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Oxidized Base Damage and Single-Strand Break Repair in Mammalian Genomes: Role of Disordered Regions and Posttranslational Modifications in Early Enzymes

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Abstract

Oxidative genome damage induced by reactive oxygen species includes oxidized bases, abasic (AP) sites, and single-strand breaks, all of which are repaired via the evolutionarily conserved base excision repair/single-strand break repair (BER/SSBR) pathway. BER/SSBR in mammalian cells is complex, with preferred and backup sub-pathways, and is linked to genome replication and transcription. The early BER/SSBR enzymes, namely, DNA glycosylases (DGs) and the end-processing proteins such as abasic endonuclease 1 (APE1), form complexes with downstream repair (and other noncanonical) proteins via pairwise interactions. Furthermore, a unique feature of mammalian early BER/SSBR enzymes is the presence of a disordered terminal extension that is absent in their *Escherichia coli* prototypes. These nonconserved segments usually contain organelle-targeting signals, common interaction interfaces, and sites of posttranslational modifications that may be involved in regulating their repair function including lesion scanning. Finally, the linkage of BER/SSBR deficiency to cancer, aging, and human neurodegenerative diseases, and therapeutic targeting of BER/SSBR are discussed.

I. Oxidative DNA Damage and Its Repair in Mammalian Cells

The sequence fidelity of the genome, essential for maintaining phenotypes of all organisms, is continuously challenged because of DNA's inherent instability, spontaneous chemical reactions, and replication errors. Moreover, all aerobic organisms continuously generate reactive oxygen species (ROS) as byproducts of respiration. ROS are also produced by cellular oxidases in response to a variety of external insults including environmental chemicals found in cigarette smoke and chemotherapeutic drugs, ultraviolet (UV) light, or ionizing radiation, and during inflammatory response.^{1–4} Intracellular ROS include superoxide anion (O_2^-), the direct product of oxidases and respiration, while hydroxyl radical ($\cdot OH$) and hydrogen peroxide (H_2O_2) are generated via Fenton reactions and enzymatic processes, respectively. The generated O_2^- may be scavenged by NO and form peroxynitrite ($\cdot OONO$). Exogenous ROS including singlet O_2^- , O_3 , and $\cdot OH$ radical are produced during radiolysis of H_2O by ionizing radiation. These reactive species produce multiple oxidative DNA damage in aerobic organisms, including oxidized DNA bases, oxidized sugar fragments, abasic (AP) sites, and single-strand breaks (SSBs). Furthermore, closely spaced SSBs, oxidized bases, or abasic (AP) sites (generated during repair) in the genome could form DNA double-strand breaks (DSBs). While unrepaired DSBs are lethal, oxidized base lesions could be mutagenic, cytotoxic, or both. Thus, ROS-induced genome damage represents the most pervasive insult to the genetic material and more than 10^5 such lesions are estimated to be generated by endogenous ROS in a mammalian cell per day.³ Thus, it is not surprising that ROS-induced genome damage has been implicated in a multitude of diseases, including cardiovascular dysfunction, arthritis, and cancer, as well as

in the aging process and age-related neurodegenerative disorders. However, DNA damage is induced sometimes on purpose, for example, for immunoglobulin gene diversification.^{5,6}

The most abundant oxidative genome damage products are oxidation products of purines, namely, 8-oxoguanine (8-oxoG) and formamidopyrimidines (FapyG and FapyA), while the common oxidized pyrimidines are thymine glycol and 5-OHU; the latter is generated via oxidative deamination of C. Except for thymine glycol and the hydantoin, these abnormal bases do not block DNA replication. The less common oxidized base lesions such as 8-oxoA, 5-formylU, and 5-OHC could also be mutagenic/toxic⁷ (Table I).

A. BER of Oxidized Bases and AP sites in Mammalian Genomes

The major process for restoring genomic integrity by repairing oxidative as well as spontaneous alkylation base damage, and AP sites and abnormal bases such as U generated by spontaneous deamination of C is the base excision repair (BER) pathway. BER proteins are involved in the repair of SSBs as well, as discussed in Section I.C. While evolutionarily conserved organisms range from bacteria to mammals, BER is a versatile DNA repair process that handles many types of damage including base damage, AP sites and their oxidation products, and DNA SSBs. BER is initiated with lesion base excision by a mono- or bifunctional DNA glycosylase (DG), and consists of the following basic steps, as also outlined in Fig. 1.

- i. *Base lesion recognition, excision, and cleavage of AP site.* Monofunctional DGs including uracil-DNA glycosylase (UDG) and methylpurine-DNA glycosylase (MPG, also named alkyladenine-DNA glycosylase or AAG) recognize and excise uracil and alkylated bases, respectively, via *N*-glycosyl bond cleavage to generate AP sites. In contrast, all oxidized bases are excised by bifunctional DGs with intrinsic AP lyase activity.^{8,9} These, in a concerted action, excise substrate bases and cleave the resulting AP sites via β or $\beta\delta$ elimination reaction.^{8,10,11} Five oxidized base-specific DGs have so far been discovered in mammalian cells, which belong to two families named after the prototypes in *Escherichia coli*: OGG1 and NTH1 in the Nth family possess β lyase activity and generate SSBs with 3' phospho $\alpha\beta$ unsaturated aldehyde (3'PUA) and 5'P termini. In contrast, NEIL1 and NEIL2 in the Nei family possess $\beta\delta$ AP lyase activity generating an SSB with 3'P and 5'P termini.¹¹ NEIL3, another paralog in the Nei family, was identified at about the same time as NEIL1 and NEIL2, based on sequence homology, and it has recently been characterized as having predominantly β lyase (and weak $\beta\delta$ lyase) activity.^{12,13} Another evolutionarily conserved DG, MYH (ortholog of *E. coli* MutY), excises the normal base A from an 8-oxoG•A pair in DNA.¹⁴ AP sites generated directly by ROS or by monofunctional DG are hydrolyzed by AP endonuclease 1 (APE1) in mammalian cells to generate an SSB with 3'OH and 5' deoxyribose phosphate (dRP) ends. Thus, the SSB products of DGs or APE1 have a 3' or 5' terminus that prevents subsequent gap-filling DNA synthesis or ligation. These blocked termini are removed in the second step of BER.
- ii. *End-processing of SSB termini to generate 3'OH/5'P.* SSB end-processing is an essential step in BER/SSBR, utilizing diverse enzymes to process various blocked termini. The two major 3' end-processing enzymes involved in mammalian BER/SSBR are APE1 and polynucleotide kinase 3' phosphatase (PNKP), which remove 3'dRP (β elimination product) and 3'P ($\beta\delta$ elimination product), respectively, generating 3'OH.¹¹ Unlike its *E. coli* homolog Xth, mammalian APE1 has extremely weak 3' phosphatase activity.¹¹ Thus, while APE1 is required for OGG1/NTH1-initiated BER, PNKP participates in NEIL-initiated BER. Furthermore, 5'dRP residue (APE1's product of AP site cleavage) is removed by

