BERing the burden of damage: Pathway crosstalk and posttranslational modification of base excision repair proteins regulate DNA damage management

Kristin Limpose, Emory University
Anita Corbett, Emory University
Paul Doetsch, Emory University

Journal Title: DNA Repair
Volume: Volume 56
Publisher: Elsevier | 2017-08-01, Pages 51-64
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.dnarep.2017.06.007
Permanent URL: https://pid.emory.edu/ark:/25593/t6hpj

Final published version: http://dx.doi.org/10.1016/j.dnarep.2017.06.007

Copyright information:
© 2017 Elsevier B.V.
This is an Open Access work distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Accessed March 11, 2019 4:26 AM EDT
BERing the burden of damage: Pathway crosstalk and posttranslational modification of base excision repair proteins regulate DNA damage management

Kristin Limpose\textsuperscript{a}, Anita H. Corbett\textsuperscript{b,d,§}, and Paul W. Doetsch\textsuperscript{a,c,d,e,§}

\textsuperscript{a}Graduate Program in Cancer Biology, Emory University, Atlanta, GA, 30322
\textsuperscript{b}Department of Biology, Emory University, Atlanta, GA, 30322
\textsuperscript{c}Department of Radiation Oncology, Emory University School of Medicine, Atlanta, GA 30322
\textsuperscript{d}Winship Cancer Institute, Emory University, Atlanta, GA 30322
\textsuperscript{e}Department of Biochemistry, Emory University, Atlanta, GA, 30322

Abstract

DNA base damage and non-coding apurinic/apyrimidinic (AP) sites are ubiquitous types of damage that must be efficiently repaired to prevent mutations. These damages can occur in both the nuclear and mitochondrial genomes. Base excision repair (BER) is the frontline pathway for identifying and excising damaged DNA bases in both of these cellular compartments. Recent advances demonstrate that BER does not operate as an isolated pathway but rather dynamically interacts with components of other DNA repair pathways to modulate and coordinate BER functions. We define the coordination and interaction between DNA repair pathways as pathway crosstalk. Numerous BER proteins are modified and regulated by post-translational modifications (PTMs), and PTMs could influence pathway crosstalk. Here, we present recent advances on BER/DNA repair pathway crosstalk describing specific examples and also highlight regulation of BER components through PTMs. We have organized and reported functional interactions and documented PTMs for BER proteins into a consolidated summary table. We further propose the concept of DNA repair hubs that coordinate DNA repair pathway crosstalk to identify central protein targets that could play a role in designing future drug targets.

Keywords

DNA Damage Base Excision Repair; BER DNA pathway crosstalk DNA repair hubs Post-translational modifications; PTMs

§Co-corresponding authors: Paul Doetsch: medpwd@emory.edu; Anita Corbett: acorbe2@emory.edu.

Publisher’s Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

7. Conflicts of Interest
The Authors declare no conflicts of interest
1. Introduction

DNA contained in both the nuclear and mitochondrial cellular compartments is subject to damage from multiple sources\(^1\text{–}^3\). Diverse classes of DNA damage are caused by exogenous sources such as UV, ionizing radiation, alkylating agents, and heavy metals\(^4\text{–}^8\). Endogenous sources of damage such as reactive oxygen species (ROS), are generated during normal metabolic functions as well as various cellular transactions\(^2,^9\). Cells have therefore evolved numerous DNA repair and tolerance pathways to protect the genome from these types of damage.

Originally, each DNA repair pathway was analyzed in isolation to define repair of a specific subtype of DNA damage. For instance, base excision repair (BER) handles non-bulky DNA base damage, nucleotide excision repair (NER) manages bulky lesions, and homologous recombination (HR) repairs double strand breaks in S phase\(^10\). As each DNA repair pathway was characterized beyond the individual biochemical steps, it became apparent that coordination between DNA repair pathways is essential for proper cellular responses to DNA damage. We refer to such coordination as pathway crosstalk. In this context, pathway crosstalk occurs when components of one, biochemically distinct DNA repair pathway influence the repair of a substrate that is corrected by a different DNA repair pathway. For example, components of NER are indispensible for efficient repair of BER substrates through interactions with several N-glycosylases that initiate BER\(^11\text{–}^13\). We focus on pathway crosstalk events primarily mediated through protein-protein interactions and extend the analysis of BER proteins to post-translational modifications (PTMs) that could affect BER activity and pathway crosstalk in response to DNA damage through multiple mechanisms. We describe several classical, as well as recently reported examples of pathway crosstalk, with an emphasis on how BER components are regulated in human cells.

Base excision repair is crucial for maintaining genome integrity through repair of non-bulky base damage in both the nuclear and mitochondrial cellular compartments\(^1,^3,^9\). As depicted in Figure 1, the N-glycosylase proteins initiate BER when they recognize and cleave a specific subset of DNA base damage leaving an apurinic/apyrimidinic (AP) site. Bifunctional N-glycosylases contain an AP lyase activity that further cleaves the DNA phosphodiester backbone resulting in single strand breaks. AP endonuclease (APEX1) performs end-cleaning duties following glycosylase AP lyase activity or cleaves the AP site following the action of a monofunctional N-glycosylase. Further processing by DNA polymerase β and subsequent ligation result in repair of the initial damage site\(^9\). Proper regulation and completion of each BER step is crucial as AP sites and single strand break intermediates created during the repair process are themselves types of DNA damage. Defining how DNA repair pathway proteins interact to ensure efficient completion of each BER intermediate step is therefore critical for understanding BER regulation within the context of genome stability.

Of note, a majority of BER crosstalk with other DNA repair pathways takes place at the initiating steps of BER. As BER glycosylases generate apurinic/apyrimidinic (AP) sites and/or strand breaks, which are themselves forms of DNA damage\(^14,^15\), proper coordination and regulation of BER glycosylase proteins is important to ensure these intermediates do not
accumulate. APEX1 (also known as APE1) also generates single strand breaks during BER and the coordinated handoff from APEX1 to downstream BER proteins must be properly regulated to avoid accumulation of BER intermediates.

One mechanism that could help coordinate DNA repair pathway regulation is PTMs of individual DNA repair proteins. Numerous examples of PTMs modulating cellular activities have been reported\textsuperscript{16–18}. However, in only a small number of cases have sites of PTMs been defined and analyzed on BER proteins. Furthermore, the biological consequence of many of these BER protein PTMs have yet to be defined. General functions of PTMs, which may be relevant to BER regulation, include, but are not limited to modulating protein-protein interactions, pathway cascade signaling, cellular localization, conformational changes, and protein stability\textsuperscript{18,19}. The research into BER protein PTM elucidation is expanding and several advances will be covered in this review. Table 1 summarizes documented PTM modifications on BER proteins together with documented BER protein interactions that aggregate data into a readily accessible resource.

