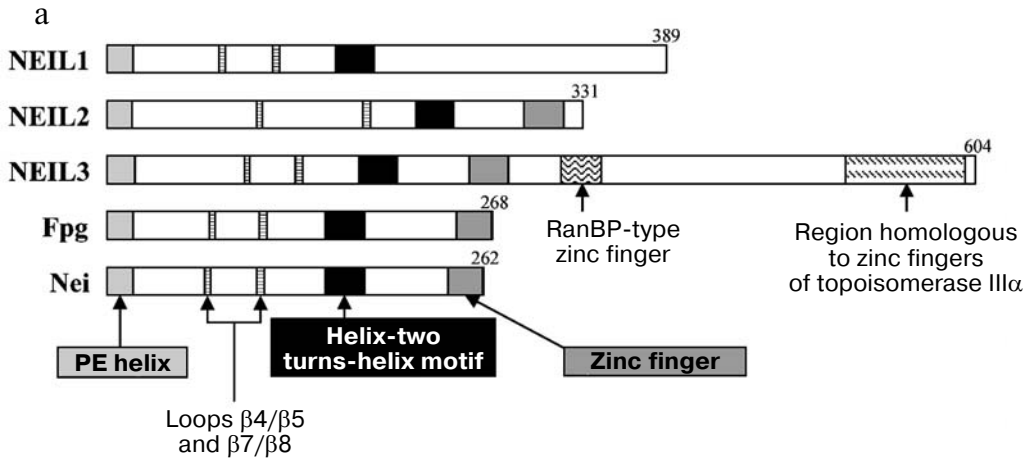
The second and the third conservative motifs are located in the short loops connecting the β-strands of the N-terminal domain. All three NEIL proteins have a Lys residue in the second conservative motif (loop β2/β3 in Scheme 2). The corresponding Lys52 and Lys56 residues (in *E. coli* Nei and Fpg proteins, respectively) are involved in the coordination of the 5′-phosphate group of the damaged deoxynucleotide [6]. The third conservative site contains amino acids that intercalate in the DNA helix, take the place of the damaged and excised base, and interact with the base opposite the damaged one [6]. All these residues (Gln69, Leu70, and Tyr71) are located in the β4/β5 loop of *E. coli* Nei protein. In the *E. coli* Fpg protein the set of residues performing the same functions is divided between the β4/β5 (Met73) and β7/β8 (Arg108 and Phe110) loops. Both variants of the arrangement of these residues can occur in sequences of NEIL proteins.

The fourth and fifth conservative motifs are a DNA-binding helix-two turns-helix motif and a zinc finger, the latter making contact with the major groove of DNA. The helix-two turns-helix motif is typical for all proteins from the Fpg/Nei superfamily including all NEIL proteins. Zinc finger motifs in NEIL proteins may vary. For example, NEIL3 protein contains a zinc finger with a sequence typical of bacterial Fpg and Nei. The NEIL2 protein contains an unusual CHCC zinc finger motif, which was shown to actually contain a zinc atom by inductively coupled plasma mass spectrometry [20]. NEIL1 contains no Cys and His residues in the positions that could form a zinc finger motif, but it has a β-hairpin corresponding to a zinc finger (see section "Structural Features of NEIL1 Protein"). The NEIL3 protein also contains a RanBP-type zinc finger motif and an extended C-terminal region homologous to the region of DNA-topoisomerase IIIα containing two GRF-type zinc fingers [14].

All sequences of Nei and NEIL proteins, on one hand, and those of the Fpg proteins from different organisms, on the other hand, differ in positions 3, 154, and 171. Nei and NEIL protein sequences contain a Gly residue in position 3, and Fpg proteins have a Leu residue. These residues are supposed to form a part of the active site in both enzymes [21]. All Fpg proteins contain a Lys154 (numbering of *E. coli* Fpg) residue, but this position is not conservative in Nei and NEIL proteins. In contrast, Nei and NEIL proteins contain a positively charged residue (Arg or Lys) at position 171 (numbering of *E. coli* Nei), which is not conservative in Fpg proteins. Both Lys154 in Fpg and Arg171 in Nei proteins are involved in the correct positioning of the zinc finger relative to the main part of the protein globule [21, 22].

Characteristics of human NEIL genes and proteins

Gene	Gene length, nucleotides	Number of exons	Coding sequence, nucleotides	Chromosomal location	Protein size, kDa
NEIL1	8258	9	1173	15q24.2	43.6
NEIL2	17 683	4	999	8p23.1	36.7
NEIL3	53 102	9	1818	4q34.3	67.7



b

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                                                    β1                                  β2
NEIL1 PEGPELHLASQFVNEACR----------------------ALVFGGCVEKSSVSRNPEVPFESSAYRISASA-  50
NEIL2 PEGPLVRKFHHLVSPFVG----------------------QQ-----VVKTGGSS--KKLQPASLQSLWLQD-  43
NEIL3 VEGPGCTLNGEKIRARVLPGQAVTGVRGSALRSPQGRALRLAASTVVVSPQAAALNNDSSQNVLSLFNGYVYSG  74
Nei   PEGPEIRRAADNLEAAIK----------------------GKPLTDVWFAFPQLKPYQSQLIG----QHVTH-  46
Fpg   PELPEVETSRRGIEPHLV----------------------GATILHAVVRNGRLRWPVSEEIYRLSDQPVLS-  50

          β2    β3              β4    β5                                        β6
NEIL1 ---RGKELRLILSPLPGAQPQQEPLALVFRFGMSGSFQLVP----------------------REELPRHAH-  97
NEIL2 TQVHGKKLFLRFDLDEEMGPPGSSPTPEPPQKEVQKEGAADPKQVGEPSGQKTLDGSSRSAELVPQGEDDSEYL 117
NEIL3 VETLGKELFMYFG----------PKALRIHFGMKGFIMINP----------------------LEYKYKN-- 112
Nei   VETRGKALLTHFSN---------DLTLYSHNQLYGVWRVVD----------------------TGEEPQT--  85
Fpg   VQRRAKYLLLELP----------EGWIIIHLGMSGSLRILP----------------------EELPPEK--  88

