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Review Article

The role of UV-DDB in processing 8-oxoguanine during base excision repair

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Recent data from our laboratory has shown that the nucleotide excision repair (NER) proteins UV-damaged DNA-binding protein (UV-DDB), xeroderma pigmentosum group C (XPC), and xeroderma pigmentosum group A (XPA) play important roles in the processing of 8-oxoG. This review first discusses biochemical studies demonstrating how UV-DDB stimulates human 8-oxoG glycosylase (OGG1), MUTYH, and apurinic/apyrimidinic (AP) endonuclease (APE1) to increase their turnover at damage sites. We further discuss our single-molecule studies showing that UV-DDB associates with these proteins at abasic moieties on DNA damage arrays. Data from cell experiments are then described showing that UV-DDB interacts with OGG1 at sites of 8-oxoG. Finally, since many glycosylases are inhibited from working on damage in the context of chromatin, we present a working model of how UV-DDB may be the first responder to alter the structure of damage containing-nucleosomes to allow access by base excision repair (BER) enzymes.

Introduction

Human DNA is under constant assault by a wide range of physical and chemical agents that can generate toxic and mutagenic lesions [1]. Major sources of genomic damage are reactive oxygen and nitrogen species (ROS/RNS), including: singlet oxygen, superoxide radical anion, hydroxyl radical, nitric oxide, hydrogen peroxide, and peroxy-nitrite. ROS/RNS are generated by normal cellular metabolism and inflammation, or by various exogenous sources such as ionizing radiation (IR) [1–3]. Oxidation induces a variety of base lesions in the DNA, which can cause genomic instability and can contribute to various human maladies including neurodegenerative disorders, aging, and cancer [4].

One of the most abundant oxidative lesions in the genome is 8-oxoguanine (8-oxoG), which is repaired by human 8-oxoG glycosylase (OGG1), through the base excision repair (BER) pathway. OGG1 is a bifunctional glycosylase that first excises the oxidized purine from 8-oxoG:C base pairs, by hydrolyzing the N-glycosidic bond between the base and sugar moiety. Subsequently, OGG1 breaks the sugar-phosphate backbone through its weak lyase activity. OGG1 is product inhibited, binding avidly to the resulting abasic site. OGG1, therefore, turns over slowly in the absence of other proteins such as apurinic/apyrimidinic (AP) endonuclease (APE1) [5,6]. If unrepaired, 8-oxoG is a premutagenic lesion that causes G:C to T:A transversions [7]. MUTYH is a monofunctional DNA glycosylase that plays a critical role in the long patch BER for excision of the nucleobase: adenine (A), which is mis-incorporated opposite 8-oxoG, during replication [8]. Adenine excision occurs within the N-terminal catalytic domain of MUTYH, while 8-oxoG recognition arises mainly in the C-terminal domain of 8-oxoG [9]. Like OGG1, MUTYH has a high affinity for abasic sites and is, therefore, product inhibited [10].

DNA glycosylases like OGG1 and MUTYH have limited ability to work on lesions embedded in nucleosomes [11]. Furthermore, as previously mentioned both proteins bind more avidly to their abasic site products than to the base damage itself causing slow enzyme turnover. Previous studies by

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several laboratories [12–14], including ours [15], have revealed that UV-damaged DNA-binding protein (UV-DDB) binds more readily to abasic sites than cyclobutane pyrimidine dimers (CPD), which raised the question of whether UV-DDB has a role in the processing of 8-oxoG and abasic sites during BER [16,17]. UV-DDB plays a major role in global genome nucleotide excision repair (GG-NER) as the initial sensor for UV-induced DNA lesions, including CPD and [6–4] photoproduct (6-4PP) [15]. UV-DDB, as part of the CUL4A-RBX E3 ubiquitin ligase, works to ubiquitylate histones surrounding the damage allowing XPC to access the lesions. Our laboratory recently discovered that UV-DDB plays a non-conventional role in stimulating the removal of 8-oxoG both biochemically and in cells [15]. This review focuses on the non-canonical role of UV-DDB as a first responder in the BER of 8-oxoG lesions [15,18–23].