We also present a network map of specific DNA repair pathway protein interactions (Figure 2) to visualize central protein-protein interaction hubs. As more DNA repair pathway crosstalk interactions are elucidated, these hubs should lead to a better understanding of how DNA damage response systems are integrated and may be valuable in future drug design to target multiple DNA repair pathways at once for clinical applications.

### 2. Nucleotide Excision Repair Crosstalk with BER Components

The canonical function of nucleotide excision repair (NER) is to eliminate bulky DNA damage, which can arise from exposure to UV radiation or certain chemical agents\textsuperscript{10}. These lesions include UV-induced cyclobutane pyrimidine dimers, pyrimidine-pyrimidone photoproducts, and bulky chemical adducts\textsuperscript{20,21}. NER also participates as a backup mechanism to base excision repair (BER) for the repair of certain oxidative induced DNA base damage\textsuperscript{22}. Thus, the interplay between multiple NER and BER components is critical to ensure efficient BER processing of base damage. Several BER glycosylase-NER protein interactions have been characterized that impact the function of key BER glycosylase enzymes in processing of their respective DNA damage substrates. Several specific examples that illustrate this interplay are described here.

#### 2.1 TDG Glycosylase

Well documented evidence that NER components can influence BER glycosylase activity comes from the analysis of the interaction between the BER thymine DNA glycosylase, TDG, and the NER XPC protein (Figure 1)\textsuperscript{23,24}. In their respective pathways, TDG recognizes G:T mismatches in DNA and excises the mismatched thymine\textsuperscript{25}. The XPC protein is involved in global genome NER (GG-NER)\textsuperscript{26}. TDG is strongly product inhibited by the AP site that is produced in DNA following thymine cleavage and APEX1 helps displace TDG from these AP sites\textsuperscript{27}. Previous work identified a physical interaction between TDG and XPC\textsuperscript{24}. To assess whether XPC is an additional factor that contributes to displacement of TDG from AP sites, APEX1 and XPC were simultaneously added to DNA-bound TDG. Individually, APEX1 or XPC stimulated moderate TDG release from DNA.

\textit{DNA Repair (Amst).} Author manuscript; available in PMC 2018 August 01.
When present together, APEX1 and XPC resulted in a 6-fold increase in TDG release from DNA compared to control reactions\textsuperscript{24}. These findings demonstrated that XPC is an additional component that triggers TDG release from DNA product. However, an exact mechanism for how XPC stimulates TDG release has yet to be defined. Furthermore, as XPC increased the ability of APEX1 to aid in TDG turnover, future studies are required to determine if XPC binds to and/or influences APEX1 activity.

2.2 NER components interact with BER substrates

Another example of BER and NER interplay includes two NER proteins, XPC and CSB. Both XPC and CSB localize to sites of oxidatively-induced DNA damage generated by laser (405 nm) excitation of a photosensitizer\textsuperscript{28}. The primary product of this reaction is the BER substrate 8-oxoguanine (8-oxoG)\textsuperscript{28}. Fluorescently tagged XPC and CSB were employed to track the localization and kinetics for both proteins within the nucleus. Upon DNA damage, XPC localized to sites of DNA damage exclusively in the nucleoplasm while CSB localized to sites of damage in both the nucleolus and the nucleoplasm\textsuperscript{28}. As the nucleolus is a site of high transcriptional activity due to ribosomal DNA\textsuperscript{29}, these results are in line with previous data assigning XPC to global genome NER and CSB to transcription coupled repair\textsuperscript{26}. In contrast, downstream NER components such as the XPA and XPB proteins were not recruited to these sites of oxidative base damage\textsuperscript{28}. This result indicates that the recruitment of XPC and CSB is independent of their respective NER functions and supports a role for XPC and CSB in influencing BER-mediated repair of oxidatively-induced DNA damage.

The NER protein, CSB, also influences binding and excision of the oxidative damage 8-oxoG by the BER glycosylase, OGG1\textsuperscript{24,30–32}. Despite a functional link to 8-oxoG repair, no direct interaction between the OGG1 and CSB proteins has been detected, suggesting that these proteins could function as part of a protein complex to ensure efficient BER function (Figure 1)\textsuperscript{33}. By analyzing the kinetics of protein recruitment to DNA damage, Menoni et al. concluded that CSB is recruited to DNA damage prior to OGG1\textsuperscript{28}. Consistent with this model, there was no change detected in either XPC or CSB recruitment to damage in cells deficient for OGG1. Thus, OGG1 is not required for recruitment of CSB to sites of oxidative DNA damage\textsuperscript{28}. XPC was recently described as a general DNA damage sensor independent of NER\textsuperscript{34}. This role for XPC is supported both by the XPC link to TDG glycosylase and nucleoplasm localization of XPC to sites of DNA damage independent of other NER components\textsuperscript{28}. How XPC may generally influence other BER glycosylases as a sensor for other BER substrates is unknown and will require further study.

2.3 NEIL Glycosylases

Another class of BER glycosylases that is modulated by CSB are the NEIL1 and NEIL2 glycosylases (Figure 1)\textsuperscript{12,13,35}. While the NEIL glycosylases have substrate specificity that overlaps with other BER N-glycosylases, they are unique in their ability to excise oxidative DNA damage from single-stranded DNA that mimics a transcription bubble\textsuperscript{36}. NEIL1 substrates include the 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 4,6-diamino-5-formamidopyrimidine (FapyA) open ring base damages\textsuperscript{13}. As both FapyG and FapyA share an intermediate structure with 8-oxoguanine\textsuperscript{37}, and as CSB impacts OGG1-mediated repair of 8-oxoguanine\textsuperscript{33}, this led to the hypothesis that CSB could impact NEIL1
activity. To address this question, levels of FapyG and FapyA damage were analyzed in the brain, liver, and kidneys of CSB−/− mice revealing that FapyA levels are increased in all three tissues relative to control mice. FapyG damage was also elevated in the brain and kidneys compared to control mouse tissues, providing evidence that CSB is required for efficient repair of NEIL1 substrates. Further analysis using NEIL1 in vitro incision assays revealed that CSB increases NEIL1-mediated incision activity up to 4-fold for FapyG, up to 2.5-fold for FapyA, and also stimulates NEIL1 AP lyase activity. The stimulation of NEIL1 by CSB was mapped to a region within the N-terminal domain of CSB (amino acids 2-341) and was independent of CSB ATPase activity. These results suggest that CSB does not mediate chromatin remodeling during repair of NEIL1 substrates. Immunoprecipitation experiments from HeLa lysates demonstrated that NEIL1 and CSB are present in the same protein complex although whether a direct interaction occurs between NEIL1 and CSB remains to be determined. An interesting question is whether NEIL1 has a role in transcription-coupled repair of oxidatively-induced DNA base damage in light of the interaction with CSB and the ability of NEIL1 to initiate repair of single-stranded DNA.