DNA polymerase β (Pol β) in mammalian cells, which possesses 5' dRP lyase activity.¹⁵ However, Pol β is ineffective if the 5' dRP residue is oxidized, as often occurs during oxidative stress.¹⁶ In such cases, the oxidized 5' dRP is displaced during gap-filling synthesis together with 2–8 additional nucleotides (nts) as an ssDNA flap, which is then removed by flap endonuclease I (FEN-1). FEN-1 is an essential enzyme normally involved in the removal of Okazaki fragment primers during lagging strand DNA replication.¹⁷ A second flap endonuclease, DNA2, that acts in concert with FEN-1 in processing long flap structures has recently been discovered in both nuclei and mitochondria of human cells.^{18,19} Several other end-processing enzymes are also involved in processing various blocked termini at SSBs directly generated in mammalian genomes; these are discussed in Section I.C.

- iii. *Gap-filling after lesion excision.* Lesion excision and termini processing usually leave a 1-nt gap at the damaged base site, which is filled in with the template strand-guided nucleotide by a DNA polymerase. Depending on the repair patch size, two types of BER have been characterized: single nt incorporation repair (SN-BER) involving replacement of only the base lesion with the parent base, and long-patch repair (LP-BER) involving repair patch size of 2–8 nt upstream of the lesion site. As already mentioned, LP-BER requires FEN-1 (and possibly DNA2) to remove the displaced DNA flap. Mammalian cells express multiple DNA polymerases that function in BER/SSBR pathways. Pol β , ubiquitous in mammalian tissues, is the primary BER polymerase and carries out SN-BER in nondividing cells, although Pol β may also participate in LP-BER in coordination with FEN-1.^{20,21} However, LP-BER generally utilizes DNA replication machinery including replicative DNA pols, Pol δ/ϵ , the sliding clamp PCNA, clamp loader replication factor-C (RF-C), DNA ligase I (LigI), and FEN-1.^{16,22,23} The complex issue of choice between SN-BER and LP-BER is yet to be completely understood although initial studies suggested that the nature of the 5'-phosphoribose terminus (normal vs. oxidized) would be a deciding factor.²⁴ However, involvement of DNA replication proteins with LP-BER strongly suggests that LP-BER could be the preferred pathway during DNA replication, irrespective of the 5' terminal group.
- iv. *Nick sealing by DNA ligases.* The sealing of the nick with 3' OH and 5' P termini by a DNA ligase to restore genomic integrity is the final step in BER/SSBR. DNA ligase III α (LigIII α) and Lig I are the major DNA ligases (in addition to Ligase IV, which is involved in DSB repair) in human cells; the former is generally associated with SN-BER and the latter with LP-BER although the distinction may not be absolute.²⁵

B. DG: The BER-initiating Enzyme

BER is unique among excision repair pathways in that the damaged bases are recognized by distinct damaged base-specific DGs. DGs are relatively small (~30–50 kDa) monomeric proteins that do not require cofactors for their activity. Although NTH1 contains an Fe–S cluster, it serves only as an architectural element to position a loop containing positively charged residues near the phosphodiester backbone of a target DNA molecule.^{26,27} Nei family proteins in *E. coli* and mammals (except NEIL1) contain Zn finger motifs, which are involved in DNA binding.²⁸ Because DGs remove base lesions that usually cause minor, or no, distortion in the DNA duplex, damaged base sensing is a major challenge for the DG, particularly for lesions such as U with subtle modifications.⁸ The difficulty is more severe in mammalian genome because the lesion is located in condensed chromatin. It should be mentioned that although DGs are involved in the recognition of base lesions owing to their affinity for a group of modified bases, other signaling factors may also be involved that have not been extensively addressed.

The mechanism of base lesion excision involves extrahelical flipping of the damaged nucleotide into the DG's recognition pocket.^{29,30} All DGs studied so far bind to the minor groove of DNA, kinking it at the site of damage, and flip the lesion nucleotide out of the major groove of DNA.^{29,30} Thus, only those lesions that could be accommodated in the binding pocket after nucleotide flipping to provide the necessary contacts and orientation for their excision are removed by the DGs. ROS produce more than 20 major oxidized base lesions that are repaired by only four (or five) DGs in human cells; thus, each DG acts on subsets of base lesions.^{4,8,31} It is likely that the plasticity of the catalytic pockets of DGs allows an induced fit of diverse substrates. It appears that DGs invariably have low turnover to compensate for their promiscuity. The properties of oxidized base-specific DGs and their preferred substrates are listed in Table II.