UV-DDB binds abasic sites and can stimulate the activities of OGG1, APE1, and MUTYH

We sought to understand the repertoire of damage bases recognized by UV-DDB. Electrophoretic mobility shift assay (EMSA) experiments performed by Dr. Sunbok Jang were used to determine the equilibrium disassociation constants (K_d) of UV-DDB to undamaged DNA, CPD lesions, tetrahydrofuran (THF), 8-oxoG:C, and 8-oxoG:A [15]. We found that UV-DDB, in the presence of magnesium (Mg^{2+}), bound to a DNA substrate containing a THF moiety with a ∼290-fold higher affinity (THF37; $K_d = 3.9 \pm 0.5$ nM), compared with non-damaged DNA (UD37; $K_d = 1108 \pm 95.5$ nM). UV-DDB also had an eight-fold higher affinity for CPD (CPD37; $K_d = 30.4 \pm 2.4$ nM) and a seven-fold increase in affinity for a 37-bp duplex containing 8-oxoG:C pair (8-oxoG37(G:C); $K_d = 159.6 \pm 12.4$ nM) and 8-oxoG:A pair (8-oxoG37(G:A); $K_d = 163.8 \pm 14.8$ nM) when compared with non-damaged DNA. These results clearly showed that UV-DDB has specificity for 8-oxoG lesions across from a C or A and abasic sites. Furthermore, the presence of Mg^{2+} increases the specificity window for the detection of damaged bases. Since OGG1 has ∼three-fold higher affinity to THF moieties than compared with 8-oxoG DNA substrates we hypothesized that UV-DDB may also work like APE1 [24] to help facilitate OGG1 turnover from abasic sites [15]. In a subsequent study using EMSA experiments, UV-DDB in the nanomolar range was found to form a co-complex with MUTYH on DNA to help dissociate MUTYH from a THF:8-oxoG DNA substrate, the product of adenine excision across from 8-oxoG [21]. A similar assay with APE1 showed that concentrations as high as 1 μM were not able to dissociate MUTYH from THF-containing DNA.

Since UV-DDB binds to abasic sites, 8-oxoG:C, and 8-oxoG:A, we next wanted to determine whether UV-DDB stimulates the activities of OGG1, APE1, and MUTYH [15,21]. Time course studies following OGG1 lyase activity in the absence and presence of UV-DDB on DNA substrates containing 8-oxoG:C, demonstrated that UV-DDB could stimulate OGG1 activity three-to-four-fold. In the same study, UV-DDB was found to stimulate APE1 incision activity at THF sites up to eight-fold [15]. Similarly, in a subsequent study, UV-DDB was found to stimulate MUTYH glycosylase activity up to seven-fold and facilitate enzymatic turnover. We also found that while 20–30-fold excess of APE1 over MUTYH was necessary to allow MUTYH to undergo multiple cycles of adenine removal, 0.5–2.5-fold excess of UV-DDB was sufficient to allow multiple cycles of MUTYH catalysis. These data indicated that UV-DDB could displace MUTYH from abasic sites allowing turnover more efficiently than APE1. Together these experiments clearly demonstrated that UV-DDB could stimulate OGG1, APE1, and MUTYH turnover, and suggested a new role for UV-DDB in helping to process 8-oxoG lesions during BER.

Single-molecule studies using a DNA tightrope assay [25–28] further revealed that UV-DDB mediates the dissociation of OGG1 and APE1 from abasic sites [15]. DNA containing one abasic site (THF) site every 2 kb was suspended between silica beads. OGG1, MUTYH, or APE1 were conjugated to Quantum dots (Qdot) (605 nm, green) and were mixed with orthogonally Qdot-labeled UV-DDB (705 nm, red). Real-time binding affinities and colocalization were observed between UV-DDB and OGG1 or APE1 at abasic sites. In separate experiments, it was found that increased concentrations of UV-DDB decreased the half-life of OGG1, MUTYH, or APE1 on THF DNA [15,21]. Interestingly, while UV-DDB increases the rate of dissociation of MUTYH from abasic sites, the DNA tightrope assay also proved that UV-DDB could increase the motility of MUTYH on DNA up to ∼three-fold. The EMSA experiments described above suggested transient complex formation between UV-DDB with MUTYH on abasic site; however, no direct interaction was seen by size exclusion chromatography. While biochemical studies and fluorescent imaging of single molecules

suggested complex formation between UV-DDB and MUTYH, atomic force microscopy (AFM) [26] was used to assess the binding characteristics of MUTYH and UV-DDB to a single 8-oxoG:A pair 30% from one end of a 538 bp duplex. AFM measures volumes, which are proportional to the molecular mass of the complexes, and we observed volumes consistent with MUTYH and UV-DDB bound together at sites of damage. Taken together these data strongly suggested that UV-DDB may be the first responder at sites of 8-oxoG paired with C or A and acts to facilitate the enzymatic turnover of OGG1, APE1, and MUTYH from their products.