Further work revealed that CSB also stimulates NEIL2 activity. Immunoprecipitation experiments from HeLa cells revealed that CSB and NEIL2 are present in the same protein complex and that the protein-protein interaction is increased following exposure to the oxidizing agent menadione. Further analysis revealed a direct protein-protein interaction between CSB and NEIL2. Incision assays of a NEIL2 substrate revealed that CSB stimulates NEIL2-mediated incision of FapyA up to 4-fold in duplex DNA and up to 3-fold for 5-hydroxyuracil present in a bubble DNA structure. However, CSB did not affect NEIL2 binding to DNA damage, suggesting that CSB may play a role in NEIL2 release from the final DNA product. Collectively, these results demonstrate that repair of BER substrate from single-stranded DNA can be coordinated through interactions between BER and NER components.

2.4 NTHL1 Glycosylase

A crucial BER glycosylase, NTHL1, repairs a large subset of oxidized DNA bases including dihydrouracil and the replication and transcription blocking base damage, thymine glycol (Tg). However, in vitro reconstitution experiments showed that purified NTHL1 has poor incision activity on an oligonucleotide containing an NTHL1 damage substrate. Based on findings that NER-deficient patient cells, which lack the XPG protein, also showed poor excision of Tg, purified XPG was added into the reconstituted NTHL1 BER in vitro system. Surprisingly, the addition of XPG stimulated NTHL1 incision and release of DNA product for both Tg and dihydrouracil substrates. The addition of other NER components, XPA or XPC, had no such stimulatory effect on NTHL1 activity. To address whether XPG endonuclease activity is required to achieve this stimulation of NTHL1, two XPG protein variants (E791A and A792V) that have no XPG nuclease activity were employed. Both variants could stimulate NTHL1-mediated base excision as effectively as wild type XPG, indicating that the XPG-dependent stimulatory effect on NTHL1 is independent of XPG nuclease function. Overall, this study demonstrates that XPG plays a critical role in NTHL1-mediated base excision of damage and release from DNA (Figure 1). Whether NTHL1 protein has an impact on XPG-mediated NER functions is not known.
2.5 AP Endonuclease (APEX1)

Platinum-based chemotherapeutics are used in the clinic as an effective treatment for multiple cancer types. A common platinum therapeutic is cisplatin, which causes intra- and interstrand crosslinks primarily between guanine bases. However, cisplatin has multiple negative side effects including peripheral neuropathy, nephrotoxicity, and an increase in cellular reactive oxygen species (ROS). NER is the main repair pathway to handle cisplatin adducts while BER initiates repair of the ROS-induced base damage.

Previous studies showed that increased protein levels of the BER protein, APEX1 (also known as APE1), protect cells against cisplatin toxicity. To assess the role of APEX1 in repair of cisplatin adducts, APEX1 levels were modulated in a neuronal tissue culture model exposed to cisplatin. In this study, knockdown of APEX1 caused an increase in the level of unrepaired cisplatin adducts. Furthermore, cisplatin adduct repair was dependent on APEX1 endonuclease activity, and this repair activity was separate from APEX1 redox functions that are critical for transcriptional regulation. Interestingly, when APEX1 was lost, an increase in the level of the NER protein, XPA, was detected. Whether this increase in XPA protein resulted from regulation at the protein or RNA level is unknown, and the exact mechanism of how APEX1 is involved in the removal of cisplatin adducts remains unclear. Thus, the level of cisplatin-DNA adducts, which are repaired by NER, increased in the absence of the BER protein, APEX1. Whether APEX1 involvement in cisplatin adduct repair is dependent on global genome NER (GG-NER) or transcription-coupled (TC-NER) NER is not known.

In support of a model where APEX1 plays a role in TC-NER of cisplatin adducts, the APEX1 and CSB proteins directly interact. CSB protein has been implicated in altering DNA conformation as well as chromatin remodeling during NER. Cells display hypersensitivity to reactive oxygen species (ROS)-generating agents, supporting a role for CSB in BER-mediated processing of oxidative DNA damage. In fact, CSB also interacts with other BER proteins, including PARP1 and FEN1, in addition to APEX1. In vitro, APEX1 endonuclease activity is increased up to 4-fold on duplex DNA and up to 6-fold on single-stranded DNA by the interaction with CSB. The larger stimulation for single-stranded DNA indicates that the CSB/APEX1 interaction might have a greater impact on damage present in transcriptionally active DNA compared to double-stranded DNA. Addition of ATP was not needed for the stimulation of APEX1 endonuclease activity by CSB, suggesting that increased APEX1 endonuclease activity is not due to CSB-mediated chromatin remodeling. While these results show that APEX1 has a role in repair of cisplatin adducts, whether APEX1 has a reciprocal impact on CSB-mediated TC-NER activity is currently unknown.

3. Non-Homologous End Joining and Homologous Recombination

Crosstalk with BER Components

Double strand breaks (DSBs) are the most deleterious class of DNA damage, and cells have evolved multiple repair pathways to repair this damage. Homologous recombination (HR) repairs DSBs in the S and G2 phases of the cell cycle when a homologous sister chromatid is present. Non-homologous end joining (NHEJ), while active in all phases of
the cell cycle, primarily repairs DSBs in the G0 and G1 phases of the cell cycle. Importantly, BER can generate DSBs as a result of excision of closely opposed base damages or from single strand break intermediates if these intermediates are encountered by the replication or transcription machinery. To avoid the accumulation of deleterious damage intermediates, BER must therefore be efficiently and precisely regulated to initiate and complete repair. In fact, the BER intermediates generated by the alkyladenine glycosylase induce more robust HR than the initial alkylation damage in vivo. The impact of BER and DSB repair pathway cross regulation and how the cell cycle phase contributes to pathway crosstalk to maintain genome stability are areas that require further study. Recent examples that illustrate the interplay between BER and DSB repair pathways are described here.

