Obstacles and Opportunities for Base Excision Repair in Chromatin

Dana J. Biechele-Speziale[1], Treshaun B. Sutton[1], Sarah Delaney*

Department of Chemistry, Brown University, Providence, RI, USA

*Correspondence to: Department of Chemistry, Brown University, Providence, RI, USA. Email address: Sarah_Delaney@brown.edu (S. Delaney)

[1] Co-first authors (in alphabetical order).

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ABSTRACT

Most eukaryotic DNA is packaged into chromatin, which is made up of tandemly repeating nucleosomes. This packaging of DNA poses a significant barrier to the various enzymes that must act on DNA, including DNA damage response enzymes that interact intimately with DNA to prevent mutations and cell death. To regulate access to certain DNA regions, chromatin remodeling, variant histone exchange, and histone post-translational modifications have been shown to assist several DNA repair pathways including nucleotide excision repair, single strand break repair, and double strand break repair. While these chromatin-level responses have been directly linked to various DNA repair pathways, how they modulate the base excision repair (BER) pathway remains elusive. This review highlights recent findings that demonstrate how BER is regulated by the packaging of DNA into nucleosome core particles (NCPs) and higher orders of chromatin structures. We also summarize the available data that indicate BER may be enabled by chromatin modifications and remodeling.

Abbreviations

8-oxoG, 8-oxo-7,8-dihydroguanine; AAG, alkyladenine glycosylase; AP, apurinic/apyrimidinic; APE1, apurinic/apyrimidinic endonuclease 1; BER, base excision repair; DDR, DNA damage response; 5'-dRP, 5'-deoxyribosephosphate; DSB, double strand break; εA, 1,N^6-ethenoadenine; FACT, facilitates chromatin transcription; Hx, hypoxanthine; MBD4, methyl-CpG domain protein 4; MMS, methyl methanesulfonate; NCP, nucleosome core particle; NEIL1, endonuclease VIII (Nei)-like glycosylase 1; NER, nucleotide excision repair; NFRs; nucleosome-free regions; NMP-seq, N-methylpurine-sequencing; NTHL1, endonuclease III homologue 1; OGG1, 8-oxo-7,8-dihydroguanine glycosylase; Pol β, polymerase beta; PTM, post-translational modification; SMUG1, single strand selective monofunctional glycosylase 1; SSBR, single strand break repair; TDG, thymine DNA glycosylase; THF, tetrahydrofuran; Tg, thymine glycol; UDG/UNG, uracil DNA glycosylase; XRCC1, X-ray repair cross-complementing protein 1

1. Introduction

To prevent the deleterious consequences of DNA damage, cells have evolved sophisticated mechanisms for DNA repair [1]. The efficiency of DNA repair pathways is essential in preventing the accumulation of DNA damage and mutations that have been shown to lead to cancer, neurodegeneration, cardiovascular diseases, and aging [2-5]. One DNA repair pathway, base excision repair (BER), is responsible for repairing modified nucleobase lesions (**Fig. 1**) [6]. Glycosylases initiate the repair and are specialized for excision of a particular lesion(s). There are both mono- and bifunctional DNA glycosylase. Monofunctional glycosylases cleave the bond that attaches the lesion to the sugar-phosphate backbone resulting in an apyrimidinic/apurinic (AP) site. Bifunctional glycosylases have an additional DNA lyase activity that incises 5′ of the AP site by β-elimination to produce a 3′-α,β-unsaturated aldehyde and a 5′-phosphate. The 3′-aldehyde can also be converted to a 3′-phosphate via a δ-elimination. AP sites are recognized by apurinic/apyrimidinic endonuclease 1 (APE1) and the backbone is incised to the 5'-side to create a 3′-hydroxyl and a 5'-deoxyribose phosphate (5'-dRP). APE1 can also process the 3′-α,β-unsaturated aldehyde resulting from the bifunctional glycosylase activity, leaving a 3′-hydroxyl group. In short-patch BER, which is the predominant pathway, polymerase β (Pol β) trims the 5'-dRP and incorporates a single deoxynucleotide at the 3'-hydroxyl to fill the gap. The DNA backbone is then sealed by a DNA ligase to restore the DNA to its original state. Under conditions where the 5'-dRP cannot be removed by Pol β, an alternate pathway, long-patch BER, is used. In long-patch BER, multiple nucleotides are incorporated by a DNA polymerase via strand displacement synthesis. The single-stranded flap of displaced DNA, which contains the modified 5'-dRP, is removed by flap endonuclease 1 (FEN1) and a DNA ligase seals the nick. Inefficiencies in the BER process lead to detrimental outcomes, as mutations in and deletions of BER enzymes have been shown to promote the formation of various cancers [7-9] and immunodeficiency disorders [10-11]. The clinical importance of BER underscores the significance of understanding the mechanisms by which these enzymes function.