UV-DDB protects cells from 8-oxoG damage

While biochemical and single-molecule studies suggested a damage sensor role for UV-DDB in the processing 8-oxoG, cellular data were necessary to prove this hypothesis. To directly address whether UV-DDB was involved in the processing of 8-oxoG in cells, we performed two types of experiments. First, we assessed whether DDB2 protects cells from 8-oxoG toxicity and if DDB2 directly participates in the removal of 8-oxoG.

Harnessing two experimental approaches, to measure 8-oxoG frequency, immunofluorescence assays, and pulse field gel electrophoresis (PFGE), we were able to show that UV-DDB played a direct role in the removal of 8-oxoG. Using U2OS cells, we found that in absence of DDB2 and OGG1 there was a significant increase (1.3 and 1.8-fold) in endogenous levels of 8-oxoG, as measured by immunofluorescence using 8-oxoG specific antibodies. In a second assay, U2OS cells were treated with $KBrO_3$ (40 mM, 1 h) to induce oxidative DNA damage and genomic DNA was isolated and digested with formaidopyrimidine DNA glycosylase (FPG), to convert 8-oxoG to single nucleotide gaps and subsequently converted to double-strand breaks (DSBs) with S1 nuclease and analyzed by PFGE. We found that loss of DDB2 causes approximately a two-fold reduction in the rate of 8-oxoG removal 4 h after treatment. DDB2 knockdown (KD) cells were also found to be significantly more sensitive to oxidative DNA damage. Together these results proved that DDB2 has a direct role in the BER of 8-oxoG [22].

Recruitment of DDB2 at telomeres containing 8-oxoG precedes OGG1

To study the recruitment of DDB2 at 8-oxoG sites, Namrata Kumar adapted a novel chemoptogenetic approach developed by our collaborators, Drs. Marcel Bruchez and Patricia Opresko [16]. A fluorogen-activating protein (FAP) binds tightly to a photosensitizer dye, di-iodinated malachite green (MG-2I), which is then excited by near-infrared wavelength (660 nm) resulting in the generation of singlet oxygen [29]. By fusing the FAP to the TTAGGG repeat binding factor 1 (TRF1) at telomeres and adding the MG-2I dye and subsequent exposure of the cells to 10-min of 660 nm light treatment resulted in about one to three, 8-oxoG lesions per telomere [16]. Using this system, we were able to show DDB2 was recruited to 8-oxoG at telomeres immediately after damage in U2OS and RPE cells confirming that recruitment of DDB2 to telomeric 8-oxoG is not cell type dependent [22]. These results were further supported by utilizing the proximity ligation assay (PLA) which is used to measure protein interactions that are <40 nm apart. First, antibodies against DDB2-tagged mCherry and TRF1 were used. A four-fold increase in PLA signal at telomeres after treatment with dye plus light indicated the recruitment of DDB2 at telomeres after 8-oxoG damage. Similar PLA studies were performed demonstrating a close interaction between DDB2 with OGG1 at 8-oxoG sites. There was a robust PLA signal from mCherry-DDB2 and OGG1-GFP at 8-oxoG sites immediately after damage, which peaked between 30 and 60 min and decreased over a period of 3 h.

The xeroderma pigmentosum (XP) causing DDB2 variant, K244E [12,30], was investigated for its ability to bind to 8-oxoG in cells. This DDB2 variant does not efficiently recognize UV photoproducts [17]. We found a two-fold reduction in the recruitment of this DDB2 K244E variant to damaged telomeres. These results indicated that the K244 residue is an important site for the stabilization of DDB2 at 8-oxoG damage. The next question was whether DDB2 was necessary for the efficient recruitment of OGG1 to 8-oxoG. Knocking down DDB2, caused a three-fold reduction in OGG1 recruitment to damaged sites within 30 min of dye and light treatment. These results demonstrated that DDB2 is required for immediate and efficient recruitment of OGG1 at 8-oxoG sites [15]. We then tested whether DDB2 retention at sites of 8-oxoG changes in the absence of OGG1 and found that DDB2 has a three-fold longer half-life ($t_{1/2}$) at the damage site and continues to re-bind unrepaired lesions.