3.1 Alternative Non-Homologous End Joining

End joining repair of DSBs can be prone to loss of genetic material as there is no template for extensive homology searching. The NHEJ pathway is subdivided into two major sub-pathways that include classical NHEJ (c-NHEJ), which is dependent on the KU70/80 proteins, and alternative NHEJ (alt-NHEJ), which uses short stretches of end resection that result in microhomology often found at chromosomal translocations. To elucidate which protein factors mediate alt-NHEJ, an RNAi library directed against DNA repair factors was screened using a fluorescent reporter assay for alt-NHEJ. Interestingly, proteins from diverse DNA repair pathways were identified as top candidates required to perform alt-NHEJ. These proteins include the BER glycosylases, NTHL1 and UNG, the mismatch repair protein, MSH6, and the crosslink repair protein, FANCA. Subsequent analyses demonstrated that knockdown of the NTHL1 and UNG glycosylases significantly decreased alt-NHEJ. Furthermore, this result was specific for alt-NHEJ, as knockdown of either glycosylase did not have a significant impact on HR or single strand annealing events as determined by reporter assays. HR and alt-NHEJ are most active in the S and G2 phases of the cell cycle. Thus, accumulation of cells in G1 upon depletion of either NTHL1 or UNG could account for the apparent decrease in alt-NHEJ. However, NTHL1 knockdown resulted in an increase in the percent of G2 cells while UNG knockdown resulted in an increase of G1 phase cells. These distinct cell cycle changes suggest that, at least in the case of NTHL1, the cell cycle status does not account for the decrease observed in alt-NHEJ activity. This result implies that the NTHL1 glycosylase plays a role in promoting alt-NHEJ, perhaps while suppressing HR. Future experiments will need to pinpoint how BER and end joining repair mechanisms coordinate their activities to ensure efficient repair of DSBs.

3.2 Homologous Recombination

Another well-known NTHL1 partner is the NER protein, XPG (Figure 1). Recent work reveals that XPG is indispensable for HR recovery from collapsed replication forks. Genomic instability can result from an inability to repair DSBs that result from these collapsed replication forks, which are normally repaired by HR. In this study, loss of XPG led to DNA damage that resulted in genomic instability. XPG is required for efficient loading of the Rad51 presynaptic filament by the BRCA2/PALB2 complex for HR following end resection. NTHL1 is also upregulated in S phase, presumably for BER glycosylase function. However, because NTHL1 appears to promote alt-NHEJ and is a binding partner for XPG, NTHL1 could modulate DSB repair pathway choice during S phase. Future
experiments will be needed to assess the functional consequences of NTHL1 protein regulation for DSB repair.

In addition, recent results reveal a reciprocal effect between the BER protein, OGG1, and the HR protein, Rad52\textsuperscript{64}. Rad52 is part of the Rad51 epistasis group that functions in presynaptic filament formation during HR\textsuperscript{64}. Previous studies in budding yeast demonstrated that Rad52 aids in strand exchange by forming a bridge between RPA-coated single-stranded DNA and Rad51\textsuperscript{65,66}. Curiously, yeast deficient in BER are further sensitized to oxidative damage when the RAD52 gene is disrupted, underscoring the importance of repair pathway crosstalk\textsuperscript{67}. Studies determined that mammalian OGG1 and Rad52 proteins directly interact, and this interaction is increased in response to oxidative stress\textsuperscript{64}. Furthermore, this interaction had reciprocal effects on the function of both the BER and HR pathways. Rad52 stimulates OGG1-mediated incision of 8-oxoG by up to 3-fold, and Rad52 promotes OGG1 release from DNA\textsuperscript{64}. Conversely, OGG1 inhibits Rad52 single-strand annealing and strand exchange activity, while another glycosylase, UNG, has no such effect\textsuperscript{64}. RNAi-mediated knockdown of Rad52 caused an increase in 8-oxoG and FapyG accumulation in genomic DNA\textsuperscript{64}. Taken together, these results demonstrate that HR proteins can impact BER activity, and, conversely, a BER protein can influence HR function. These findings raise the question of whether BER glycosylases have reciprocal effects on the efficient function of the DNA repair pathways that interact with BER such as NER. Future research will be required to investigate BER protein regulation and understand how BER proteins influence the activities of other repair pathways.

Pathway Crosstalk Conclusions—To illustrate and examine DNA repair pathway crosstalk, we utilized the STRING protein-protein interaction network (www.string-db.org)\textsuperscript{68}. Various types of protein interactions and databases are included in the STRING analysis. Visualization of the interaction map is straightforward; each protein is represented by a node (circle), while a protein interaction is represented by an edge (line) (Figure 2). We included a panel of DNA repair proteins from five repair pathways (BER, NER, MMR, NHEJ, and HR), which yielded three main clusters after Kmeans clustering analysis with a high confidence (0.700) minimum interaction score.

By examining the three different clusters, one can appreciate the breadth of coordination not only within a specific pathway but also between DNA repair pathways (Figure 2). For example, the red cluster highlights BER. Dashed lines indicate crosstalk with the black supercluster containing components of NER, MMR, and NHEJ. One could postulate that a BER interaction may, in fact, influence the HR pathway (yellow) by modulating a common interaction highlighted within the black supercluster. As DNA repair is a tightly regulated process, perturbations in pathway crosstalk may have untoward consequences for multiple DNA repair pathways. Therefore, understanding the nuances of regulation at the protein level by identifying central interaction hubs could be an effective approach to identifying new targets for therapeutic development, or for predicting how a patient may respond to existing chemotherapeutic options that target DNA repair.

To detect central interaction hubs, we propose a two-fold approach of assessing 1) the total number of edges that a node has, and 2) the number of dashed edges per node. In this way,
interaction hubs for a specific pathway, or a hub that impacts the greatest number of interactions between pathways can be identified. For example, OGG1 has six solid edges to denote interactions with other BER components while nine dashed edges represent crosstalk with various components of the supercluster for a total of fifteen edges. Furthermore, many MMR proteins have interactions connected to HR. One could hypothesize that dysregulation at the protein level anywhere along this string of interactions could impact the functions of BER, MMR, and/or HR simultaneously. The same logic can be applied along any node-edge pathway.

Another striking observation is the large number of interactions that appear to be coordinated through the MMR proteins MLH1, MSH6, and MSH3 (Figure 2). These proteins emerge as central coordinators between certain BER proteins and HR. For example, an interesting case emerges with the MSH6 protein. MSH6 is strongly implicated in promoting alt-NHEJ. From the STRING interaction network, one can see that MSH6 interacts with RAD51 as well as components of the BER pathway (Figure 2). Whether MSH6 can promote alt-NHEJ while suppressing HR through the interaction with RAD51 remains to be determined. Alternatively, whether MSH6 interaction with BER components aids in the suppression of HR has not been investigated. The fact that NTHL1 is also a top candidate for promoting alt-NHEJ, suggests that BER and MMR could potentially influence HR functions.