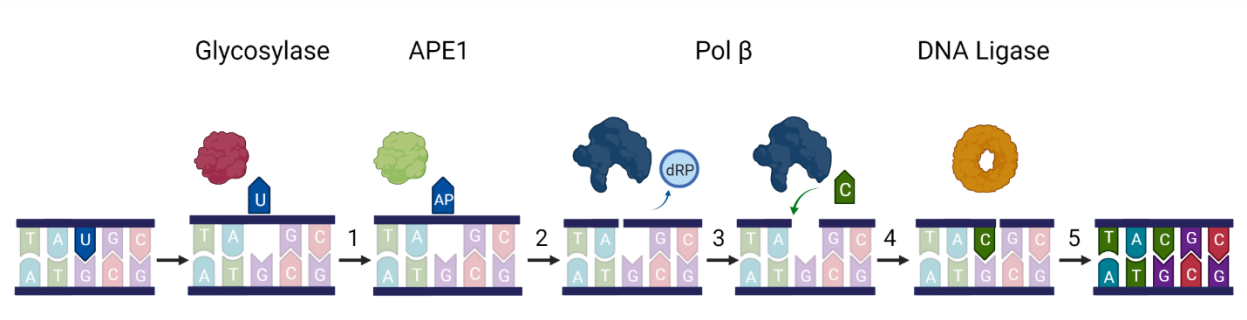

Fig. 1. The BER pathway using uracil as a representative nucleobase lesion. The short-patch pathway is depicted. The lesion is removed by a uracil-specific monofunctional glycosylase to generate an AP site (Step 1). APE1 recognizes the AP site and incises the sugar-phosphate backbone to the 5′-side (Step 2). Pol β removes the 5′-dRP (Step 3) and incorporates the correct nucleobase (C) at the 3′-termini (Step 4). Lastly, DNA ligase catalyzes formation of a phosphodiester bond, completing the repair event (Step 5). In long-patch BER (not shown), Pol β incorporates multiple nucleotides in Step 4, followed by removal of the displaced flap of DNA by FEN1 and DNA ligase activity. Created with BioRender.com.

The BER pathway has been studied extensively using oligonucleotide DNA substrates, and this research has been paramount in determining the molecular mechanisms and substrate

specificities of the BER enzymes. However, eukaryotic DNA is packaged into chromatin, which is composed of repeating units called nucleosome core particles (NCPs) (**Fig. 2**). NCPs consist of a protein core around which 145-147 base pairs (bp) of DNA are wrapped in approximately two superhelical turns [12]. The protein core contains two copies each of histones H2A, H2B, H3, and H4 with a 2-fold axis of symmetry known as the dyad axis. Each histone contains a highly structured globular core and an N-terminal disordered tail that is subject to post-translational modification (PTM) [13] and proteolytic clipping [14]. Intra- and inter-nucleosomal interactions of these histone tails are also required for formation of higher order chromatin structures [15].

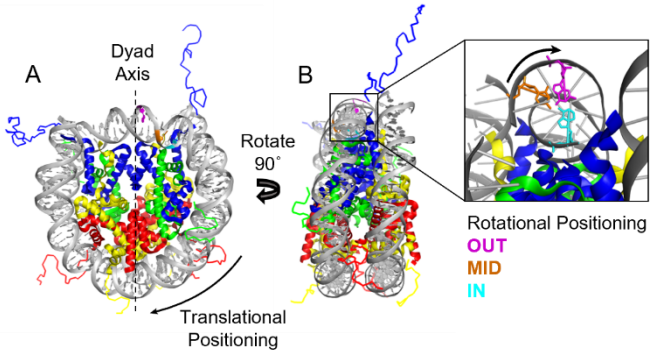

Fig. 2. A representative crystal structure of an NCP (PDB ID: 1kx5). (A) DNA is wrapped around a histone octamer core, which consists of two copies each of the four histones: H2A (yellow), H2B (red), H3 (blue), and H4 (green). The dyad axis is depicted as a dashed line. (B) Side view of the NCP. Insert: rotational positionings are highlighted as magenta (OUT), orange (MID), and cyan (IN).