C. SSBR: A DG-independent Variant of BER

Repair of ROS-induced SSBs shares the last three steps of the BER pathway, namely, end cleaning, gap-filling, and nick sealing, although SSBR could involve additional end-processing enzymes to remove various termini produced by ROS.

- i. *Diverse end-processing for SSBR.* The end-processing of SSBs has recently been shown to be more versatile than previously suggested. The most common block at ROS-induced SSB is 3' phosphoglycolate (or 3' phosphoglycolaldehyde), which is removed by APE1.^{36,37} Tyrosylphosphodiesterase 1 (TDP1), another 3' end-processing enzyme, cleaves Top1 (Tyr)-cross-linked to 3'P at the strand break generated by abortive topoisomerase 1 (Top1) reaction. TDP1 also processes 3' phosphoglycolate at DSBs.³⁸ The resulting 3'P is subsequently removed by PNKP,³⁹⁻⁴² which also phosphorylates the 5'OH generated at an SSB. A unique 5' blocking group is formed as intermediates during abortive DNA ligation, namely, adenylate linked to the 5'P terminus at an SSB via a 5'•5' pyrophosphate bond. Aprataxin releases 5'AMP to restore the 5'P terminus.^{43,44} Some blocked 3' termini could also be removed by ERCC1/XPF nuclease, a functional homolog of the yeast Rad1/Rad10 complex proposed for this role in the budding yeast.⁴⁵ The various end-processing enzymes involved in BER/SSBR and their substrates are listed in Table III.

Unlike repair of simple nicks, which may be generated by nucleases or caused by replication inhibition and usually contain 3'OH and 5'P, repair of most ROS-induced SSBs involves gap-filling repair synthesis. Polβ, which could carry out LP-BER in collaboration with FEN-1, likely provides the major DNA polymerase activity for SSBR, particularly in nonreplicating cells.^{20,21}

- ii. *Role of XRCC1 and PARP-1 in BER/SSBR.* XRCC1 and PARPs are other key proteins with indirect roles in BER/SSBR. While XRCC1 acts as a scaffold to recruit BER proteins for excision or strand break repair, PARP acts as an SSB sensor protein.⁴⁶ XRCC1 physically interacts with NEILs, PNKP, APE1, and other BER proteins Polβ and LigIIIα, implicating it in the first step of BER.^{11,32} Moreover, persistence of 5'-OH and 3'-P termini at SSBs in XRCC1-deficient cells underscores the latter's role in end-processing.⁴⁷ PARPs, expressed in mammalian cells but absent in *E. coli* or yeast, constitute a superfamily with regulatory functions in various cellular processes including development.^{48,49} Only PARP-1, -2, and -3 are involved in DNA repair.⁵⁰ PARP-1 and -2 are activated by SSBs when they transfer ADP-ribose moiety from NAD to a variety of proteins including themselves. PARP-2 may serve as a backup for PARP-1 because inactivation of both *PARP 1* and *2* genes in mice is lethal, while these are individually dispensable for viability.^{51,52} These proteins play structural and regulatory roles in SSB repair by acting as sensors and by recruiting other repair

proteins to the strand break site.⁵³ However, their direct involvement in damage processing during BER has not been demonstrated so far. PARP-3 has recently been implicated in DNA DSB repair.⁵⁴

D. BER/SSBR in Mammalian Mitochondria

The mitochondria are the major site of ROS generation in aerobic organisms and thus their genomes are continuously exposed to oxidative damage. Furthermore, lack of protective histones makes the mitochondrial DNA more susceptible to oxidative damage than the nuclear genome. Repair of oxidized bases and SSBs via both SN-BER and LP-BER has been established in mammalian mitochondria.^{55–57} The early mitochondrial BER proteins, all encoded by nuclear genes, are usually isoforms of the nuclear proteins, generated due to alternate RNA splicing or proteolytic cleavage. Among the DGs, OGG1, NTH1, and NEIL1 have been shown to localize in the mitochondria.^{58–60} We have shown that an N-terminal truncation product of APE1 is present in mammalian mitochondria.⁶¹ Recent studies have shown the presence of TDP1 and aprataxin in mitochondria.^{62,63} In contrast to the sharing of early BER proteins between nucleus and mitochondria, DNA polymerase γ (Pol γ), the only DNA polymerase in mammalian mitochondria, is required for both mt genome replication and repair.⁶⁴ A splice variant of nuclear LigIII α functions similarly in both mt genome replication and repair.²⁵ Although mitochondrial BER/SSBR was initially thought to be a simple process involving only a few essential enzymes, recent studies have demonstrated the presence of several additional nuclear BER/SSBR components in mitochondria.⁶⁵

II. Complexity and Sub-pathways of BER/SSBR

Complete nuclear BER/SSBR that requires only four or five enzymes could be demonstrated *in vitro*. However, recent studies have revealed that BER is far more complex, involving a network of distinct cell cycle dependent as well as genome region-specific repair sub-pathways and could also involve several non-BER proteins.^{66,67}

A. Preferred and Backup Sub-pathways

The overlapping substrate specificity of DGs and the lack of strong phenotypes associated with their individual deficiency suggest that these enzymes have preferential and backup functions. Mouse mutants individually lacking OGG1, NTH1, NEIL1, or MYH and the cells derived thereof are viable, while combined deficiency of two DGs (e.g., NEIL1 and NTH1 or OGG1 and MYH) strongly increases cancer susceptibility.^{68–73} We postulate that these enzymes with the unusual plasticity of their catalytic pockets can carry out excision of diverse damaged bases in DNA, which is consistent with their catalytic inefficiency. However, the choice of the DG-initiated BER sub-pathway for the same lesion may also depend on the cellular state.