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DDB2 mediates XPC recruitment to telomeric 8-oxoG, while XPA is independent of DDB2

During NER, XPC is recruited to UV-induced photoproducts by DDB2, and XPA arrives later at these damage sites in both GG-NER and transcription-coupled repair (TCR) [31–33]. To assess whether XPC or XPA were also recruited to 8-oxoG by DDB2, GFP-tagged- XPC or XPA were examined in U2OS-TRF-FAP cells treated with dye and light. XPC and XPA were recruited within 30 min post dye and light treatment, at 8-oxoG sites. Intriguingly in DDB2 KO cells, XPC accumulation was decreased by three-fold and XPA recruitment was not affected, suggesting that XPC is recruited downstream of DDB2 and XPA recruitment is independent of DDB2. These results indicated that XPC stabilization at 8-oxoG is dependent on timely disassociation of DDB2 and recruitment of OGG1 [22]. We further demonstrated that XPA processes 8-oxoG through TCR, by treating cells with transcription inhibitor THZ1, and revealing a two-to-three-fold reduction in XPA recruitment within 30 min after dye and light treatment, and no effect on XPC recruitment. Strikingly, knocking down OGG1 using siRNA, XPA recruitment decreased five-fold. We also showed that XPA recruitment was dependent upon CSB.

DDB2 triggers OGG1 recruitment to densely clustered 8-oxoG lesions

Chromatin structure can impact the efficiency of oxidative DNA damage repair [9]. To further investigate the role of DDB2 in 8-oxoG recognition at densely clustered lesions in non-telomeric sequences, a photosensitizer (Ro 19-8022) in combination with 405 nm laser pulse was used to locally induce 8-oxoG lesions at high density in specific sub-nuclear regions [22]. Recruitment of GFP-DDB2, OGG1-GFP, or XPC-GFP to 8-oxoG in three different cell lines was observed using real-time live-cell imaging. OGG1 and DDB2 were not recruited to single-strand breaks efficiently. Furthermore, using a neddylation (NEDD8) inhibitor (NAEi) to inactivate UV-DDB, we observed a significant reduction in OGG1 recruitment. Similarly using CSN5i, an inhibitor of the COP9 signalosome (CSN), DDB2 was greatly depleted and a significant reduction in OGG1 recruitment to 8-oxoG sites was also observed. Taken together these findings suggested that DDB2 mediates OGG1 recruitment to 8-oxoG sites irrespective of genomic location. Blocking CUL4A-RBX1 ubiquitin E3 ligase complex (CRL^{DDB2}) or ubiquitylating and degrading DDB2 reduces OGG1 recruitment to 8-oxoG, indicating the involvement of CRL^{DDB2} during 8-oxoG repair at lesions at densely clustered lesions in genomic DNA [22].

DDB2 mediates chromatin decompaction at telomeric 8-oxoG sites

It has been shown that DDB2 plays a major role in chromatin decompaction for better lesion accessibility [34,35]. To assess whether DDB2 plays a similar role in the processing of 8-oxoG, telomere 3D volumes were measured using confocal microscopy in the presence and absence of DDB2. While the telomeres were shown to increase in size after 8-oxoG damage in the presence of DDB2 in both U2OS and RPE cells, DBB2 KO cells showed no such chromatin decompaction. These findings clearly indicated that DDB2 binding mediates local chromatin restructuring, allowing 8-oxoG lesions to be accessible to other downstream repair proteins.

Conclusions

Studies described in this brief review demonstrate conclusively that DDB2 is the initial damage sensor and initiates 8-oxoG processing in chromatin immediately after damage. Biochemical studies revealed that UV-DDB stimulated the activities of OGG1, APE1, and MUTYH. Cell experiments clearly demonstrated that DDB2 is recruited to 8-oxoG immediately after damage, preceding OGG1 recruitment, and is not cell type dependent. We also learned that UV-DDB could facilitate XPC to 8-oxoG sites, suggesting that both UV-DDB and XPC act as early recognition factors in 8-oxoG repair. Additionally, we also found that in the absence of OGG1, UV-DDB was associated with CUL4A, and evidence for chromatin decompaction at 8-oxoG sites was dependent upon DBB2. These findings helped us establish that UV-DDB has a direct role in 8-oxoG processing through BER as shown in Figure 1. Surprisingly, another NER factor, XPA, was shown to be recruited to 8-oxoG sites in telomeres through a process that was dependent upon transcription, OGG1, and CSB, but was independent of DDB2.