Nucleobase orientation in an NCP can be described in two ways: (1) rotational positioning, referring to the location of the backbone relative to the histone core (inward towards the histones (IN), outwards towards solution (OUT), or in between these two (MID)), and (2) translational positioning, referring to the location of the nucleobase relative to the dyad axis (**Fig. 2**). Notably, DNA sequences that bind to the histone core in a predictable and reproducible manner with the same rotational and translational positioning are called nucleosome positioning sequences. Both naturally occurring and positioning sequences selected from DNA libraries have been identified and are reviewed in [16].

It is also important to highlight that NCPs are dynamic structures [17-21]. The DNA can transiently and spontaneously unwrap from the histone core. This unwrapping can expose occluded nucleobases, especially near the entry/exit regions where this phenomenon is most pronounced [18]. Unwrapping can also be further modulated by histone PTMs and histone tail clipping [21-25]. In addition to DNA unwrapping, the protein core is dynamic with histone exchange and deposition of histone variants [26-27].

The packaging of DNA into chromatin poses a significant physical barrier for nuclear processes that must access DNA, including BER. In this review, we summarize recent findings of how the BER pathway is modulated by the packaging of DNA into NCPs and higher-order chromatin structures. We also discuss the available data that indicate BER may be enabled by chromatin remodeling, histone PTMs, and histone variants to maintain genomic integrity.

2. BER on Positioned NCPs

There is a significant body of literature that has examined BER on NCPs. When assembling NCPs for these biochemical experiments, a positioning sequence such as alpha-satellite DNA, 5S ribosomal DNA, or Widom 601 DNA is typically used in order to provide a homogeneous substrate population. Lesions can be incorporated into the DNA using a variety of techniques. A modified nucleobase can be installed during chemical DNA synthesis using site-specific techniques to yield DNA with the lesion at a single and well-defined location. Alternatively, global techniques can yield a population of DNA with lesions in a variety of rotational and translational positions. Depending on the lesion, these global techniques can utilize chemical DNA synthesis [28] or a DNA polymerase [29] for incorporation. Sources of histones can be recombinantly expressed proteins or those isolated from a biological source.

Results from these experiments using NCPs as substrates provide information about the ability of BER to occur on the most fundamental unit of packaging in chromatin. The overall conclusion from these studies is that there are multiple factors that can impact the activity of BER enzymes such as the geometric orientation of the lesion (i.e., rotational and translational positioning), DNA sequence, NCP dynamics, PTMs, histone variants, chromatin remodelers, and even the presence of other BER enzymes (**Fig. 3**). Below we summarize each step of BER and how these factors influence repair on NCPs.