                  β6        β7        β8
NEIL1 -----------LRFYTAPPGPRLALCFVDIRRFGRWDLGGK-----WQPGRGP------------------CV 136
NEIL2 ERDAPAGDAGRWLRVSFGLFGSVWVNDFSRAKKANKRGDWRDPSPRLVLHFGGGGFLAFYNCQLSWSSSPVVTP 191
NEIL3 -----------GASPVLEVQLTKDLICFFDSSVELRNSMESQQRIRMMKEL--------------------DV 154
Nei   -----------TRVLRVKLQTADKTILLYSASDIEMLTPEQLTTHPFLQRVGP------------------DV 129
Fpg   -----------HDHVDLVMSNGKVLRYTDPRRFGAWLWTKELEGHNVLTHLGP------------------EP 132

NEIL1 LQEYQQFRESVLRNLADKAFDRPICEALLDQRFFNGIGNYLRAEILYRLKIPPFEKARSVLEALQQHRPSPELT 210
NEIL2 TCDILSEKFHRGQALEALGQAQPVCYTLLDQRYFSGLGNIIKNEALYRAGIHPLSLGSVLSASRREVLVDHVVE 265
NEIL3 CSPEFS--FLRAESEVKKQKGRMLGDVLMDQNVLPGVGNIIKNEALFDSGLHPAVKVCQLTDEQIHHLMKMIRD 226
Nei   LDPNLTPEVVKERLLSPRFRNRQFAGLLLDQAFLAGLGNYLRVEILWQVGLTGNHKAKDLNAAQLDALAHALLE 203
Fpg   LSDDFNGEYLHQKCAK---KKTAIKPWLMDNKLVVGVGNIYASESLFAAGIHPDRLASSLSLAECELLARVIKA 203

NEIL1 LSQKIRTKLQNPDLLELCHSVPKEVVQLGGRGYGSESGEEDFAAFRAWLRCYGMPGMSSLQDRHGRTIWFQGDP 284
NEIL2 FSTAW-------------------LQGKFQGRPQ-------------HTQVY------------------QKEQ 289
NEIL3 FSILF-------------------YRCRKAGLA-----------LSKHYKVYKR----------------PN-- 252
Nei   IPRFS--------------------YATRGQVDENKHHG------ALFRFKVFHR----------------DGEP 236
Fpg   VLLRS--------------------IEQGGTTLKDFLQSDGKPGYFAQELQVYGR----------------KGEP 242

NEIL1 GPL-APKGRKS---RKKKSKATQLSPEDRVEDALPPSKAPSRTRRAKRDLPKRTATQRPEGTSLQQDPEAPTVP 354
NEIL2 CPAGHQVMKEAFGPEDGLQ--RLTWWCPQCQPQLSEEPEQCQFS                              331
NEIL3 CGQ-CHCRITV---CRFGDNNRMTYFCPHCQKENPQHVDICKLPTRNTIISWTSSRVDHVMDSVARKSEEHWTC 322
Nei   CER-CGSIIEK---TTLSS--RPFYWCPGCQH                                          262
Fpg   CRV-CGTPIVA---TKHAQ--RATFYCRQCQK                                          268

NEIL1 KKGRRKGRQAASGHCRPRKVKADIPSLEPEGTSAS                                       389
NEIL3 VVCTLINKPSSKACDACLTSRPIDSVLKSEENSTVFSHLMKYPCNTFGKPHTEVKINRKTAFGTTTLVLTDFSN 396

NEIL3 KSSTLERKTKQNQILDEEFQNSPPASVCLNDIQHPSKKTTNDITQLSSKVNISPTISSESKLFSPAHKKPKTAH 470

NEIL3 YSSPELKSCNPGYSNSELQINMTDGPRTLNPDSPRCSKHNRLCILRVVRKDGENKGRQFYACPLPREAQCGFFE 544

NEIL3 WADLSFPFCNHGKRSTMKTVLKIGPNNGKNFFVCPLGKEKQCNFFQWAENGPGIKIIPGC               604
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a) Location of characteristic structural motifs in DNA glycosylases from the Fpg/Nei superfamily (Fpg and Nei from *E. coli*, NEIL1, NEIL2, and NEIL3 from human). b) Protein sequence comparison of Fpg and Nei from *E. coli* and NEIL1, NEIL2, and NEIL3 from human. Marked: PE-helix (black letters on light gray background), helix-two turns-helix motif (white letters on black background), and zinc finger motif (white letters on dark gray background). Short loops between β-strands involved in DNA binding and intercalation into double helix are underlined; β-strands of the NEIL1 N-terminal domain are indicated by the arrows above the respective regions of the sequence. In the NEIL3 sequence, a RanBP-type zinc finger motif is underlined with a thick line, and the sequence homologous to zinc fingers of topoisomerase IIIα is underlined with a dashed line. The alignment was obtained with the ClustalW2 program [91]

Scheme 2

DISTRIBUTION OF NEIL PROTEINS

While DNA glycosylases from the Nth superfamily are found in practically all groups of bacteria, archaea, and eukaryotes, the distribution of proteins from the Fpg/Nei superfamily is more limited. These proteins are absent from archaea but are found in bacteria and eukaryotes, which indicates that a common Fpg/Nei ancestor existed before the separation of bacteria, archaea, and eukaryotes, while the loss of this superfamily occurred in the archaean branch of evolution. Sequences highly homologous to those of *E. coli* Fpg protein are found in almost all bacterial taxa, whereas sequences close to the primary structure of Nei proteins are found only in a very small number of taxa belonging to Proteobacteria, Firmicutes, and Cyanobacteria (Fig. 1). Additionally, a small number of taxa of Actinobacteria, Acidobacteria, Proteobacteria, and Planctomycetes types show sequences close to Nei but different to the extent of treating them as separate Nei2 and Nei3 proteins (Fig. 1). Only Nei2 and Nei3 proteins from *Mycobacterium tuberculosis* have been biochemically characterized. Their substrate specificity is wider than that of the *E. coli* Nei enzyme [23, 24]. Some bacterial members of the superfamily annotated in databases as Fpg do not belong either to the Fpg or to the Nei group according to their primary structure (marked as "Fpg(?)" in Fig. 1).

The distribution of superfamily Fpg/Nei proteins among eukaryotic organisms is also uneven. One group of homologs, namely the MMH (MutM homolog; MutM is an alternative name of Fpg protein [25]) proteins, is found in plants and fungi, but some species even in these kingdoms lack them. For example, no sequences of *MMH* are found in the completely sequenced genomes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, even though they are present in the genomes of other yeasts such as *Candida*, *Debaryomyces*, *Pichia*, and *Yarrowia*. NEIL1, NEIL2, and NEIL3 proteins do not belong to the MMH group and are found only in animals (Fig. 1). Moreover, despite the fact that the *NEIL1* gene is present in the genome of the most primitive multicellular animals such as Placozoa (*Trichoplax adhaerens*) and coelenterates (*Nematostella vectensis*, *Hydra magnipapillata*), and, therefore, existed in their common ancestor, it is not detected in completely sequenced genomes of the evolutionary branch which includes nematodes (*Caenorhabditis elegans*) and insects (*Drosophila melanogaster*). NEIL2 sequences are found only in deuterostomes (chordates and echinoderms), and NEIL3 is found only in chordates. NEIL2 and NEIL3 groups are more similar to the bacterial Nei group than to the eukaryotic groups NEIL1 and MMH (Fig. 1).