Our initial discovery that OGG1 and NTH1 are active only with the duplex DNA while NEILs are more active with ss DNA substrates that mimic replicating or transcribing sequences led us to hypothesize that NEILs preferentially function in repair during DNA replication and/or transcription.⁶⁶ We subsequently characterized the role of NEIL2 in preferential repair of transcribed genes, where NEIL2 functionally interacts with RNA polymerase II.⁷⁴ Unlike NEIL2, NEIL1 is induced in S-phase cells and functionally interacts with DNA replication proteins including PCNA, replication protein A (RPA) FEN-1, and Werner's helicase (WRN), which suggests that NEIL1 is preferentially involved in replication-coordinated BER.^{33,34,75–78}

B. Role of Noncanonical Proteins

Several noncanonical proteins have been shown to be involved in BER, adding another dimension to the BER complexity. However, their precise *in vivo* functions in BER/SSBR are yet to be unraveled. We showed that NEIL2 interacts with YB-1, a Y-box binding protein, and it was suggested that YB-1 may be required for the fine-tuning of repair.⁷⁹ NTH1 and APE1 were also shown to interact with, and be stimulated by, YB-1.^{80,81} Recently, Banerjee *et al.* showed association of NEIL2 with hnRNP-U, an RNA-binding protein, and its role in transcription-coupled BER.⁷⁴ HMGB1 has been implicated in SSBR involving Pol β .⁸² Tumor suppressor protein p53 interacts with APE1 and Pol β , and stimulates BER *in vitro*.⁸³ P53 was also shown to play a role in UV radiation-induced DNA damage repair.⁸⁴ Jaiswal *et al.* showed inhibition of both SN-BER and LP-BER or LP-BER alone by the adenomatous polyposis coli (APC) and by cyclin-dependent kinase inhibitor p21 genes, respectively.^{85,86} The growing list of noncanonical proteins underscores the paradigm that BER/SSBR *in vivo* is far more complex than *in vitro* repair demonstrated with minimal components.

C. Repair Interactome: Preformed Complexes Versus Sequential Recruitment

The prevailing view of BER is that it comprises a sequence of steps with individual repair enzymes carrying out reactions independently of one another. This concept was initially proposed based on X-ray crystallographic studies, as a “hand-off” or “passing the baton” process, wherein the repair product of each enzyme in the BER pathway is handed over to the next enzyme, primarily based on differential bending of DNA in each intermediate step.^{35,87,88} However, our recent studies as well as others’ have shown that early BER/SSBR enzymes (e.g., NEIL1, APE1) stably interact with most downstream repair components including DNA ligase, via their common interacting domain.^{11,32,77,78,89} Furthermore, NEIL1 and NEIL2 immunoprecipitates (IP) from human cells contain the SN-BER proteins PNKP, Pol β , LigIII α , and XRCC1, as well as LP-BER-specific DNA replication proteins including PCNA and FEN-1,^{11,32,76,77} with which the NEILs also interact in a pairwise fashion in the absence of DNA. Direct interaction of NEILs with LigIII α , the last enzyme in SN-BER, indicates that the repair is regulated by the initiating DG, which appears to act as a hub protein. This has led us to propose a new BER paradigm in which collaboration of multiple proteins in a coordinated fashion involving dynamic protein–protein interactions enhances repair efficiency. Although the *in vivo* role of sequential engagement of individual BER proteins versus coordinated action of a preformed BER complex is not determined yet, we propose that the preformed BER complexes repair endogenous base lesions, while repair via hand-off mechanism by sequential recruitment could occur for induced DNA damage. Further characterization of the dynamics of such preformed “BERosomes” is required to unravel the precise repair processes.

III. Nonconserved Terminal Extensions in Mammalian Early BER Proteins

Mammalian DGs possess unique structural features absent in their homologs in lower organisms because of the presence of a nonconserved extension at the N or C terminus (reviewed in Ref. 90). These might have been acquired during evolution via terminal fusion of a non-BER gene (Fig. 2). The sequence alignment of human (h) NTH1 with its prototype Nth in *E. coli* and NTHs in other lower organisms clearly defines hNTH1’s unique N-terminal extension. hNTH1 was also shown to have reduced activity compared to *E. coli* Nth prototype, which appears to be due to the inhibitory role of the N-terminal tail.^{27,91} This extension reduces the rate of product release without affecting base excision or AP lyase activities.⁹² Similarly, the crystal structure of the catalytically active deletion construct of hNEIL1 (lacking 56 C-terminal residues) indicated that hNEIL1 has C-terminal extension (~100 residues), which is absent in the *E. coli* prototype Nei.^{90,93} Furthermore, PONDR, the

predictor of naturally disordered regions in proteins, and other modeling studies showed that the terminal extensions in mammalian DGs are mostly disordered. A sequence comparison of hMYH and its prototype MutY in *E. coli* shows the presence of N-terminal disordered segment in the former.^{90,94} Similar disordered terminal sequences may also be present in other human DGs, for example, UNG2 and TDG. In the case of APE, the nonconserved N-terminal segment (~65 residues) in hAPE1, absent in the *E. coli* prototype Xth, appears to be mostly disordered. Although the unfolded sequence generally exists at the N or C terminus, this could also exist internally as in hNEIL2, where it may serve as a linker of two domains.⁹⁰

The size range of disordered extensions in early BER proteins is 50–100 residues, with few exceptions, for example, hOGG1 and human Pol β , which have short (~10 residues) disordered segments at both termini.⁹⁰