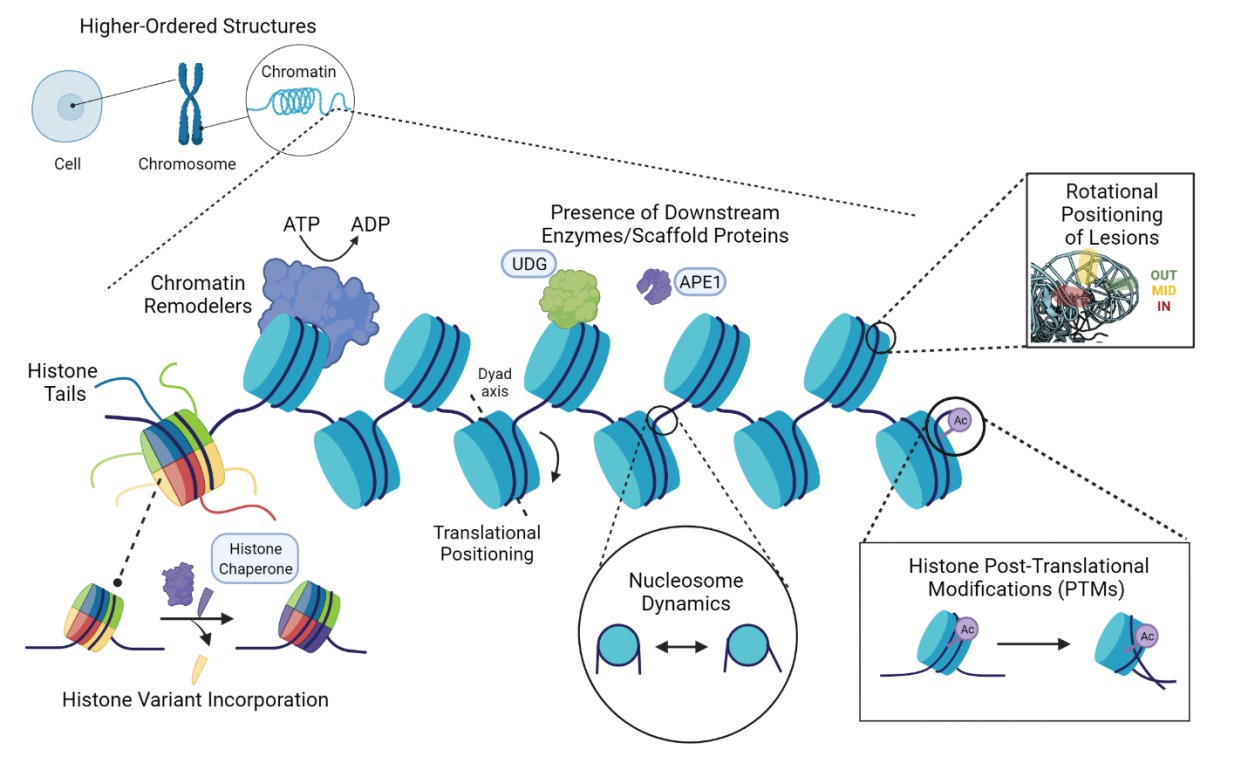

Fig. 3. Graphical depiction of factors that influence BER enzyme activity on chromatin: histone variant incorporation, histone tails, lesion positioning, histone PTMs, nucleosome dynamics, chromatin remodelers, and presence of downstream enzymes and/or scaffold proteins. For more details see text. Created with BioRender.com.

2.1. DNA Glycosylase Activity on NCPs

Glycosylases are considered the first line of defense against DNA lesions. There are eleven known mammalian DNA glycosylases that each recognize and remove one or more lesions. A glycosylase initiates BER by recognizing a target lesion, flipping the lesion into its active site, and cleaving the glycosidic bond (**Fig. 1**, Step 1) [30-34]. The activity of several glycosylases has been examined in NCPs.

Glycosylase activity has been shown to be modulated by both rotational and translational positioning of the target lesion. For uracil DNA glycosylase (UDG/UNG) [29, 35-39], alkyladenine glycosylase (AAG) [38, 40-42], 8-oxo-guanine glycosylase (OGG1) [28, 43], endonuclease III homologue 1 (NTHL1) [44-46], and endonuclease VIII (Nei)-like glycosylase 1 (NEIL1) [47], glycosylase activity is generally higher for OUT facing lesions than for sterically occluded IN and MID lesions. Regarding translational positioning, lesions are generally less efficiently removed when located near the dyad region and more efficiently removed in the entry/exit regions, in some cases regardless of rotational positioning [28-29, 39-44]. However, for methyl-CpG domain protein 4 (MBD4), high activity was observed near the dyad region and near one entry/exit region but only limited activity at the other entry/exit region [48]. Activity of UNG has also been reported to be only minorly impacted by rotational positioning [49-50], and instead predominantly determined by local DNA structure [50]. Some of the differences may be due to the varied experimental conditions and the complexity of reconstituted NCPs assembled using different positioning sequences, base pair composition, and source of histone proteins that differ in the level and type of PTMs. However, even with the experimental conditions are identical, the identity of the lesion can impact glycosylase activity. For example, AAG is able to remove 1,N^6-ethenoadenine (εA) from NCPs but not hypoxanthine (Hx) located at the same geometric position [38], lesions that are both substrates for AAG in unpackaged DNA [51].

The modulation of glycosylase activity in NCPs by a variety of histone modifications has been examined. MBD4 activity is enhanced in the presence of hyperacetylated histone octamers [48]. OGG1 activity is enhanced 4 to 5-fold on NCPs containing the H2A.Bbd variant [51] and 2-fold on NCPs containing hyperacetylated H2B [43]. A dramatic increase in OGG1 activity is observed upon addition of the chromatin remodeler SWI/SNF [52]. UDG activity is enhanced in the presence of APE1 [53] or when the NCPs contain the H2A.Z or macroH2A variants [39], and this enhancement is rotationally dependent. Single strand selective monofunctional glycosylase 1 (SMUG1) is also facilitated by H2A.Z and macroH2A variants [39]. Absence of the histone H2B tail leads to increased AAG activity in the entry/exit region due to DNA unwrapping [42], while removal of the H3 tail alters the DNA periodicity in the NCP and leads to suppressed AAG activity at some locations that are not prone to unwrapping [42].