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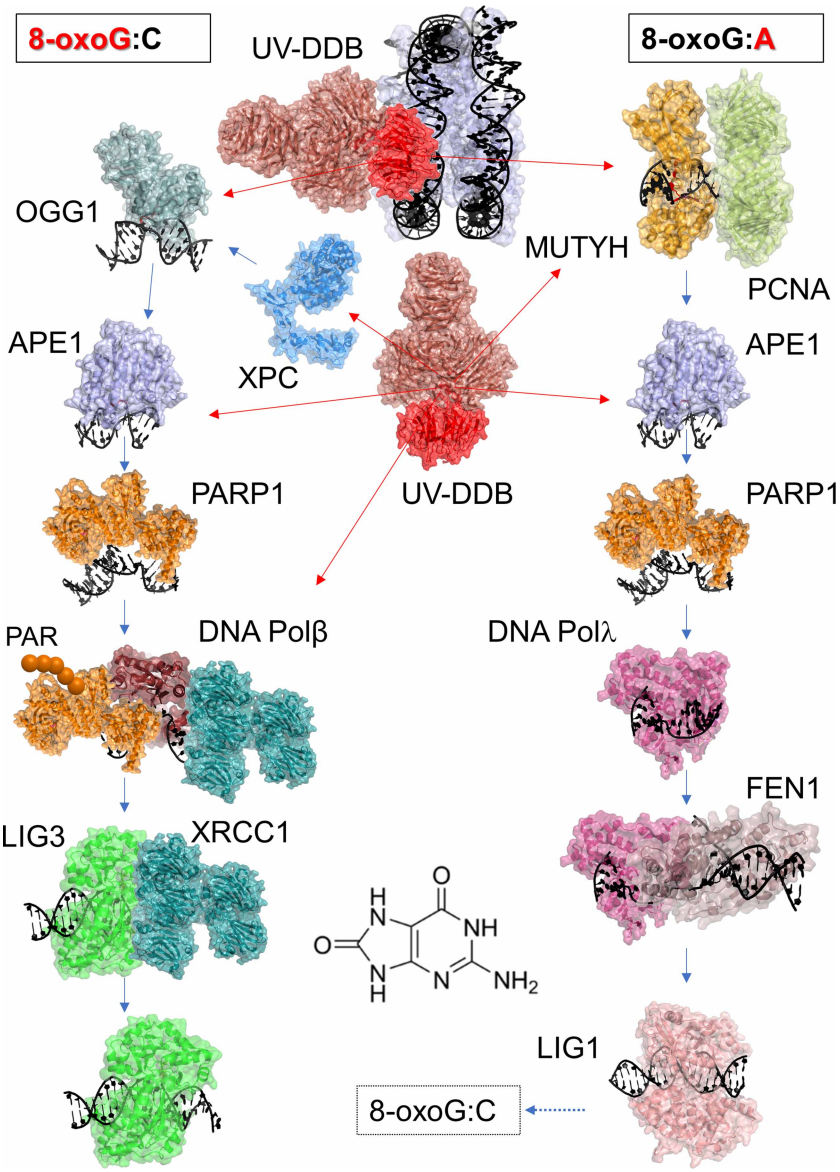

Figure 1. Role of UV-DDB in 8-oxoG processing in human cells.

8-oxoG:C processing (left), UV-DDB (red; DDB1 light red, DDB2 dark red) is the first responder at the site of damage in a nucleosome (light purple), helping to recruit OGG1 (purple) to the site of damage. XPC (blue) is also recruited by UV-DDB and together they help OGG1 turnover. APE1 (violet) is recruited by UV-DDB to help process the resulting abasic site. PARP1 (orange) binds to the subsequent nick activating its poly(ADP)-ribose activity (spheres), which helps recruit DNA Polβ (maroon) and XRCC1 (turquoise) to remove the sugar moiety and fill in the resulting one base gap. XRCC1 recruits LIG3 (green) which seals the nick. *8-oxoG:A processing*, MUTYH (yellow) is recruited by either UV-DDB or PCNA (light green) and the A is removed leaving an abasic site. UV-DDB, and not APE1 helps to turnover MUTYH. APE1 nicks the abasic site, recruiting PARP1. DNA Polλ (pink) is recruited and through strand displacement synthesis fills in a several base patch, FEN1 (brown) removes the 5′ flap, and LIG1 (salmon) seals the nick resulting in an 8-oxoG:C, which is then be processed as shown in the left side of the figure. PDB IDs for structures used: nucleosome (6R8Y), UV-DDB (4E5Z), OGG1 (3IH7), APE1 (5DFF), PARP1 (4DQY, modeled wit nicked DNA), DNA Polβ (1BPZ), XRCC1 (3K77), LIG3 (3L2P), MUTYH (4YPH), PCNA (2HII), DNA Polλ (1XSL), FEN1 (5UMP), and LIG1 (1X9N).