A. Functions of Disordered Terminal Extensions

The nonconserved, mostly disordered terminal peptide segments of early BER/SSBR proteins in mammals implicate these in important functions including damage sensing, protein–protein interactions, repair regulation via posttranslational modifications, and nuclear localization signal (NLS).^{8,90} Furthermore, the disordered regions provide an opportunity for alternative splicing without perturbing the structured regions.⁹⁵ Disorder also provides size advantage in a polypeptide by providing a common interface for multiprotein binding and sites of covalent modifications. Thus, disorder may help higher organisms limit protein size and reduce intracellular crowding.⁹⁶

- i. *Protein–protein interactions.* As mentioned earlier, the “hub” proteins such as NEIL1 or APE1 with multiple interacting partners (>10) utilize the disordered segment as a common interaction interface.⁹⁷ The “hub” protein complexes have been shown to be widely present in higher eukaryotes, invariably via interaction with disordered regions.⁹⁸ The crucial role of intrinsic disorder in hub proteins has been reviewed elsewhere.^{99,100} Although it is intriguing how NEIL1 or APE1 could simultaneously bind multiple proteins with high affinity and specificity via a small common interaction interface, this phenomenon is quite common for mammalian “hub” proteins with disordered structures.^{8,90} Further, disorder-mediated interactions may confer advantages over order-mediated interactions because rapid interconversion among diverse conformers allows for formation of dynamic complexes.¹⁰¹ In addition, disorder-mediated interactions have steric advantages as they provide a large surface area for binding interface for wrapping around partners, resulting in stronger specificity.⁹⁶
- ii. *Subcellular localization.* Organelle localization signals including NLS and mitochondrial transport signal (MTS) are typically contained in short disordered segments (generally <20 residues). The classical NLS consists of seven basic residues and the bipartite NLS has two strings of basic residues separated by a short intervening sequence.¹⁰² Almost all NLS regions with mostly basic residues are disordered.¹⁰³ We mapped the NLS of hAPE1 to the disordered N-terminal 20 residues, the deletion of which markedly diminishes its translocation to the nucleus.¹⁰⁴ Our preliminary studies of GFP-fusion polypeptide of truncated NEIL1 suggest the presence of putative NLS at the disordered C-terminal region (Hegde ML and Mitra S, unpublished observation). Similarly, the disordered N-terminal tail in hNTH1, UNG2, and TDG contains putative NLS and MTS.^{91,105,106} Taken together, these studies show that subcellular distribution of many human BER proteins is mediated through signals localized in their disordered regions.

- iii. *Target DNA scanning.* The ability of early BER/SSBR proteins to locate and then bind to substrate lesions in a large pool of DNA should strongly impact their repair efficiency. Such target DNA search could be achieved via facilitated diffusion comprising four mechanisms, namely, one-dimensional (1D) sliding, hopping, 3D search, and intersegmental transfer. An efficient search mechanism involves a combination of these different modes.¹⁰⁷ Recent studies have shown that the most efficient and rapid scanning of the DNA for the target site involves 80% hopping and intersegmental transfer and 20% sliding by the DNA-binding proteins, which invariably contain a disordered terminal extension or a disordered linker for multidomain or multi-subunit proteins.^{108–110} Furthermore, nearly 70% of DNA-binding proteins have such disordered tails compared to about 25% for non-DNA-binding proteins.^{109,111} Another unique characteristic of the disordered tails in DNA-binding proteins is clustering of positively charged residues in the distal region, which were shown to be important for the scanning. Mutating such residues in HOXD9 and NK-2 markedly decreases the scanning efficiency.^{109,112} Similar results were obtained when the N-terminal segment in these proteins were deleted, suggesting that the initial scanning is mediated by a nonspecific, mostly electrostatic transient DNA binding via the basic disordered segment, which is followed by target DNA sequence binding by the active site.^{109,113}

In light of these studies, we examined hNEIL1's C terminus, which possesses most of the characteristics required for DNA scanning, including the presence of clustered basic residues. Our recent biochemical studies using C-terminal deletion mutants of NEIL1 showed that the C terminus is important for NEIL1's substrate scanning and efficiency of damage recognition, via its nonspecific DNA binding (Hegde ML and Mitra S, unpublished data). Although limited information is available on the role of disordered regions in other early BER proteins, we predict a similar situation for these proteins as observed for NEIL1 or APE1.

IV. Posttranslational Modifications in Early BER Proteins

Posttranslational modifications of proteins, including acetylation, phosphorylation, ubiquitylation, ADP-ribosylation, sumoylation, and methylation, play a critical role in diverse cellular processes including DNA repair.¹¹⁴ Such covalent modifications may have multiple physiological effects on these proteins, including stability, interaction with DNA or other proteins, organelle targeting, and enzymatic activity.¹¹⁵ Furthermore, the modification sites are often localized in disordered regions, for example, in the N-terminal segment in hAPE1,^{116,117} N and C termini of p53,¹¹⁸ and the C-terminal region in hNEIL1 (Bhakat KK, Hegde ML and Mitra S, unpublished data).

A. Acetylation and Phosphorylation Modulate Repair Activity

The functions of DNA repair proteins are generally regulated via their acetylation or phosphorylation. These modifications could also regulate protein stability, interaction, and intracellular distribution (reviewed in Ref. 115). Our laboratories and others identified and characterized acetylation of hAPE1 at Lys6 or Lys7, Lys25, Lys27, and Lys31,^{116,119} Lys 6 and 7 acetylation strongly affects APE1's transcriptional regulatory functions.¹¹⁶ APE1 stably interacts with Y-box-binding protein 1 (YB-1) and acetylation further enhances its binding with YB-1 both *in vivo* and *in vitro*,⁸⁰ leading to the activation of the multidrug resistance (*MDR1*) gene. Thus, it appears likely that acetylation-mediated conformational change in the disordered N-terminal segment (~40 residues), which is dispensable for APE1's endonuclease activity, modulates protein–protein interactions. The physiological importance of APE1 acetylation became more evident with our findings that the levels of acetylated APE1 are increased in response to a variety of cellular stresses, or changes in

intracellular Ca^{2+} , or bacterial infection to gastric epithelial cells.^{120,121} We also showed that hNEIL2 is acetylated at Lys49 and Lys153 both *in vitro* and in cells.¹²² Acetylation of Lys49 located in the disordered region inactivated NEIL2's base excision and AP lyase activity while acetylation of Lys150 had no effect. We thus proposed that acetylation of Lys49 could act as a regulatory switch in NEIL2.¹²² TDG is acetylated at Lys70, 94, 95, and 98 in the N-terminal region, within the disordered segment (~100 residues).¹²³