2.2 APE1 Activity on NCPs

When an AP site is present in DNA, either from DNA glycosylase removal of a damaged nucleobase or spontaneous depurination, APE1 recognizes these AP sites and incises the sugar-phosphate backbone 5′ to the AP site, resulting in a single strand break (**Fig. 1**, Step 2) [53-54]. APE1 is essential for cell viability as it is responsible for more than 95% of AP site processing in mammalian cells [55].

In several kinetics studies, the activity of APE1 has been shown to be rotationally dependent, with OUT lesions being cleaved faster than IN or MID lesions [36, 56-57]. The binding of APE1 to AP sites has also been shown to be dependent on rotational position [56], and its activity is enhanced in NCPs containing H3 acetylated at lysine 56 (H3K56Ac) [58]. This enhancement is reversed

with the addition of the deacetylase SIRT6, confirming that H3K56Ac is the source of the enhancement. This PTM is known to enhance NCP dynamics and unwrapping of DNA in the entry/exit regions [59]. In other work, incision at tetrahydrofuran (THF) sites, a chemically stable AP site analog which is a known substrate for APE1 [60], was found to be rotationally dependent when using purified APE1 protein [61]. However, when HeLa whole cell extracts were used as the source of repair enzymes, the rotational dependence was diminished. Subsequent experiments identified the E3 ubiquitin ligase HECTD1 as a chromatin remodeling factor that facilitated access to the THF sites to diminish the impact of the rotational position of the THF site [61]. NCPs containing tailless histones were shown to not significantly increase UDG or APE1 activity [62].

In the context of packaged DNA, it is also notable that histone tails can participate in DNA damage and repair by reacting with AP sites. AP sites are known to be chemically labile, especially at elevated pH, and they can be further destabilized in NCPs. AP sites generated using a photolabile precursor undergo strand scission ~100-fold more rapidly in NCPs than in unpackaged DNA [63-64]. The strand scission is initiated by crosslink formation between the AP site and a histone tail lysine residue. A subsequent elimination reaction leads to a DNA-protein crosslink containing cleaved DNA [65-66]. The rate of AP site strand cleavage in NCPs is independent of rotational positioning since the histone tails can access AP sites via both the major and minor groove [64]. Methylated histone tails lead to a small increase in AP site strand cleavage by increasing the rate of the elimination reaction [67]. Histones have also been shown to enhance the AP lyase activity of OGG1 by crosslinking with the 3′-phospho-α,β-unsaturated aldehyde, although this activity is suppressed in the presence of APE1 [68].

2.3 DNA Pol β Activity on NCPs

Following APE1 activity, Pol β utilizes its lyase activity to remove the 5'-dRP and incorporate, via a phosphoryl transfer reaction [69-71], the correct nucleobase to fill the gap (**Fig. 1**, Steps 3/4). Pol β is the smallest of the X family of DNA polymerases and has been shown to bend the DNA template 90° during dNTP incorporation on oligonucleotide substrates [72]. Interestingly, it has been suggested that an OUT facing gap in an NCP may already be sufficiently “pre-bent” to facilitate Pol β binding [45].

Several studies investigating Pol β activity on NCPs concluded that the gap filling activity of Pol β is either inhibited on NCPs [35, 73-75] or decreased relative to glycosylase and APE1 activity [45], while its lyase activity is unaffected by the NCP [73]. Acetylation of H3K56 and H3K14 has been shown to decrease gap-filling activity near the dyad region whereas activity in the entry/exit region is unaffected [75]. Removal of the H3 tail did not alter Pol β’s activity on NCPs [62]. Repair patch size has been shown to be limited to one nucleotide in an NCP (i.e., short-patch BER), whereas in the linker region between NCPs, multi-nucleotide extension can occur (i.e., long-patch BER) [76]. Another study reported that Pol β is not altered in the presence of APE1 [37], whereas Pol β gap-filling activity is enhanced on NCPs in the presence of DNA Ligase IIIα-XRCC1 (LigIIIα-XRCC1) [45], the chromatin remodeler SWI/SNF [52], and the chromatin architectural factor HMGB1 [74]. These results suggest that chromatin remodelers or the disruption of the NCP may be necessary for gap filling by Pol β.

2.4 DNA Ligase Activity on NCPs

A DNA ligase completes the final step of BER by catalyzing the formation of a phosphodiester bond between the 3'-OH and the 5'-phosphate at the gap [77-78] (**Fig. 1**, Step 5). The crystal

structure of human DNA ligase I (Lig I) shows that it encircles the DNA substrate [79] in order to position the DNA nick at its catalytic core. However, it is not known if or how this encircling occurs on NCPs.