A distinct subset of BER proteins is coordinated with various components of NER. One example is the interaction between NTHL1 and XPG. As previously noted, recent studies demonstrate that XPG is indispensable for proper HR, and that this function is independent from the NER functions of XPC. In turn, XPG is also crucial for catalytic turnover of the NTHL1 glycosylase. Thus, protein dysregulation of any of these DNA repair components has the potential to affect more than one DNA repair pathway. For example, dysregulation of NTHL1 could ultimately affect the efficiency of HR through unregulated interactions with XPG. Examination at the protein level will provide a starting point for investigating the overall impact of DNA repair protein dysregulation and pathway crosstalk.

4. Post-translational Modifications Affecting Function and Protein Levels of BER Components

Many of the crosstalk examples described depend on DNA damage induced protein-protein interactions. One mechanism of regulating DNA repair is through reversible posttranslational modifications (PTMs) of repair proteins, which could directly impact protein activity or protein-protein interactions (Figure 3). While there are a limited number of PTMs annotated on BER proteins, even fewer of these PTMs have been assigned a biological function. However, recent studies have begun to elucidate how a small subset of PTMs regulates BER proteins. Table 1 lists PTMs that have been identified on individual BER proteins and includes results from proteome-wide mass spectrometry analyses to identify phosphorylated and acetylated peptides. As many BER glycosylases are cell cycle regulated, PTM modification by CDK/cyclin proteins could play a role in modulating BER function in a cell cycle-dependent manner. Some of the best-characterized
examples of BER modifications are discussed below. We focus on results reported since our group last discussed this topic in 2010.  

4.1 TDG Glycosylase  

A classic and striking example of BER regulation by a PTM is SUMO modification of the G:T-mismatch glycosylase, TDG. TDG has multiple functions which include BER glycosylase activity and transcription regulation, that are mediated by interactions with the CBP/p300 complex. In BER, substrates for TDG include the G:T and G:U mismatched base pairs resulting from deamination of cytosine or 5-methylcytosine. TDG also functions in DNA demethylation through excision of 5-formylcytosine and 5-carboxylcytosine. However, TDG is strongly product inhibited by the resulting AP site from TDG-mediated glycosylase activity, raising the question of how TDG is removed from DNA following TDG catalytic activity. One mechanism of eliminating this AP product inhibition is through interaction with the NER protein, XPC, as discussed in the pathway crosstalk portion of this review.  

In addition to interaction with XPC and APEX1, TDG is modulated by a SUMO1 or SUMO2/3 modification, which regulates TDG turnover. A structural approach was employed to probe the mechanism by which SUMO modification triggers TDG turnover. The crystal structures for unmodified TDG and TDG modified by SUMO1 and SUMO2/3 were resolved to provide insight into the impact of SUMO modification on TDG structure. TDG modification by SUMO triggered TDG turnover, and did not influence the structure of the TDG core domain, which contains the glycosylase activity. Sumoylation occurs within the C-terminus of TDG, and the resulting C-terminal structural rearrangement involves non-covalent interactions with water. The conformational change causes protrusion of an α-helix that sterically clashes with the DNA phosphate backbone and causes release of TDG from the DNA. These results imply that TDG is only sumoylated following TDG glycosylase activity in order to activate release of TDG from product DNA.  

In contrast to this model, another study suggested that TDG can be modified by SUMO as free protein not bound to DNA. Unbound TDG and TDG in complex with DNA containing a TDG substrate are sumoylated to approximately the same extent in vitro. Furthermore, TDG bound to undamaged DNA and bound to DNA containing a TDG substrate is SUMO modified at similar rates. TDG sumoylation does not influence TDG excision activity against 5-formylcytosine or 5-carboxylcytosine substrates. These results confirm previous findings that TDG is sumoylated to relieve product inhibition. However, as unbound TDG can also be sumoylated, these results suggest that TDG sumoylation may have a distinct function in addition to relief of TDG product inhibition.  

SUMO modification of TDG is regulated in a cell cycle-dependent manner. When cells are synchronized in S phase, the steady state level of TDG decreases. In contrast, in G2 phase and mitosis, both unmodified TDG and sumoylated TDG increase relative to other phases of the cell cycle. Furthermore, TDG and TDG-SUMO protein fluctuation is dependent on proteasomal degradation, as treatment with the proteasome inhibitor, MG132, increases the steady state levels of both TDG and TDG-SUMO. As the steady state level of TDG-
SUMO is regulated in a cell-cycle dependent manner, and SUMO modification impacts TDG turnover, these results suggest that TDG-mediated repair is also cell cycle dependent.

Evidence that chemotherapeutic agents affect TDG modification comes from studies of TDG acetylation by CBP/p300. Acetylation of TDG weakens the interaction of TDG with APEX1\(^{77}\). Thus, TDG acetylation may coordinate TDG BER glycosylase function and transcriptional activity\(^{83}\). A critical deacetylase, SIRT1, removes the acetyl group from TDG\(^{77}\). Upon de-acetylation, TDG displays increased excision activity of a G:T substrate. In fact, TDG de-acetylation stimulates TDG release from an AP site, demonstrating that together, XPC, APEX1, sumoylation, and acetylation all influence TDG release from DNA product. The opposite effect for TDG acetylation was observed in the case of excision of the chemotherapeutic–induced DNA base damage, 5-flourouracil (FU), from DNA. Acetylated TDG displayed enhanced FU:G excision activity, in contrast to slower excision for G:T nucleotide pairs. Therefore, acetylation of TDG results in opposing effects on substrate excision of a chemotherapeutic. Thus, TDG acetylation status within tumor cells could potentially impact clinical efficacy of 5-flourouracil. To date, TDG is the best characterized example of how the intersection of PTMs and protein interactions coordinate efficient BER repair of DNA damage.

### 4.2 NTHL1 Glycosylase

Sumoylation of the *Saccharomyces cerevisae* DNA N-glycosylase, Ntg1, is another PTM that was recently reported. The Ntg1 protein is a functional ortholog of the human NTHL1 glycosylase\(^{84,85}\). Recent work revealed that Ntg1 is sumoylated in response to both oxidative stress and the alkylating agent methyl methanesulfonate (MMS)\(^{84,85}\). Consistent with an evolutionarily conserved function of this modification, human NTHL1 is also SUMO modified in response to oxidative stress\(^{85}\). Sumoylated Ntg1 is enriched in the nuclear but not the mitochondrial cellular compartment\(^{84}\). Whether this SUMO modification mediates nuclear localization or occurs in the nucleus is not known. To understand the functional consequence of sumoylation of Ntg1, the sites of SUMO modification on Ntg1 were mapped, and an Ntg1 variant that could not be SUMO modified was created (Ntg1ΔSUMO)\(^{85}\). This variant provides one of the first tools to examine the functional consequences of SUMO modification of BER proteins in future studies.