Acetylation of TDG by CBP/p300 indirectly deregulates TDG-coupled repair by releasing CBP/p300 from the DNA-bound complex, leading to reduced interaction with APE1 and suppressing APE1-dependent repair, and could contribute to genomic instability.¹²³ Acetylation of hOGG1 occurs at Lys338 and Lys341 within its short disordered C terminus, and the modification increases OGG1's turnover by reducing its affinity for the product AP site.¹²⁴ Moreover, oxidative stress increases the level of acetylated OGG1, most likely as a result of ROS-induced activation of p300. We have speculated that OGG1 acetylation provides a mechanism for rapid cellular response without requiring its *de novo* synthesis when enhanced repair is promptly needed for handling increased lesion load in cell genomes after exposure to oxidative stress. This is further supported by our observation that the repair of 8-oxoG is correlated with the level of acetylated OGG1.¹²⁴

APE1 was also shown to be phosphorylated *in vitro* and in cells by protein kinase C.^{117,125,126} APE1 can also be phosphorylated *in vitro* by casein kinase I and II (CKI and CKII) at various sites^{126,127}; CKII-mediated phosphorylation abolished DNA repair activity *in vitro*, while phosphorylation by CKI or PKC had no such effect.¹²⁶ APE1 phosphorylation by CKII also enhances transactivation of the AP-1 transcription factor.¹²⁸ XRCC1 in human cells is phosphorylated by CKII and is required for its stability and efficient BER/SSBR.¹²⁹ More recently, Huang *et al.* found that APE1 was phosphorylated at its Thr233 residue by threonine kinase CDK5, a paralog of CDK2/4.¹³⁰ CDK5 is expressed in neurons and is speculated to be involved in cell death triggered by uncontrolled DNA replication. A high level of phosphorylated Thr233 was observed in brain tissues from Parkinson's and Alzheimer's patients,¹³⁰ and inactivation and degradation of APE1 by phosphorylation and the subsequent ubiquitylation may profoundly affect the severity of the diseases. Furthermore, phosphorylation of the DGs, UDG,^{131,132} MYH,¹³³ and OGG1¹³⁴ has been reported to modulate their repair function. Thus acetylation and phosphorylation of early BER/SSBR proteins play a key role in regulating their repair functions and modulating their interactions with other repair proteins.

B. Ubiquitylation and BER Protein Turnover

Ubiquitylation has been demonstrated to play a role in regulating the steady-state level of BER/SSBR enzymes in mammalian cells by directing their degradation and turnover.¹³⁵ Ubiquitin is a highly conserved small protein (~8.5 kDa) whose covalent addition to a target protein as a polymer by ubiquitin ligases promotes the latter's degradation by the 26S proteasome.¹³⁶ However, ubiquitylated proteins may also be recycled by the removal of ubiquitin by deubiquitylases (DUBs) or ubiquitin-specific proteases.^{137,138} Proteins may be ubiquitylated with a single ubiquitin moiety (monoubiquitylation) or with multiple ubiquitin molecules (polyubiquitylation). Polβ is ubiquitylated by the E3 ubiquitin ligase Mule at Lys 41, 61, and 81, and substitution of these Lys with Arg increased Polβ stability.¹³⁵ Furthermore, Mule-depleted cells contain higher levels of Polβ and increased DNA repair activity. It was recently demonstrated that APE1's ubiquitylation, which is enhanced by phosphorylation at Thr233,¹³⁹ could act as a signal for regulating the stability, subcellular localization, and gene regulatory functions of APE1.^{117,137} The mammalian nuclear UDG, UNG2, is also ubiquitylated in cell. Blocking its nuclear export using a nuclear export inhibitor prevents its ubiquitylation and subsequent degradation.¹⁴⁰ This suggests that translocation of proteins from the nucleus to the cytoplasm is required for their turnover. In

a distinct situation, PCNA is both mono- and polyubiquitylated, thus mediating translesion DNA synthesis and error-free lesion bypass, respectively.^{141,142}

V. BER/SSBR Deficiency in Human Diseases

Maintaining genomic integrity through DNA repair is essential for the functioning and survival of an organism. Hence, defective or deficient repair leading to accumulation of unrepaired genome damage has been associated with a range of human disorders including cancer susceptibility, aging, and various neurodegenerative diseases. While compelling evidence exists linking deficiency in SSBR proteins to human diseases,¹⁴³ there is some controversy regarding a similar association with the early BER proteins, presumably due to the backup role of BER sub-pathways as discussed earlier.