Two homologs of NEIL proteins were found in mimiviruses possessing a very large (~1.2 million base pairs) DNA genome and parasitizing on amoebae [26]. One of them is homologous to NEIL1, and the other has an N-terminal domain homologous to NEIL3 and a C-terminal domain homologous to NEIL2.

The evolutionary history of the DNA glycosylase Fpg/Nei superfamily and NEIL proteins in particular was accompanied by repeated disappearances of their encoding genes from certain branches. This may be due to the fact that the known functions of the Nei, NEIL, and MMH proteins are actually duplicated by other DNA glycosylases that belong to the other structural superfamily Nth. Narrow phylogenetic distribution of Nei sequences indicates a probable horizontal *nei* gene transfer between unrelated groups of bacteria. However, the repeated duplication of the ancestral superfamily Fpg/Nei gene with following functional divergence is apparent. This may indicate that some members of the superfamily (possibly NEIL proteins) perform a specialized function that is not redundant in relation to other DNA glycosylases.

KEY SUBSTRATES AND POSSIBLE FUNCTIONS OF NEIL ENZYMES

The discovery of NEIL DNA glycosylases raised a question about the possible functions of these proteins. Some facts suggest that NEIL proteins may have a role in DNA repair that is different from that of other DNA glycosylases. First, the known spectrum of the NEIL enzyme substrates largely overlaps with the OGG1 and NTHL1 substrate spectra. Second, the sporadic occurrence of NEIL proteins in nature suggests an inessential function for these proteins in eukaryotic cells. Third, the phenotype of animals deficient in NEIL1 protein is not associated with genomic instability, in contrast to the phenotype of most mutants in other genes of the repair system.

Of all NEIL proteins the NEIL1 protein is the closest to the prokaryotic enzyme Nei according to its enzymatic functions. NEIL1 catalyzes a reaction proceeding by the mechanism of βδ-elimination with a Schiff base as an intermediate and a 3′-phosphate-containing final product [9]. The optimum for the enzymatic activity of NEIL1 is at pH 9.5 and 100-150 mM NaCl [12].

The substrate specificities of the DNA glycosylases NEIL1 and Nei largely overlap. DNA glycosylase NEIL1 is able to catalyze the removal of oligonucleotide substrates such as oxidized pyrimidine bases (Fig. 2, I-VI) as well as some oxidized purine bases (Fig. 2, VII-XIII). However, it exhibits a very low activity towards substrates containing 8-oxoguanine (8-oxoGua; Fig. 2, X) [9, 11, 12, 14, 27, 28]. The efficiency of removal of different damaged bases by the NEIL1 enzyme varies greatly. For example, the removal of guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp) is about 100 times faster than that of ThyGly or 5-hydroxycytosine [28]. Additionally, the enzyme excises the *S*-diastereomer of Sp faster than its *R*-diastereomer. This indicates a specific architecture