Several studies indicate that LigIIIα may require nucleosome disruption to complete BER at some positions. Small amounts of LigIIIα-XRCC1 are able to ligate OUT facing nicks in an NCP, but when the concentration of LigIIIα-XRCC1 is increased to facilitate ligation of IN sites, the moderate levels of ligation are accompanied by disruption of the NCP [45]. Another study investigated the efficiency of ligation on IN nick sites located 26 or 46 bp from the dyad axis [80]. An increase in ligation at the site further from the dyad supports the hypothesis that spontaneous unwrapping plays a role in completing BER by LigIIIα-XRCC1. Notably, gel shift assays confirmed that while LigIIIα-XRCC1 does disrupt the NCPs as part of the repair event, the structural changes are reversible as demonstrated by addition of competitor DNA to bind LigIIIα-XRCC1.

Unlike LigIIIα-XRCC1, it has been shown that it is not essential for Lig I to disrupt the nucleosome to ligate nicks [81]. Using a 218 bp DNA sequence to assemble a nucleosome containing one nick near the dyad axis, one in the entry/exit region, and a third nick in the linker region, it was shown that Lig I has the highest activity in the linker region with slightly slower kinetics observed at the other two nick sites. This ligation occurs without nucleosomal disruption or sliding. Interestingly, when a shorter 154 bp DNA sequence was used to assemble an NCP, ligation at a nick near the dyad axis was ~1,000 times slower than at the same site in the 218 bp NCP [81]. When the N-terminal histone tails are removed, the level of ligation activity is comparable to that observed for the 218 bp NCP. The authors attribute this difference to the histone tails binding to intra-core NCP DNA when the shorter sequence is used, but in the presence of longer linker DNA the tails “reposition” to bind the extra-core linker DNA and allow Lig I access to the dyad region.

3. BER in Higher Orders of Structure

3.1 Chromatin Structure and Characteristics

While many studies have revealed detailed mechanisms of BER in the context of NCPs, eukaryotic DNA is further packaged into chromatin which consists of nucleosome arrays, where many nucleosomes are “strung together” via linker DNA **(Fig. 4)**. Depending on the length of the linker DNA between each nucleosome and any histone modifications, this chromatin further condenses into some dynamic tertiary structure. When compared to NCPs alone, chromatin is significantly more complex. The added presence of linker DNA and the linker histone H1, as well as the ability of H1 to rapidly exchange between sites [82, 83], may pose significant barriers to genomic processes, including BER. Because the packaging of DNA occludes it from DNA-binding proteins that must act on it, understanding how the genome is organized *in vivo* is crucial to elucidating how BER is regulated.

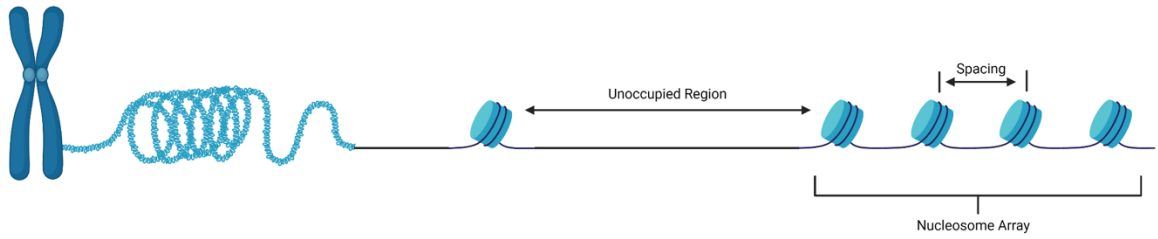

Fig. 4. Hierarchy of packaging of DNA into NCPs. Different aspects of chromatin structure and organization are highlighted, where (1) an unoccupied region indicates a stretch of DNA that is consistently unoccupied by nucleosomes (i.e., transcription start sites and promoter regions), (2) nucleosome spacing is the distance between two adjoined nucleosomes, and (3) a nucleosome array consists of many NCPs that are adjoined by linker DNA. Created with BioRender.com.