To explore whether sumoylation of Ntg1 is required for a proper response to DNA damage, yeast with wild type or Ntg1ΔSUMO were reconstituted into BER/NER deficient cells, and these cells were treated with MMS. In the absence of MMS, there was no discernable difference in growth between strains expressing control or the Ntg1ΔSUMO variant\(^{85}\). Surprisingly, yeast expressing Ntg1ΔSUMO grew better than the control cells with wild type Ntg1 when challenged with MMS. However, four days post MMS exposure, yeast containing wild type *Ntg1* caught up to the growth of yeast expressing Ntg1ΔSUMO. This result implies that sumoylation of Ntg1 may play a role in DNA repair or coordinating a DNA damage response in response to DNA damage. Additional experiments defining the site(s) of human NTHL1 sumoylation, and how the phosphorylation at S71 (Table 1) influences NTHL1 activity will be a focus of future studies. Further work to understand how...
damage-dependent sumoylation of Ntg1 coordinates this growth phenotype will need to be performed to gain a fuller understanding of BER crosstalk with cell cycle progression.

4.3 UNG2 Glycosylase

The UNG glycosylases (UNG1 and UNG2) are the primary enzymes tasked with removing uracil from duplex DNA\(^ {86}\). UNG1 is predominately localized to the mitochondria, while UNG2 is primarily responsible for removing uracil from nuclear DNA\(^ {3,87}\). Uracil is mutagenic if left unrepaired and is particularly problematic once the cell undergoes replication. Therefore, understanding how the cell coordinates repair of uracil during S phase is critical to defining mechanisms that protect genome integrity. UNG2 binds to PCNA and RPA suggesting that UNG2 can process uracil in single stranded DNA during replication\(^ {86,88,89}\). The steady-state level of UNG2 is regulated in S phase by phosphorylation of three residues, S23, T60, and S64\(^ {86}\). Phosphorylation at these sites is mediated in a cell cycle-dependent manner by the CDK4/cyclinD complex\(^ {86}\). UNG2 S64 is also modified in late S phase/early G2 phase by the CDK2/cyclinA and CDK1/cyclinB complexes\(^ {86}\). Phosphorylation at S23 increases UNG2 association with both RPA and replicating chromatin, and also influences UNG2 catalytic turnover. Conversely, phosphorylation at T60 and S64 creates a phosphodegron triggering ubiquitination and proteolytic degradation in late S phase and early G2\(^ {86}\). Taken together these findings provide a model for how UNG2 is regulated and degraded during S phase in response to specific PTMs. As UNG2 also repairs uracil in all phases of the cell cycle, how additional PTMs regulate UNG2 activity in other phases of the cell cycle is a key question. In fact, the PHOSIDA database annotates multiple uncharacterized phosphorylation and acetylation sites within UNG1/2 (Table 1), which could influence the biological function of UNG1/2. Future studies will need to determine the biological function of these uncharacterized UNG PTMs.

4.4 MUTYH Glycosylase

MUTYH is a DNA N-glycosylase that excises adenine from A:8oxoG mispairs. Consistent with this function, deletion of the human MUTYH glycosylase is linked to colon cancer\(^ {90-92}\). Like other BER components\(^ {93}\), MUTYH is regulated by ubiquitination\(^ {90}\). In vitro, MUTYH is modified by the E3 ligase, Mule, in conjunction with the E2 enzymes H5b, H5c, and H7\(^ {90}\). MUTYH ubiquitination occurs within a domain comprised of amino acids 475-535. Lysines in this region were systematically analyzed to abolish MUTYH ubiquitination and to explore the biological consequences of MUTYH ubiquitination. MUTYH that could not be ubiquitinated showed an increase in protein steady state levels and altered interaction with chromatin\(^ {90}\). In cells deficient for the Mule E3 ligase, which have increased MUTYH levels, a decrease in the mutation frequency of the HPRT gene was observed as compared to control cells. Conversely, when Mule was overexpressed in cells, which have decreased MUTYH levels, an increase in HPRT gene mutations was detected. Therefore, the regulation of a BER glycosylase at the protein level influences mutation frequencies in mammalian cells. MUTYH protein regulation through specific PTMs may therefore serve as a regulatory paradigm for how other BER glycosylases could be influenced by PTMs that modulate steady state protein levels.
4.5 OGG1 Glycosylase

Another key player in repair of oxidative DNA damage is OGG1, which is primarily responsible for excision of 8-oxoguanine base damage. A common variant of OGG1 present in the genome is the polymorphism coding for OGG1 S326C. The presence of the S326C OGG1 variant predisposes carriers to multiple cancer types, but how this variant responds to oxidative stress in vivo had not been defined. Recent work reveals that in response to physiological oxidative stress, the OGG1 S326C variant loses glycosylase activity. Employing a prediction program for disulfide bridge formation, a potential for increased disulfide bond formation in the OGG1 S326C variant as compared to wild type OGG1 was identified. One of the predicted inter- or intra-protein disulfide bridges includes amino acid 326. Thus, disulfide bridge formation and the resulting loss of glycosylase activity could impair OGG1 activity and cause increased mutation frequencies. This model for gain of a disulfide bridge could explain how the OGG1 S326C polymorphism predisposes to cancer. This study adds disulfide bridges to the list of functionally important modifications to BER protein variants.

4.6 AP Endonuclease (APEX1)

Another a key protein in BER, APEX1, contains multiple PTMs that affect both the endonuclease and transcription regulatory functions of the protein. Documented PTMs detected in APEX1 include phosphorylation, acetylation, ubiquitination, and S-nitrosylation (Table 1). However, S-glutathionylation also occurs in response to the altered redox state of the cell. Glutathionylation, addition of a glutathione group, occurs at cysteine residues and three candidate cysteines are located in the redox responsive domain of APEX1. As cysteine modifications include disulfide bridges, S-nitrosylation, and S-glutathionylation, understanding the competition between different PTMs and how they impact APEX1 function is crucial. APEX1 undergoes reversible glutathionylation in vitro. Glutathionylation of APEX1 occurs on C99, and inhibits APEX1-mediated AP site cleavage by 90%. In fact, modified APEX1 does not form stable complexes with AP-DNA. HeLa cells exposed to mildly toxic doses of hydrogen peroxide also show APEX1 glutathionylation suggesting that this modification could be relevant in cells and potentially in vivo. Determining how the cellular redox environment impacts APEX1 modification in non-transformed cell lines will be important to define how PTMs of BER proteins influence genome stability.