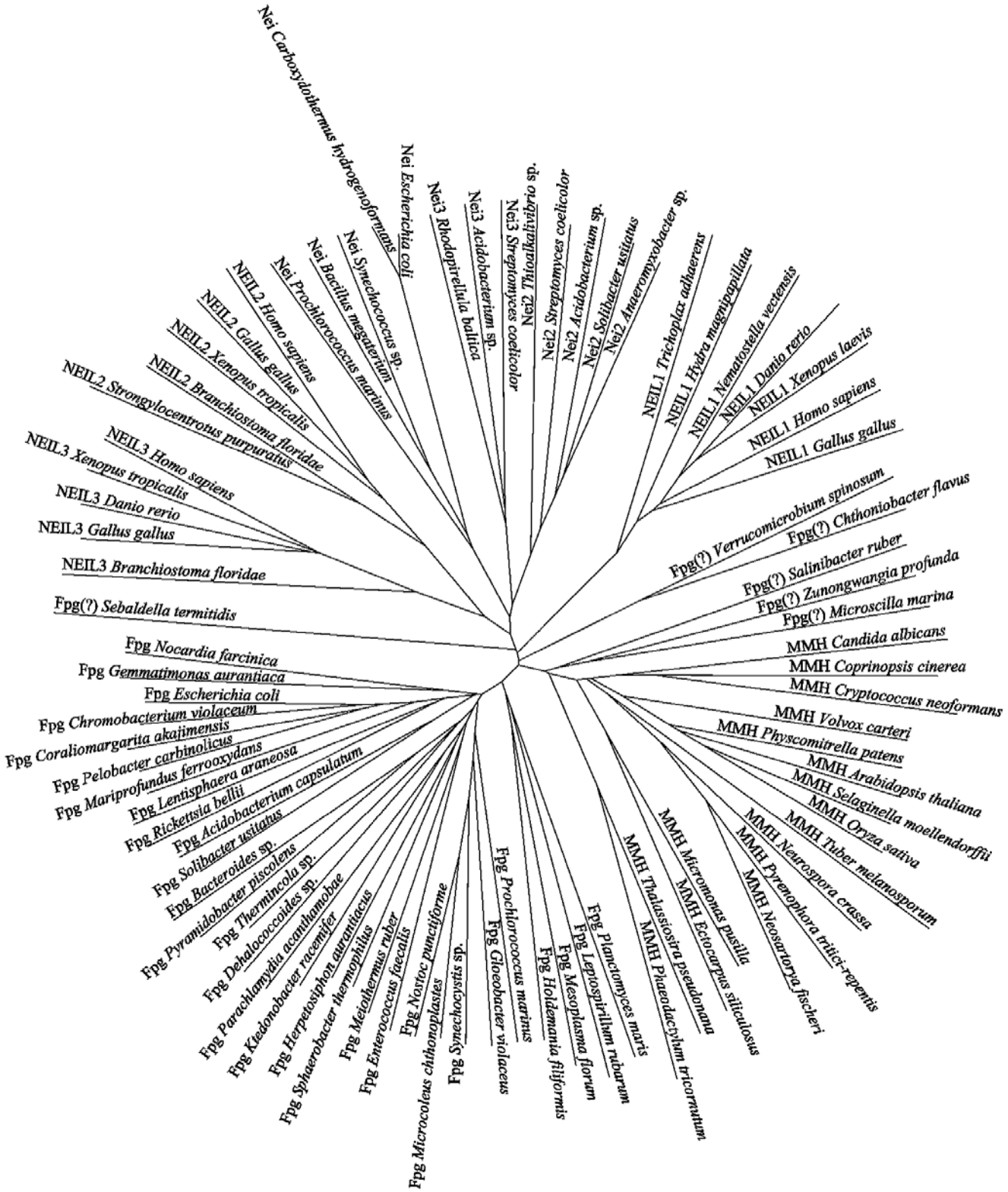

Fig. 1. Tree of polypeptide sequences of DNA glycosylases from the Fpg/Nei superfamily. One representative sequence for each taxonomic class is presented from sequences available in the GenBank, RefSeq, PDB, Swiss-Prot, PIR, and PRF databases. The PHYLIP software package was used to build the tree [92].

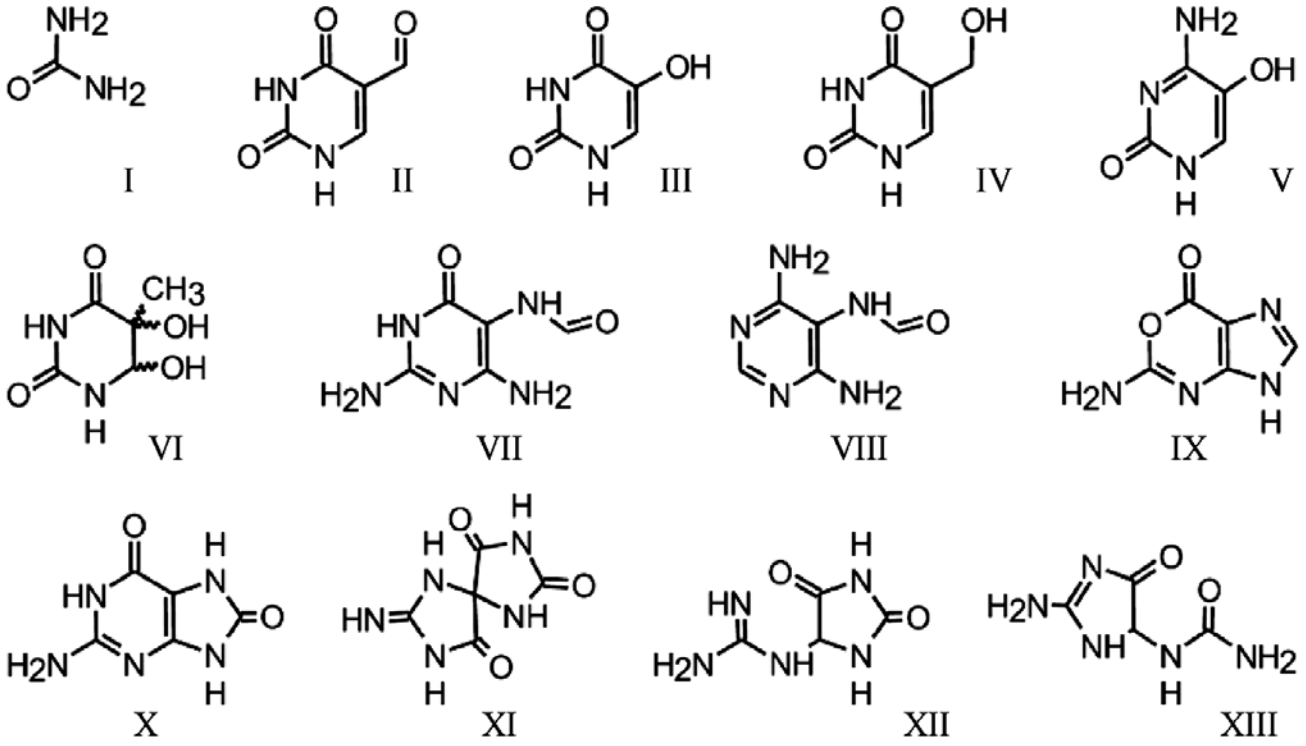

Fig. 2. Some known damaged bases removed from DNA by NEIL1 enzyme. I-XIII correspond to urea, 5-formyluracil, 5-OH-Ura, 5-hydroxymethyluracil, 5-hydroxycytosin, ThyGly, FapyAde, FapyGua, oxanine, 8-oxoGua, Sp, Gh, and iminoallantoin, respectively.

of the active site capable of distinguishing between these two stereoisomers [28]. It should be noted that the NEIL1 enzyme can cleave substrates containing ThyGly and 5-hydroxyuracil (5-OH-Ura) in single-stranded DNA and also has a unique DNA glycosylase activity with respect to Ura and Thy bases in noncanonical pairs with Cyt [11]. The substrate specificity of the NEIL1 enzyme depends on the DNA base opposing the damaged base. Thus, the Gh and Sp bases are more effectively removed when paired with Thy, Gua, or Cyt [28], and 8-oxoadenine (8-oxoAde) can only be removed from pairs with Cyt [29].

Despite the wide range of substrate bases removed from oligonucleotides by the NEIL1 enzyme, studying its activity on high molecular weight DNA containing multiple oxidized bases due to γ-irradiation showed that from all damaged purine bases only formamidopyrimidine derivatives of Gua and Ade (FapyGua and FapyAde) were removed in significant quantities [27]. The enzyme also removes ThyGly and 5-hydroxy-5-methylhydantoin, although significantly worse than formamidopyrimidines, and is almost inactive with respect to other damaged bases [27]. DNA from the *NEIL1*$^{-/-}$ mice cells accumulates (5′*R*)- and (5′*S*)-8,5′-cyclo-2′-deoxyadenosine residues. This damaged deoxynucleoside is removed by the nucleotide excision repair system rather than by BER, which indicates possible involvement of NEIL1 in nucleotide excision repair [30].

The NEIL2 enzyme efficiently cleaves substrates containing 5-OH-Ura, 5,6-dihydrouracil, 5,6-dihydrothymine, 5-hydroxycytosine, ThyGly, and, less effectively, 8-oxoGua (opposing Cyt and Ade) [10, 31]. DNA containing AP sites is an excellent substrate for NEIL1 and NEIL2.