Genome organization is broadly thought about in terms of nucleosome positioning and occupancy. Nucleosome positioning indicates where NCPs are located on a given stretch of genomic DNA. Several studies have mapped nucleosome positioning, revealing that the majority of the human genome shows flexibility in positioning, while only a small fraction exhibit well-positioned nucleosome arrays that typically emanate from the nucleosome-free regions (NFRs) found at transcriptional start sites of active genes [84]. Inactive promoters, however, were not shown to exhibit a depletion of nucleosomes or a well-positioning of nucleosomes, indicating that the transition of an inactive promoter to an active promoter signals an eviction of nucleosomes surrounding the transcription start site [84]. In addition to positioning, nucleosome spacing is important in defining chromatin structure, which is determined by the length of linker DNA that separates NCPs **(Fig. 4)**. Linker DNA length is highly variable in chromatin (~20-90 bp), and recent studies have shown that linker DNA plays an important role in internucleosomal interactions and the subsequent folding of arrays into the tertiary structure of chromatin, indicating that nucleosome spacing plays an important role in chromatin accessibility to DNA binding proteins [85].

Another important characteristic of chromatin structure is nucleosome occupancy, which indicates the fraction of cells in a population that display a given region of DNA that is occupied by a histone octamer. Intrinsic DNA sequence has been shown to play a central role in determining the occupancy of nucleosomes *in vivo*. Many promoter and transcription factor binding sites exhibiting low or no occupancy, which is hypothesized to be encoded in the genome [86]. Evidence has recently emerged indicating that NFRs in mouse embryonic stem cells are occupied by “fragile” nucleosomes that inhabit regions at distinct genomic landmarks that were previously thought to be depleted of NCPs, showing a preference for nucleosomes at exon/intron junctions [87]. While the dynamic nature of nucleosome positioning and occupancy and its impact on forming a tertiary chromatin structure has been shown to affect transcription [88,89], better chemical biology tools and approaches for studying BER on higher orders of chromatin structure are necessary to elucidate the mechanisms by which chromatin regulates BER.

3.2 BER in Chromatin

Given the complexity of chromatin, genomic processes should be studied in systems that closely mimic its structure and organization to better understand how they are regulated *in vivo*. Nucleosome arrays offer a more complex *in vitro* substrate to study BER, and at least twelve nucleosomes are required in one array to fold and condense in a manner that resembles native chromatin [90]. However, to study BER in nucleosome arrays, experimental challenges must be overcome. The most significant challenge is modifying nucleobases in a controlled manner in long stretches of DNA. At least 165 bases (145-150 to wrap around the histone octamer and 20-25 for the linker) are needed per nucleosome. For an array consisting of twelve nucleosomes, ~2,000 bases would be required.

The first report of BER on the 30-nm chromatin fiber, a hypothesized first hierarchical level of chromatin folding, used a twelve-nucleosome array assembled from linearized plasmid DNA [91]. The DNA was chemically deaminated using sodium bisulfite under conditions such that 1-2

cytosines were converted to uracil. Activity of UDG and APE1 were reported to be faster at lesions in linker regions but both enzymes also access lesions in nucleosomal DNA in a manner consistent with the predicted rotational positioning of the lesions. However, Pol β is significantly inhibited at most sites in the array. Addition of the ISW1 and ISW2 chromatin remodeling complexes from yeast enhance nucleotide incorporation by Pol β suggesting that while the initial steps of BER may occur efficiently on folded nucleosome arrays, chromatin remodeling may be required for the latter steps.

In other work, a “plug and play” approach has been reported to create designer chromatin with site-specifically modified DNA [92]. A nicking endonuclease was used to excise short fragments of unmodified DNA and replace them with synthetic oligonucleotides containing the desired lesion. This technique was used to study the combined catalytic activities of UDG and APE1 on the 30-nm chromatin fiber and it was found that internucleosomal interactions regulate BER. Activity of UDG and APE1 is inhibited by up to 20-fold or accelerated by 5-fold in compact chromatin depending on the positioning of the lesion relative to the dyad axis when compared to unpackaged and NCP substrates. Interestingly, this study also demonstrated that in nucleosome arrays incubated with a higher concentration of Mg^{2+} to simulate heterochromatin formation, a lesion located in the linker DNA region was processed at a 5-fold increased rate when compared to unpackaged DNA. This result suggests that heterochromatin may promote the initial steps of BER in linker and nucleosome-free regions of DNA *in vivo*.

Furthermore, genome-wide mapping of alkylation damage, repair, and mutagenesis in yeast found that BER of alkylation damage is significantly regulated by chromatin. This study utilized N-methylpurine-sequencing (NMP-seq) to map methyl methanesulfonate (MMS)-induced alkylation damage [93]. Efficiency of BER towards 7-methylguanine lesions was demonstrated to be significantly modulated by rotational and translational positioning of the lesion as well as by nucleosome organization. The highest mutation density occurred within strongly positioned NCPs immediately following the nucleosome depleted region, and faster repair was shown in nucleosome depleted regions. Further, this effect was found to be asymmetric to the dyad axis, which is frequently observed in human cancers [94, 95], and modulated by histone PTMs.