A. Cancer

Varying degrees of cancer proneness have also been demonstrated with BER/SSBR defects. A connection between a repair defect and cancer risk is established for the hMYH.^{144,145} The genetic locus of OGG1 (3p26.2) is frequently lost in various human cancers, including lung and renal cell cancer.^{146,147} Polymorphic variants of OGG1 (S326C and R46Q) have also been identified within the population that demonstrate mild reductions in enzymatic activity.^{148,149} However, there are conflicting epidemiological reports regarding the association of these variants with cancer susceptibility.¹⁵⁰ While an increase in the levels of 8-oxoG and mutation frequency was evident in OGG1-null mice, no significant enhancement of spontaneous tumor incidence was observed.^{68,151,152} Interestingly, the NEIL1-knockout mouse was found to exhibit clinical features distinctly similar to the human metabolic syndrome, with extensive fat deposits in various tissues.⁷³ As already mentioned, because of the backup functions of mammalian DGs, mice lacking a single DG do not show a significant phenotype, and only when two or more DGs are simultaneously eliminated do symptoms appear. For example, when both MYH and OGG1 were deleted, the resulting double-knockout animal (unlike the single knockouts) exhibited an increased predisposition to spontaneous tumorigenesis, particularly lung and ovarian tumors, and lymphomas.¹⁵³ It is likely that single DG-null animals possess compensatory mechanisms for base damage tolerance that prevent disease manifestation,¹⁵⁴ which could explain why the association of BER defects with cancer proneness appears less compelling. Furthermore, specific environmental factor(s) may foster the observed disease susceptibility in DG-deficient animals/cells.¹⁵⁵

In contrast to the weak association of DGs with cancer risk, deficiencies in downstream BER/SSBR proteins, for example, Pol β , APE1, or XRCC1, as well as FEN1 or a DNA ligase (I or III), lead to embryonic lethality in mice, suggesting an absolute requirement for the BER process during embryonic development.^{156–159} Furthermore, inactivation of Pol β mutations has been strongly associated with cancer. Genetic analysis revealed that an astonishing ~30% of human tumors express Pol β variants,¹⁶⁰ many of which (e.g., K289M and I260M) exhibit reduced polymerase fidelity.¹⁶¹ APE1 and XRCC1 variants have also been found in numerous epidemiology studies to associate with specific human cancers^{162,163}; however, definitive conclusions could not be drawn because of the small sample sizes.^{164,165}

B. Neurodegenerative Diseases

More than 200 neurological disorders with diverse etiologies and genetic characteristics have been reported so far in humans, many of which have been linked to inherited or acquired defects in one of the DNA repair pathways, leading to imbalance in damage and repair capacity.^{166–169} This is consistent with accumulation of oxidative genome damage in

the neurons for the majority of such diseases including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis (ALS), and various ataxias. Mutations or altered expression of BER (e.g., OGG1, XRCC1^{170–172}) and SSBR (e.g., TDP1, aprataxin, PNKP^{39,43,173,174}) proteins has been observed in humans predisposed to various hereditary neurodegenerative diseases. Association between the XRCC1 Arg399Gln polymorphism and ALS risk was reported.¹⁷⁵ A common Ser326Cys polymorphism in OGG1 with reduced enzyme activity and increased risk of cancer was shown to be weakly associated with HD and ALS,¹⁷⁶ but not with AD or PD.^{171,177,178} Asp148Glu polymorphism in APE1 with lower repair activity, was linked to increased ALS risk.¹⁷⁹ Further, defects in end-processing proteins aprataxin and TDP1 have been shown to be associated with ataxia with oculomotor apraxia1 and spinocerebellar ataxia with axonal neuropathy, respectively.^{173,180,181} A recent study linked mutations in PNKP to autosomal-recessive disease characterized by severe neurological abnormalities including microcephaly, early-onset intractable seizures, and developmental delay (denoted MCSZ¹⁷⁴).

Decreased repair capacity for oxidative genome damage was also observed in brain cell extracts in sporadic neurodegenerative diseases constituting more than 80% of most disease incidences, whose causes are not known. Weissman *et al.*¹⁶⁹ showed that the significant BER deficiencies in the brains of AD patients are due to limited DNA base damage processing by DGs and reduced repair synthesis by Pol β . We recently showed that transition metals iron (Fe) and copper (Cu) that invariably accumulate in neurons in these diseases significantly inhibit the activity of NEIL1 and NEIL2 at physiological levels in both neuronal cells and *in vitro*.^{182,183} These metals affect both base excision and AP lyase activities of NEILs and inhibit NEIL1's interaction with downstream repair proteins including Pol β , and FEN-1, further inhibiting the overall repair. Inhibition of NEILs by Cu involved oxidation of cysteines as well as structural changes via direct binding. The lack of OGG1 inhibition under similar conditions suggests binding specificity of the NEILs and excludes metal ions' direct binding to DNA. These results showed for the first time that the Fe/Cu overload associated with neurodegenerative diseases could be a "double whammy" by increasing oxidative genome damage load and, at the same time, inhibiting its repair. Many other BER/SSBR proteins are also inhibited by transition metals. Whiteside *et al.* showed that Cd and Cu inhibit both phosphatase and kinase activities of PNKP with human cell extracts and recombinant protein.¹⁸⁴ Furthermore, elevated Fe levels cause reduction in FEN-1 and LigIII activities because of the interference of repair protein binding to their DNA substrates.¹⁸⁵

Interestingly, curcumin, a natural spice component with both metal chelation and reducing activities, was able to reverse the metal-induced inhibition of NEILs both *in vitro* and in neuronal cells, suggesting its therapeutic potential.^{182,183}

C. BER/SSBR as Cancer Therapeutic Targets: Are We at a Crossroad?

Cellular sensitivity to DNA-damaging agents, such as ionizing radiation and radiomimetic drugs, is generally believed to reflect their DNA repair competence. For example, high expression of APE1 in tumor tissues is linked to their resistance to radiation and chemotherapy.^{186–188} Therefore, research targeting BER proteins, in particular APE1, for sensitizing cancer cells has been intensified in recent years.^{86,189–191} Among BER/SSBR proteins, PARP-1 was shown to be an effective therapeutic target where PARP-1 inhibitors strongly enhanced susceptibility to radiation (IR)/chemotherapy of BRCA1-negative human breast cancer cells, which was proposed to be due to synthetic lethality.^{192,193} Resistance of tumor cells to radiation and radiomimetic drugs results from efficient repair of induced DNA DSBs via homologous recombination (HR), which involves BRCA1. In the absence of HR, PARP-1 inhibitors were proposed to target SSBR, also induced by radiation and drugs.¹⁹⁴