The overlapping substrate specificity of NEIL enzymes and other DNA glycosylases raises a question: why do vertebrates need this functional duplication that the lower eukaryotes can exist without? On one hand, the NEIL proteins can be assumed to form a “second line” of DNA defense that is necessary upon low activity of the key BER enzymes. For example, the kinetic parameters of the reaction of 8-oxoAde removal from 8-oxoAde:Cyt pairs catalyzed by the NEIL1 DNA glycosylase are similar to the parameters of the reaction catalyzed by the OGG1 enzyme [29]. But the following steps of repair initiated by OGG1 and NEIL1 enzymes differ: in the first case the APEX1 AP-endonuclease is required after the action of DNA glycosylase, and in the second case the polynucleotide kinase 3′-phosphatase is involved (Scheme 1). On the other hand, NEIL enzymes may play a special role in certain types of cells or tissues at specific phases of the cell cycle or at certain stages of development. For example, the NEIL1 enzyme can functionally substitute for the NTHL1 DNA glycosylase in liver cells where the amount of the latter is small [32].

There is some evidence suggesting the importance of NEIL enzymes for repair of damaged DNA structures different from its canonical B-form. A characteristic feature of NEIL1 and NEIL2 enzymes, distinguishing them from other DNA-glycosylases, is their ability to process DNA containing damaged bases in a bubble – a double-stranded 5-20-nucleotide-long segment that is not stabilized by complementary bonds [33]. The NEIL2 enzyme can remove damaged bases of all the recognized types from bubble substrates, whereas NEIL1 enzyme is able to do it only for 5-OH-Ura and Gh [33, 34]. It is often assumed in the literature that this ability may be crucial for establishing the role of NEIL enzymes to repair of damaged bases located in partially melted DNA in the transcription bubble formed by RNA polymerase, or in a moving replication fork. However, the issue of competition for such DNA between NEIL enzymes and components of transcription and replication systems has not yet been investigated. In addition, in the full cycle of BER the lack of DNA polymerase access to the undamaged DNA strand (for example, due to its binding with other proteins) can facilitate ligation of the gap in the damaged strand without inclusion of dNMP, which leads to a single nucleotide deletion [34]. Based on these considerations, we conclude that the biological significance of NEIL enzyme activity against bubble substrates is unclear.

NEIL1 enzyme is active against mono-adducts of psoralens as well as adducts of psoralens with an oligonucleotide forming triple-helix DNA (repair intermediates of psoralen-containing interstrand cross-links), suggesting the involvement of NEIL1 in this process [35, 36]. It is also known that NEIL1 effectively excises damaged bases from DNA segments located on the surface of nucleosomes, but it poorly removes damaged bases adjacent to histone core [37].

The NEIL1 enzyme is active toward damaged bases located near DNA breaks. Such substrates containing so-called tandem lesions are usually poorly cleaved by other DNA glycosylases. For example, if there is a 5-OH-Ura residue at a distance of 2-4 nucleotides from the 5′-side of a single-stranded break, then both 5-OH-Ura repair by DNA glycosylase NTHL1 and single-stranded break repair by DNA polymerase β and DNA ligase IIIα are greatly impaired. DNA glycosylases NEIL1 and SMUG1 (single-strand selective monofunctional uracil-DNA glycosylase 1) remove 5-OH-Ura from such substrates and initiate repair, and it should be noted that NEIL1 enzyme does it much more efficiently [38, 39]. The same situation is observed for 8-oxoGua repair at the 5′-side of a single-stranded break: the damaged base is removed much more effectively by NEIL1 enzyme than by OGG1, although NEIL1 activity is much lower than OGG1 activity with 8-oxoGua in completely double-stranded DNA [38]. The NEIL1 enzyme is also able to cleave tandem lesions containing two neighboring residues of 5,6-dihydrouracil. The cleavage efficiency is the same for both damaged positions [40].

NEIL protein functions are not solely limited to their glycosylase activity. For example, NEIL1 and NEIL2 proteins were shown *in vitro* to be functional dRP-lyases. Efficiency of dRP-lyase catalysis by these enzymes is comparable to that of DNA polymerase β in kinetic parameters and the ability to replace DNA polymerase β in the reconstruction of Ura, AP sites, and 8-oxoGua repair systems [41].

Analysis of NEIL3 protein function has long been impeded by the difficulty of its isolation [42]. Recently it has been definitively shown that the NEIL3 enzyme also possesses bifunctional DNA-glycosylase activity [17, 43]. Substrates for this enzyme include Sp, Gh, FapyGua, and FapyAde bases, but not 8-oxoGua. In this case preferable substrates are single-stranded DNA and bubble structures. Expression of NEIL3 protein in *E. coli* cells deficient in various combinations of the DNA glycosylases Fpg, Nei, Nth, and MutY improves survival of bacteria after H_2O_2 treatment and reduces the frequency of spontaneous mutagenesis and FapyGua level in DNA, which indicates the important role of the NEIL3 enzyme in damaged base repair [17, 43].

STRUCTURAL FEATURES OF NEIL1 PROTEIN

The spatial structure of the NEIL1 protein was determined by X-ray analysis of the human protein (Protein Data Bank (PDB) code 1TDH [44]) and of the mimivirus protein (PDB codes 3A42 and 3A45 [45]) in the free state and of the mimivirus protein in a complex with a DNA molecule containing (3-hydroxytetrahydrofuranyl-2)-methylphosphate, which is an uncleavable analog of the AP site (PDB code 3A46 [45]) (Fig. 3). The structures of both proteins are quite consistent with known structures of Fpg and Nei, though the length of the corresponding loops, α-helixes, and β-strands may vary for different proteins. Moreover, the NEIL1, Fpg, and Nei proteins share the same structural elements, which are involved in DNA binding as indicated by X-ray analysis. The most interesting structural feature found in NEIL1 proteins is the β-hairpin in the C-terminal domain (known as a zincless finger). It corresponds to zinc finger motifs in Fpg and Nei proteins but is not homologous to them at the level of primary structure and does not coordinate a zinc atom.

Since the NEIL1 protein structure in complex with DNA containing a damaged base has not been experimentally established, possible interactions in the active site of the protein were computationally modeled. The NEIL1 protein complexes were modeled both with well-recognizable substrates (*R*- and *S*-stereoisomers of spiroiminodihydantoin, 5*R*,6*S*- and 5*S*,6*R*-stereoisomers of ThyGly) and with a poor substrate (8-oxoGua) [46].