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Outlook

Our recent studies [15,21,22] have given us important insights about the crucial role of UV-DDB as an early damage sensor in 8-oxoG processing. While the experiments discussed in this review provide compelling evidence for a direct role of UV-DDB in 8-oxoG processing, many questions need to be addressed by future experiments. For example, how UV-DDB modifies the chromatin structure for accessing the lesions is a topic of importance. It will be of interest to know whether DDB2 regulates chromatin decompaction specifically at heterochromatin or whether it plays a role in the processing of 8-oxoG in euchromatin. DDB2 alone or working with DDB1 and the CUL4A E3 ubiquitin ligase may co-operate with other chromatin remodelers such as PARP1 and histone modifiers like RSC, FACT, ISWI to facilitate lesion accessibility and 8-oxoG repair. Future work is needed to understand how post-translational modifications (PTMs) can alter this process, in the context of other lesions, and the communication between PARP1 and UV-DDB at 8-oxoG site repair is a topic of interest. It is known for example that PARP1 mediates PARylation of UV-DDB, which suppresses subsequent UV-DDB auto-ubiquitination and degradation [36]. The potential interaction of UV-DDB with PARP1 and XRCC1-Lig3 during the nick-sealing process should also be studied (Figure 1). The fact that APE1 cannot efficiently displace and turnover MUTYH, suggests UV-DDB plays a vital role in the removal of 8-oxoG:A and loss of DDB2 could increase spontaneous mutations and/or cell death. Interestingly, DDB2 knock-out mice suffer a high frequency of internal tumors and die prematurely [18,37,38] Finally, an interesting question is whether proteins involved in TCR-NER preferentially accumulate at 8-oxoG in euchromatin, if so, it would explain why we observed recruitment of XPA to sites of 8-oxoG that was transcription-dependent. To this end, it will be interesting to determine whether other downstream TCR -NER factors such as CSA, CSB, TFIIH, XPF-ERCC1, and XPG arrive to help process 8-oxoG. We also believe that owing to its high affinity for abasic sites, UV-DDB might stimulate the activities of other nine mammalian DNA glycosylases including SMUG1, AAG, and TDG. Future experiments addressing these unresolved questions will provide more insights into the synergistic role of DNA repair pathways in processing 8-oxoG repair.

Perspectives

- Maintaining genome stability after oxidant injury is essential for normal cell functioning.
- The nucleotide excision repair proteins, UV-DDB, XPA, XPC, CSA, and CSB play an essential role in the processing of 8-oxoG during BER.
- Future work should focus on how post-translation modification can alter chromatin structure to provide proper lesion access during base excision repair.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

AN, SR, and BVH contributed equally to the writing of this article.

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Abbreviations

6-4PP, (6-4) photoproduct; 8-oxoG, 8-oxoguanine; AFM, atomic force microscopy; APE1, apurinic/apyrimidinic (AP) endonuclease; BER, base excision repair; CPD, cyclobutane pyrimidine dimers; CSA, Cockayne syndrome

group A; CSB, Cockayne syndrome group B; DSBs, double-strand breaks; EMSA, electrophoretic mobility shift assay; FPG, formaidopyrimidine DNA glycosylase; IF, immunofluorescence assays; MUTYH, mutY DNA glycosylase; NER, nucleotide excision repair; OGG1, human 8-oxoG glycosylase; PFGE, pulse field gel electrophoresis; PLA, proximity ligation assay; RNS, reactive nitrogen species; ROS, reactive oxygen species; THF, tetrahydrofuran; UV-DDB, UV-damaged DNA-binding protein; XPA, xeroderma pigmentosum, complementation group A; XPC, xeroderma pigmentosum complementation group C.

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