However, we propose an alternative basis for drug sensitization by PARP inhibitors. Based on the underlying assumption that SSBs do not trigger cell death until they are converted into DSBs via DNA replication and that DSBs activate apoptotic signaling, we suggest that in the absence of HR, the DSBs that are produced as replication intermediates are repaired via the alternate end joining (Alt-EJ) pathway, which is distinct from classical nonhomologous end joining (NHEJ), by requiring SSBR proteins including PARP-1, LigIII α , and XRCC1, but not Ku, DNA-PKcs, LigIV/XRCC4, etc.¹⁹⁵ Unraveling the detailed molecular mechanisms of BER/SSBR and their regulation has significant ramifications for future cancer therapy where not only PARP1 inhibitors but also other BER/SSBR proteins could be targeted to sensitize cancer cells, particularly when the surviving cells develop resistance to PARP inhibitors.

VI. Conclusions and Future Perspectives

Although BER/SSBR is universally conserved, the mammalian BER/SSBR has multiple sub-pathways, sometimes involving noncanonical proteins, commensurate with the structural complexity of the genome and its organization. Our recent studies have led us to propose a new paradigm for BER/SSBR whose key features are as follows: (a) The DG controls the BER sub-pathway by pairwise interaction with most downstream proteins via the disordered common interaction interface that is located in one of the polypeptide termini; (b) essentiality of common interface-mediated interaction for efficient in cell oxidized base repair is shown by its trans-dominant negative activity; (c) the DGs could be present in cell as megadalton complexes containing both repair and noncanonical proteins to direct repair via distinct sub-pathways; (d) the BER complex enhancement by oxidative stress could involve acetylation and other covalent modifications of DGs and other BER proteins. Contrary to the early perception of BER being the simplest among the DNA repair pathways, it is now clear that many profound questions are still unresolved including: (1) lesion scanning in chromatinized DNA, (2) repair of oxidized bases in heterochromatin versus euchromatin, (3) the role of the plethora of proteins involved in chromatin unfolding and remodeling and the impact of their posttranslational modifications. Future studies should also focus on a comprehensive characterization of the role of noncanonical proteins in the repair of endogenous versus induced oxidized bases, including chromatin-modifying enzymes involved in oxidized base repair. Understanding how DNA repair deficiency occurs and affects cellular functions could provide a rational therapeutic basis for ameliorating the genotoxic consequences in neurodegenerative diseases and for sensitizing cells for cancer therapy by blocking DNA repair.

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Fig. 1. Schematic representation of mammalian BER/SSBR pathways for repair of oxidized bases, AP sites, and SSBs. The BER and SSBR pathways converge at the end-processing step. The gap-filling step may involve synthesis of 1 nt (SN-BER/SSBR) by Polβ or 2–8 nt (LP-BER/SSBR) by Polδ/ε or Polβ in collaboration with FEN-1. Other details are in the text.

Fig. 2.

Disordered terminal extensions in human (and other mammalian) early BER/SSBR proteins that are absent in their *E. coli* prototypes (*not drawn to scale*). In many cases, disordered segments were deleted for X-ray crystallographic structure analysis, which are consistent with PONDR prediction.^{8,90}

TABLE I

Common Oxidized Bases Induced by Reactive Oxygen Species (ROS)

Normal DNA bases			
1. Adenine	2. Guanine	3. Cytosine	4. Thymine

Common oxidized base lesions			
Formamidoadenine (FapyA)	8-Oxoguanine	5-Hydroxycytosine (5-OHC)	Thymine glycol

8-Oxoadenine (8-OxoA)	Formamidoguanine (FapyG)	5-Hydroxyuracil (5-OHU)	Dihydrothymine

	Guanidinohydantoin	5-Formyluracil (5-FormylU)

Oxidative modifications are shown in *gray*.

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Oxidized DNA Base-Specific DNA Glycosylases in Mammalian Cells and Their Properties

TABLE II

Enzyme/property	OGG1	NTH1	NEIL1	NEIL2	NEIL3
Type	Nth type				
AP lyase	β Elimination	β Elimination	$\beta\delta$ Elimination	$\beta\delta$ Elimination	β (weak $\beta\delta$) elimination
Product	3' dRP	3' dRP	3' P	3' P	3' dRP/3' P
Downstream Enzyme	APE1	APE1	PNKP	PNKP	APE1/PNKP
DNA substrate	Only duplex	Only duplex	Duplex, ss, bubble	Duplex, ss, bubble	Duplex, ss, bubble
Conserved motif	HhH	HhH	H2TH	H2TH	H2TH
Presence of Zn finger	None	None	Zn less finger	Zn finger	Zn finger (2)
Fe-S cluster	None	Fe-S cluster	None	None	None
Catalytic residue	Lys249	Lys212	Pro1	Pro1	Val1
Cell cycle-dependent expression	None	None	S-phase specific	None	Unknown

Compiled from Refs. 8,9,11–13,28,32–35.

TABLE III**DNA End-Processing Enzymes Involved in BER/SSBR**

Enzyme	Substrate	Product
APE1	3' dRP (3' PUA)	3' OH
	3' phosphoglycolate	
	3' phosphoglycolaldehyde	
PNKP	3' P	3' OH
	5' OH	5' P
Po1 β	5' dRP	5' P
TDP1	Top (Tyr) linked 3' P	3' P
Aprataxin	5' adenylate•5' P	5' P

Their substrates and products are indicated.