5. Conclusions

As illustrated in Figure 1 and analyzed in Figure 2, functional interactions between proteins from different DNA repair pathways can influence efficient BER responses to DNA damage. Figure 3 highlights the mechanisms that can achieve pathway crosstalk, including protein-protein interactions and PTMs that coordinate function to ensure rapid and efficient repair of DNA damage. Individually, each of these sub-areas is a rapidly evolving field, but studies suggest that repair biological outcomes depend on the extent of overlap of each of these components (Figure 3). Comprehensive studies to uncover further mechanisms of regulation that achieve pathway crosstalk are warranted. What remains unclear is the extent to which BER proteins impact the function of other DNA repair pathways. Most research to date in...
the DNA repair field has focused on biochemical elucidation of pathways, identifying new disease predisposing single nucleotide polymorphisms (SNPs) in DNA repair genes, and transcriptional regulation of DNA repair genes. Although each of these areas has value in contributing to the depth and breadth of DNA repair knowledge, a relatively neglected area has been defining how diseases are influenced by dysregulation of DNA repair proteins at the protein level. For example, responses to chemotherapeutics are often influenced by the DNA repair protein status of a patient’s tumor cells\textsuperscript{104,105}. Thus, there is a need to investigate DNA repair dysregulation at the protein level and to understand how DNA repair pathway crosstalk occurs in order to optimize treatment strategies. Furthermore, how efficient DNA repair is coordinated via protein-protein interactions and PTMs in order to influence repair activities requires future studies. As BER coordinates multiple interactions with other DNA repair pathways, we propose BER as a starting point for addressing how DNA repair can be dysregulated at the protein level, and how such dysregulation can influence other DNA repair pathways.

We also propose that by focusing on central interaction hubs (Figure 2), a directed effort at future drug design and DNA repair protein screening will open avenues previously unexplored for responses to chemotherapeutics. For instance, if BER and/or MMR proteins are dysregulated through altered interactions, PTMs, or steady-state protein levels to promote alt-NHEJ while suppressing HR, could this scenario impact clinical responses to chemotherapeutics? An analogous scenario is found in breast cancer patients with a germline mutation in the \textit{BRCA1/2} genes that results in inefficient HR function\textsuperscript{106,107}. As a consequence of decreased HR function, cells are sensitive to PARP inhibitors as PARP1 and BRCA1 are synthetically lethal\textsuperscript{108,109}. Conversely, if a patient does not have a \textit{BRCA1/2} mutation, but instead has suppressed HR as a consequence of dysregulated proteins in BER and/or MMR, this raises the issue of whether that patient would be sensitive to PARP inhibitors. Thus, patient tumors could also be screened for specific interactions or protein dysregulation as a potential biomarker for tumor responsiveness to chemotherapeutics. As studies reveal additional functional interactions within and among the various DNA repair pathways, the interaction networks displayed in Figure 2 will need to further evolve to accurately reflect this new information. As a consequence, pathway crosstalk through PTMs and protein-protein interactions may reveal a potential therapeutic avenue to sensitize a tumor previously thought to be unresponsive to certain treatment options. As cells are constantly “BERing” the burden of DNA damage, we must expand our knowledge of DNA repair in order to ultimately improve human health.

**Acknowledgments**

We would like to acknowledge the helpful discussions and guidance from Dr. Nicholas Seyfried’s lab (Emory University) in preparing the STRING analysis for Figure 2. Support for this work was provided by NIH grant ESES011163 (PWD), Emory University School of Medicine (PWD), the Winship Cancer Institute of Emory University (PWD), and by NIH grant GM058728 (AHC).

**Abbreviations**

\begin{itemize}
  \item [BER] Base Excision Repair
  \item [NER] Nucleotide Excision Repair
\end{itemize}
MMR Mismatch Repair
HR Homologous Recombination
NHEJ Non-homologous End Joining
PTMs Post-translational Modifications

References


DNA Repair (Amst). Author manuscript; available in PMC 2018 August 01.


DNA Repair (Amst). Author manuscript; available in PMC 2018 August 01.


208. Warbrick E, Lane DP, Glover DM, Cox LS. Homologous regions of Fen1 and p21Cip1 compete for binding to the same site on PCNA: a potential mechanism to co-ordinate DNA replication and repair. Oncogene. 1997; 14:2313–2321. [PubMed: 9178907]


Figure 1. Key human N-glycosylases and APEX1 interactions with components of other DNA repair pathways enhance BER activity

In the initiating steps of BER, a damage substrate is detected, and an N-glycosylase cleaves the damaged base, leaving an apurinic/apyrimidinic (AP) site. Bifunctional glycosylases cleave the phosphodiester backbone and create a single-strand break, while monofunctional glycosylases require APEX1-mediated cleavage of the phosphodiester backbone. Further processing results in repair of the initial damage site. Functional interactions with BER proteins in this review are depicted, with an emphasis on the initiating steps of BER. Pathway crosstalk of BER proteins (red) at the initiating steps of BER includes interactions with components of the NER (blue), HR (yellow), and alt-NHEJ (purple) pathways. We highlight recent advances that provide insight into BER functions, and the interactions displayed are discussed in this review.
Figure 2. Human base excision repair protein interactions with other DNA repair pathway components

The STRING functional protein association network and Kmeans clustering reveal how DNA repair components interact. A circle represents a node (protein), while edges (lines) indicate protein interactions. Solid edges denote interactions within the same cluster (pathway), while dashed lines are pathway crosstalk interactions that take place between proteins of different clusters. The line thickness is related to the strength of data that supports a particular interaction. Three clusters of interactions were generated and color-coded according to the canonical function of the protein— a BER cluster (red); a supercluster comprised of MMR (grey), NHEJ (light grey), NER (black), and an HR cluster (yellow). The MMR/NER/NHEJ supercluster was generated by the STRING algorithm during Kmeans clustering. The STRING map demonstrates that DNA repair pathways dynamically interact with each other as demonstrated by the dashed edges.
Figure 3. Maintenance of genomic integrity requires the integration of multiple factors for DNA repair in response to damage.

Mechanisms resulting in pathway crosstalk include the induction of functional protein-protein interactions (red) or PTMs (blue). A field that requires further analysis is how these individual areas intersect to result in pathway crosstalk, as illustrated by the overlap in the Venn diagram. Pathway crosstalk results in functional consequences that allow for a rapid and efficient DNA repair response to damage.
### Table 1

**Human BER proteins undergo posttranslational modifications (PTMs)**

A panel of BER proteins is represented with characterized PTMs and PTMs reported in the PHOSIDA database (www.PHOSIDA.com)\(^{69,70}\). PHOSIDA is a phosphorylation and acetylation database derived from experimental analysis of proteins from different species and conditions. Functional interactions characterized for human BER proteins are included to demonstrate the breadth of BER protein associations.