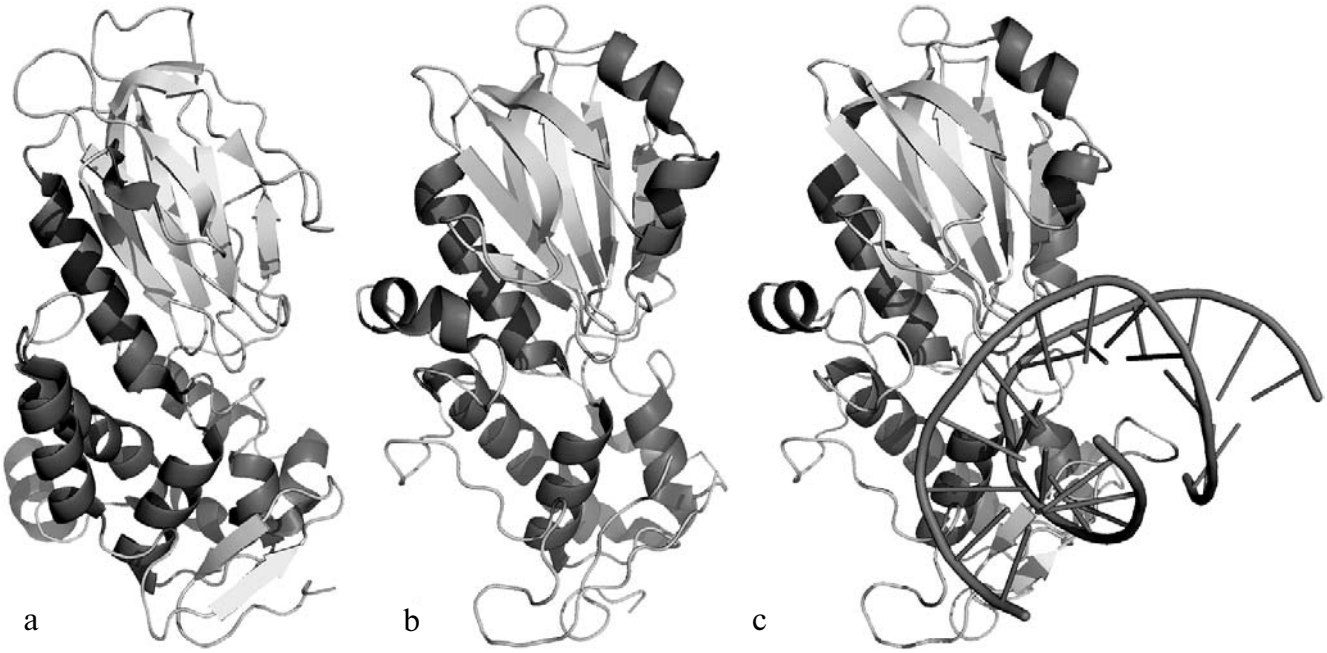

Fig. 3. Structure of DNA glycosylase NEIL1: human (a), mimivirus in the free state (b), and mimivirus in a complex with DNA containing (3-hydroxytetrahydrofuranyl-2)-methylphosphate (c). The PyMOL program was used to visualize the structures (www.pymol.org).

The simulation suggested that good substrates of NEIL1 have common features: the presence of a six-membered heterocycle and the formation of a characteristic set of hydrogen bonds between its constituent amide group and the active site of the enzyme. The 8-oxoGua base can form significantly fewer hydrogen bonds, and the purine heterocycle cannot fully fit in the shallow and relatively narrow active site of the enzyme [46].

INTERACTIONS OF NEIL PROTEINS WITH OTHER PROTEINS

As with many other DNA glycosylases, the activity and functions of NEIL proteins may depend on their interactions with other proteins. For example, the repair was shown to be stimulated by the joint action of two DNA glycosylases that do not stably interact with each other: the NEIL1 enzyme has a weak 8-oxoGua-removal activity, but it can stimulate the OGG1 enzyme activity and catalyze the βδ-elimination of the resulting AP site [47]. Increased activity is due to higher affinity of NEIL1 enzyme for AP sites and higher AP lyase activity compared to the OGG1 enzyme, which prevents OGG1 from re-binding to the reaction product after its release. In this case the interaction of NEIL1 and OGG1 is purely functional, and no stable complex is formed between these proteins [47]. A similar mechanism of stimulation of OGG1 DNA glycosylase is described for the APEX1 AP endonuclease [48].

There are several examples of regulation of the NEIL proteins by formation of stable multicomponent complexes with other proteins involved in BER. In particular, NEIL1 binds to the flap-endonuclease FEN1, and this stimulates the enzymatic activity of NEIL1. This interaction is mediated by the C-terminal domain of the NEIL1 protein outside the region of homology with prokaryotic Fpg and Nei DNA glycosylases. It is also mediated by the C-terminal domain of FEN1 unique to mammals [49]. Moreover, NEIL1 forms a complex with the adapter protein XRCC1 that coordinates different stages of BER. The affinity of NEIL1 to XRCC1 is low in the binary complex, but it is much higher in the presence of other BER proteins, which are included in a multiprotein complex formed around XRCC1. The NEIL1 protein was also shown to interact with DNA polymerase β and DNA ligase IIIα in this complex [50]. NEIL2 also interacts with XRCC1 by binding to its BRCT1-domain [51].

The NEIL1 and NEIL2 proteins associate with the replication factor PCNA, stimulating the NEIL1 activity and increasing the efficiency of its binding to the substrate, but this process has no effect on NEIL2 [52]. As in the case of FEN1 protein, this interaction is mediated by the C-terminal domain of NEIL1 and the interdomain linker of PCNA, which serves as binding site for many proteins. The C-terminal domain of NEIL1 does not have any canonical PCNA-binding motifs, but it has a peptide homologous to the PCNA-binding peptide of DNA polymerase δ [52]. The interaction of NEIL1 with FEN1 and PCNA also indicates the possibility of NEIL1

participating in the short-patch BER pathway. In addition, NEIL1 and NEIL2 proteins are known to interact with the PCNA-like protein complex 9-1-1. In the case of NEIL1 this interaction stimulates the activity of the enzyme [53].

NEIL2 interacts with transcription activating protein YB1 (Y-box binding protein 1 – a protein that binds Y-boxes in promoters of genes of major histocompatibility complex), which stimulates the DNA glycosylase activity of NEIL2. YB1 was detected in the complex, which, besides NEIL2, also includes DNA polymerase β and DNA ligase IIIα. Under oxidative stress YB1 moves from the cytoplasm to nucleus accompanied by NEIL2 enzyme activation. Under suppression of *YB1* gene expression by RNA interference, the activity of NEIL2 enzyme in cell extracts decreases [54].