<table>
<thead>
<tr>
<th>BER Protein</th>
<th>Activity</th>
<th>Level of Regulation</th>
<th>Post-translational Modifications (Literature)</th>
<th>PHOSIDA Post-translational Modifications (Human(^{69,70}))</th>
<th>Functional Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG1/2</td>
<td>Uracil DNA Glycosylase</td>
<td>Phosphorylation at T6 and T126(^{110}), Phosphorylation at S23, T60, S64(^{110})</td>
<td>Phosphorylation at S12, S14, S23, S40, S54, S55, S58, S63, S64, S67, T31, T60, T51 Acetylation at K286, K5, K295</td>
<td>Glycosylase activity increased by APEX1(^{111}); Associated with APEX1, XRCC1(^{112}), PCNA(^{46}), and PPM1D(^{110})</td>
<td>Associated with APEX1, XRCC1, PCNA, and ERα(^{14} )</td>
</tr>
<tr>
<td>SMUG1</td>
<td>–</td>
<td>Phosphorylation at S241</td>
<td>Glycosylase activity increased by APEX1(^{113})</td>
<td>–</td>
<td>Association with hHR23(^{115}), XRCC1(^{116}), and ERα(^{144} )</td>
</tr>
<tr>
<td>MPG</td>
<td>3-methyl adenine DNA Glycosylase</td>
<td>Acetylation(^{114})</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NTHL1</td>
<td>Endonuclease III DNA Glycosylase</td>
<td>Sumoylation(^{85})</td>
<td>Phosphorylation at S71</td>
<td>Glycosylase activity increased by APEX1(^{117}); Associated with APEX1, XRCC1(^{118}), and YB-1(^{118})</td>
<td>–</td>
</tr>
<tr>
<td>NEIL1</td>
<td>Endonuclease VIII DNA Glycosylase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Associated with FEN1(^{119}), XRCC1(^{120}), PCNA(^{121}), Pol ε(^{121}), CSB(^{17}), RPA(^{122}), and PARP1(^{113})</td>
</tr>
<tr>
<td>NEIL2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Interacts with p300(^{24,125}), XRCC1(^{118}), and YB-1(^{126})</td>
</tr>
<tr>
<td>NEIL3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Associated with RPA(^{127})</td>
</tr>
<tr>
<td>OGG1</td>
<td>8-Oxoguanine DNA Glycosylase</td>
<td>Phosphorylation at S326(^{128-129}), Acetylation at K41 and K338(^{130}), Nitrosylated(^{131})</td>
<td>–</td>
<td>Glycosylase activity increased by APEX1(^{132}); Associated with XRCC1(^{113,114}), SIRT3(^{135})</td>
<td>–</td>
</tr>
<tr>
<td>MUTYH</td>
<td>A-G Mismatch DNA Glycosylase</td>
<td>Phosphorylation at S524(^{136,137}) Ubiquitination(^{90})</td>
<td>–</td>
<td>Associated with APEX1(^{138}), PCNA(^{138}), RPA(^{138}), MSH6(^{139}), and 91-1 complex(^{140})</td>
<td>–</td>
</tr>
<tr>
<td>MBD4</td>
<td>–</td>
<td>Phosphorylation at S112</td>
<td>–</td>
<td>–</td>
<td>Associated with MLH1(^{141}), and HDAC1(^{142},) DMNT3B(^{143})</td>
</tr>
<tr>
<td>TDG</td>
<td>G-T Mismatch DNA Glycosylase</td>
<td>Acetylation(^{13}) and Sumoylation(^{151})</td>
<td>–</td>
<td>Glycosylase activity increased by APEX1(^{27}); Associated with CBP/ p300(^{92}), DMNT3A(^{144}), DMNT3B(^{145}), RXR/ RAR(^{45}), SCR(^{46}), p73a(^{147},) p63y(^{147}), ERα(^{148},) RAD9(^{149}), XPC(^{150}), NEIL1/NEIL2(^{150}), SIRT1(^{157})</td>
<td>–</td>
</tr>
<tr>
<td>BER Prote in</td>
<td>Activity</td>
<td>Level of Regulation</td>
<td>PHOSIDA Post-translational Modifications (Human)</td>
<td>Functional Interactions</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>----------</td>
<td>---------------------</td>
<td>-----------------------------------------------</td>
<td>------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>APEX1</strong></td>
<td>AP Endonuclease</td>
<td>Acetylation at K27, K31, K32, K3598,152,153, Phosphorylation at T22396; Glutathionylation at C99102; Ubiquitination at K24, K25, K27134</td>
<td>Phosphorylation at S128, K196</td>
<td>Associated with UNG1111, SMUG111, NTHL1132, OGG1132, TDG135, MUTYH138, XRCC1135, TP53136, HSP70137, PCNA138, LIG1139, FEN1138, 139, WRN160</td>
<td></td>
</tr>
<tr>
<td><strong>APEX2</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Associated with PCNA161</td>
<td></td>
</tr>
<tr>
<td><strong>XRCC1</strong></td>
<td>X-ray Repair Cross Complementing Group 1</td>
<td>Phosphorylation162,163</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>PARP1</strong></td>
<td>Poly (ADP-ribose) Polymerase</td>
<td>Phosphorylation mapping176, S372/T373177, Y907178 Sumoylation at K486179,180 and K203181; Acetylation at K498, K505, K508, K521, and K524182 Méthylation at K508183, K528184 Ribosylation mapping185</td>
<td>Acetylation at K96, K104, K130, K547, K550, K599, K620 Phosphorylation at T94, S176, S178, S273, S464, S781, S863</td>
<td>Enzyme activity increase by ROS186; Associated with LIG1179,187, XRCC1182,188, POL183, OGG1186, XPA190, BRCA1191, DNA-PKcs192, CHK1192, SIRT1193, SIRT6194,195, CDK5196, TET1197, CTCF198, P21199, TPMPRESS:ERG fusion200, and numerous other proteins201-203</td>
<td></td>
</tr>
<tr>
<td><strong>FEN1</strong></td>
<td>Flap Endonuclease</td>
<td>Phosphorylation at S187201 Sumoylation at K168205 Ubiquitination at K354206 Acetylation at K354, K375, K377, K380208 Méthylation at R192207</td>
<td>Acetylation at K80, K267, K375 Phosphorylation at S197, S363, T195, T364</td>
<td>Associated with PCNA89, 208, 209, WRN210, CDK1210, CDK2210, NEIL1119, LIGI211, p305212, APEX1113, FANCA213, APC214, MUS81215, 9-1-1 complex216, E2F217</td>
<td></td>
</tr>
</tbody>
</table>