CSB protein (Cockayne syndrome group B), which is non-functional in Cockayne syndrome complementation group B, was shown to form a complex with the enzyme NEIL1 and stimulate its activity [55]. The cells of $CSB^{-/-}$ mice have increased levels of FapyGua and FapyAde, and the efficiency of their repair is reduced in extracts of these cells [55]. NEIL1 protein also interacts with DNA helicase WRN, which stimulates NEIL1 activity on bubble substrates [56]. This interaction is mediated by the C-terminal domain of NEIL1 protein and the C-terminal domain of WRN, which is common for DNA helicases of the RecQ family. Interaction between WRN and NEIL1 proteins increases under oxidative stress in cells, and WRN protein deficiency in cells leads to elevated levels of 8-oxoGua, FapyGua, and FapyAde. However, there is no additive effect with a deficit of WRN and NEIL1, which indicates the joint functioning of these proteins in the same repair pathway [56].

COVALENT MODIFICATIONS AND INHIBITION OF NEIL PROTEINS

Covalent modification of proteins is a well-studied way of regulating their functions. There are many examples of covalent modifications of DNA glycosylases, but the consequences of such modifications are known for only a few cases. Posttranslational covalent modifications of NEIL proteins are virtually unstudied. It is only known that the CBP/p300 acetyltransferase acetylates Lys49 and Lys153 residues of NEIL2 with Lys49 modification inactivating the enzyme [57]. NEIL2 inactivation was also shown to occur *in vitro* after pyridoxal-5′-phosphate (a form of vitamin B_6) treatment [58]. Inactivation occurs through covalent modification of the Lys50 ε-amino group, which once again emphasizes the role of the β2/β3 loop that contains the Lys49 and Lys50 residues in regulating NEIL2 activity.

Some nonsynonymous polymorphisms of *NEIL2* gene lead to the synthesis of a protein containing a PEST motif – a peptide enriched with Pro, Asp/Glu, and Ser/Thr residues, often serving as a recognition site for the ubiquitinylation system. However, the ubiquitinylation of NEIL proteins has not yet been shown [59].

Inhibition of NEIL proteins by heavy metal ions should be noted as a noncovalent interaction of NEIL proteins that alters their activity. The mechanisms of inhibition of DNA repair by heavy metals remain poorly studied. Examples of co-mutagenic activity of some heavy metal ions were described – for example, they acted synergistically with reactive oxygen species, greatly increasing mutation rates [60, 61]. One of the mechanisms of such co-mutagenicity for Cd^{2+}, Zn^{2+}, and Cu^{2+} may be suppression of NEIL1 enzyme activity as well as the activity of its bacterial homologs – the Fpg and Nei proteins [62, 63].

FEATURES OF *NEIL* GENE EXPRESSION

Participation of NEIL proteins in processes connected with development or tissue-specific functions is quite possible. The level of *NEIL* gene expression in embryonic mouse brain cells varies with time. *NEIL1* and *NEIL2* gene expression begins no later than the 16th day of embryonic development and increases with time [64]. In brain cells of adult mice the expression level of these genes is high at any age, and *NEIL1* expression continues to increase with age [65]. In contrast, *NEIL3* expression starts abruptly on the 12th-13th day of embryonic development in the subventricular zone and dentate gyrus, which are parts of the brain where neural stem cells and progenitor cells are located. During the subsequent formation of the brain *NEIL3* gene expression lowers significantly, and in the adult brain it is only observed in the layer V of the cerebral cortex [65]. Increased *NEIL3* gene expression is observed in hematopoietic cells, some tumor tissues, and some normal tissues during organogenesis. This may indicate a connection between NEIL3 protein functions and high proliferative potential of cells [66, 67]. Interestingly, decrease in *NEIL3* gene expression by RNA interference inhibits replication of human immunodeficiency virus in such cells [68].

Hybridization analysis of mRNA from different organs of adult mice showed a high expression of the *NEIL1* gene in liver, pancreas, and thymus [9, 11, 69]. An intermediate level of expression was observed in brain, spleen, prostate, and ovaries. The expression was relatively low in testis and leukocytes. In most tissues the expression varies slightly with age [9, 11, 69]. A high level of *NEIL2* gene expression is detected in skeletal muscle and testis, and in brain and heart muscle the expression is intermediate [10]. Other analyzed tissues showed a very low level of *NEIL2* gene expression.

Expression of *NEIL* genes may vary within a single organ. For example, while only an intermediate level of

NEIL1 expression is observed in spleen on the average organ-wide, the expression level is significantly higher in B-lymphocytes of spleen germinal nodules, where rapid cell division occurs, along with somatic hypermutation and immunoglobulin class switching. In *NEIL1*$^{-/-}$ mice the number of B-lymphocytes is decreased in germinal centers and the level of somatic hypermutation is lowered, leading to a weakening of the immune response [70].

It was shown that *NEIL* gene expression and localization of their protein products might depend on the phase of the cell cycle. *NEIL1* expression increases in S-phase approximately 4-fold compared with G1 and G2 phases, while the content of *NEIL2* mRNA does not substantially change through the cell cycle [10]. During mitosis NEIL1 protein is localized in the centrosome and in condensed chromosomes [71]. In interphase fibroblasts, a significant fraction of NEIL2 protein is localized in the cytoplasm and is associated with microtubules [72]. NEIL3 protein in lymphocytes is mainly localized in the nucleus regardless of cell cycle phase [66].

Oxidative stress caused by glucose oxidase treatment of a cell culture increases levels of *NEIL1* mRNA in human cells due to activation of transcription factors c-Jun and CREB/ATF2, binding sites of which are found in the promoter of the *NEIL1* gene [73]. *NEIL2* gene expression is regulated by two antagonistically acting elements of its promoter: the positive regulatory element in the region from –206 to +90 and the negative element in the region from +49 to +710 [74]. Oxidative stress caused by glucose oxidase treatment reduces the activity of the positive regulatory element; this regulation depends on the presence of potential binding site for factor NFκB/Sp-1 at position –104 [74]. However, human *NEIL1-NEIL3* genes are not induced and the levels of the corresponding proteins are not increased in γ-irradiated lymphoblastoid cells from TK6 line [75]. *NEIL1* gene expression in lymphocytes from human peripheral blood does not change during hypoxia and during transition from hypoxia to normal partial oxygen pressure [76].

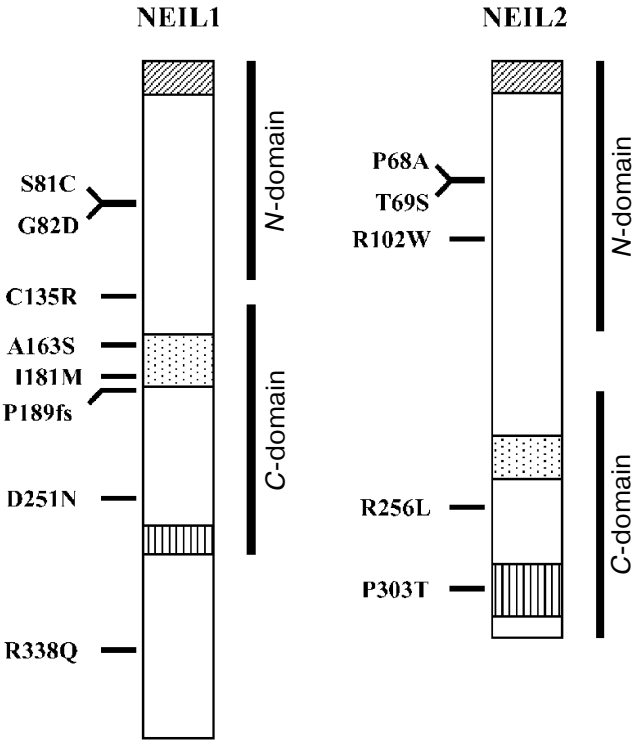

Fig. 4. Known polymorphic variants of NEIL1 and NEIL2 proteins found in humans. P189fs is a protein variant caused by mutation in the Pro189 codon residue that leads to reading frame shift.

POLYMORPHISM OF *NEIL* GENES

Currently a large body of information indicates that defects in DNA repair system are connected with cancer. Repair enzyme inactivation may lead to accumulation of DNA lesions, increasing the mutation frequency in tumor suppressor genes and protooncogenes and elevating the risk of cancer. Thus, polymorphisms and mutations in DNA glycosylase genes can be risk factors for cancer, and the genes are regarded as tumor suppressor genes [3].

There is evidence of an important tumor suppressor role of NEIL proteins. From the polymorphic variants of NEIL1 protein occurring in the population (Fig. 4), the C135R variant has a significantly lowered DNA-glycosylase and AP-lyase activities [77], and the G82D variant of NEIL1 has a lowered DNA glycosylase enzyme activity but increased AP-lyase activity [78]. G82D polymorphism was identified in cases of carcinogenesis in patients with early sclerotic cholangitis – a chronic liver disease characterized by inflammation and destruction of bile-excreting tracts and frequent development of cholangiocarcinoma [78]. Two polymorphisms and three mutations of the *NEIL1* gene were revealed in patients with stomach cancer [79]. One of these mutations (ΔE28) leads to the loss of activity of NEIL1, while another disturbs the splicing of *NEIL1* pre-mRNA, which results in a partial loss of the nuclear localization signal and the active enzyme remains in the cytoplasm.

Nine alleles of *NEIL2* associated with colorectal cancer were identified in the study of inherited mutations in repair genes [80]. *NEIL2* genotype g.4102971CC turned out to be a risk factor for development of squamous cell carcinoma of the oral cavity and nasopharynx [81]. The chromosomal region 8p23.1, which contains the *NEIL2* gene, has a commonly inversed segment with length of about 4 million base pairs. *NEIL2* gene expression was shown to depend on the orientation of this segment as well as on polymorphisms in the promoter region [82, 83]. Loss of heterozygosity in the *NEIL3* locus is often observed in hepatocellular carcinoma [84].

EFFECTS OF INACTIVATION OF *NEIL* GENES

The consequences of *NEIL1* gene inactivation in the whole organism have been studied in mouse models, but the results of these studies are mixed. *NEIL1*$^{-/-}$ mice were reported to develop metabolic syndrome, which is a combination of obesity, hypertension, and hyperinsulinemia [85]. However, later studies did not confirm this observation but showed *NEIL1*$^{-/-}$ mice to exhibit a decreased immune response [70, 86]. Interestingly, the *NEIL1* gene is located near the *Adip5* chromosomal quantitative trait locus, which contributes to the accumulation of body fat [87]. The difference between these results may reflect different interactions of the *Adip5* locus with integrated *NEIL1* gene inactivation construct with various mouse genotypes. The reduction of *NEIL1* mRNA by antisense oligonucleotides leads to an increase in spontaneous and induced mutagenesis in the *HPRT* gene by 3- and 7-8-fold, respectively, with a predominant accumulation of mutations in A-T pairs [88].

The joint inactivation of the *NEIL1* and *NTHL1* genes, the protein products of which remove oxidized pyrimidine bases, leads to the development of tumors in lungs and liver [86]. These tumors contain a characteristic GGT→GAT mutation in the 12th codon of the *RAS* gene that distinguishes them from the GGT→GTT mutations caused by the presence of the 8-oxoGua residue in the second position of this codon [89, 90]. Several tissues of *NEIL1*$^{-/-}$ *NTHL1*$^{-/-}$ mice exhibit elevated FapyGua and FapyAde, but the level of 8-oxoGua does not change. These data suggests that enzyme NEIL1 does not play a significant role in 8-oxoGua repair *in vivo*.

During the eight years since the discovery of NEIL DNA glycosylase, the substrate specificity of these enzymes and protein–protein interactions in the process of DNA repair involving these enzymes have been thoroughly studied. Structural studies started with determining the structure of NEIL1 protein and that of its complex with DNA will undoubtedly continue. Since the NEIL2 and NEIL3 sequences contain unique motifs, one can expect the determination of NEIL2 and NEIL3 tertiary structures to facilitate the characterization of these novel structural elements. The analysis of NEIL protein complexes with other repair proteins is also of great interest. However, the biochemical and structural data is not sufficient for determining the exact function of NEIL proteins in cells. To solve this problem, we must thoroughly investigate the regulation of *NEIL* gene expression, the localization, stability, and activity of NEIL enzymes depending on type of cells and tissues, developmental stage, cell cycle phase, and external influences. Moreover, we need to conduct a detailed analysis of the phenotype of organisms deficient in *NEIL* genes or carrying their mutant variants. Further study of this interesting group of proteins can be expected to uncover new aspects of the DNA repair process.

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