Another study mapped genome-wide 8-oxo-7,8-dihydroguanine (8-oxoG) damage in yeast using a “Click-code-seq” method [96]. In this technique, a glycosylase and APE1 are used to excise 8-oxoG throughout the genome and create nucleotide gaps. A DNA polymerase is then used to incorporate an alkynylated nucleoside at the gap. Taking advantage of the selective reaction between alkynes and azides, an azido-modified code sequence is ligated to the alkynylated DNA via a click reaction. Thus, the locations of the original 8-oxoG sites are labeled with a code sequence that can be revealed using next-generation sequencing. This study found that 8-oxoG damage persists in heterochromatin and is more easily repaired in euchromatin, and that the linker region of DNA harbored 16% less 8-oxoG than NCP dyad regions, indicating that histone-DNA interactions inhibit repair. Moreover, nucleosomes harboring histone PTMs (H3K4me3 and H3K36me3) lead to decreased 8-oxoG abundance when compared to unmodified nucleosomes.

The observed increase in alkylation-associated mutation density in DNA occupied by strongly positioned NCPs as well as differential persistence of 8-oxoG across heterochromatin, euchromatin, and post-translationally modified NCPs underscore the importance of studying BER on higher orders of chromatin structure. These results suggest that to prevent mutations and disease, there must be mechanisms that allow BER to access packaged DNA.

3.3 Facilitation of BER by Post-Translational Modifications and Chromatin Remodeling

Despite the significant barrier chromatin poses to DNA repair enzymes, it also has a critical role in regulating the DNA damage response through chromatin remodeling, variant histone exchange, and histone PTMs. While these chromatin-level responses have been directly linked to the regulation of other DNA repair pathways including double strand break (DSB) repair [97-99] and nucleotide excision repair (NER) [100-102], how these responses may regulate BER remain elusive. Recently, several studies have begun to piece together how certain PTMs and chromatin remodeling complexes may help BER overcome the chromatin barrier in reconstituted nucleosome arrays and *in vivo*.

While histone modifications have been shown to play an important role in transcription and other genomic processes, very few studies have proven such roles exist in the context of BER *in vivo* due to challenges associated with chemically modifying chromatin in cells. One study investigated histone acetylation of H3 tails in nucleosome arrays containing site-specific DNA lesions and demonstrated a relationship between histone acetylation and BER in higher order chromatin structures [103]. This study used 12-mer nucleosome arrays with homogenous H3K18ac and H3K27ac histone modifications and measured the combined UDG and APE1 repair activity of a site-specific uracil lesion to demonstrate that acetylation modulates BER. When compared to unmodified arrays, H3K18ac resulted in an increase in DNA cleavage when incubated with UDG and APE1 while H3K27ac demonstrated the opposite effect. Interestingly, the initial rate of uracil incision is similar for each array despite cleavage being significantly lower for H3K27ac arrays. This result could be explained if histone acetylation influences the distribution of substrate populations within each compact array, where in one population uracil is more accessible than in the other. Given that the acetyltransferase CBP/p300 acetylates both H3K18 and H3K27 positions, CBP/p300 may play a vital role in the BER pathway that merits future investigation.

Another study found that human cells contain an activity that facilitates glycosylase-mediated removal of lesions in NCPs [104]. DNA glycosylase NTHL1 was shown to efficiently excise thymine glycol (Tg) lesions from unpackaged DNA and NCPs that were incubated with nuclear extracts from human cells. Importantly, NTHL1 was also able to excise sterically-occluded Tg lesions upon addition of Mg^{2+} and ATP, and these NCPs are intact following the excision of Tg. When tested in unpackaged DNA containing Tg lesions, addition of Mg^{2+} and ATP does not significantly alter Tg excision by NTHL1, which indicates that there is a factor(s) in human cell extracts that facilitate NTHL1-mediated excision of Tg in NCPs. The factor is proposed to be different than most known chromatin remodeling complexes as size exclusion chromatography revealed that the fractions that contain known chromatin remodeling complexes do not exhibit the same stimulatory activity as fractions with the factor, which is of relatively low molecular weight. These results indicate that cells possess a factor that can promote initiation of BER in chromatin that has not yet been associated with glycosylase stimulation.

Chromatin remodelers have also been implicated in facilitating BER. One study found that ALC1, a chromatin remodeler in the SNF2 superfamily of ATPases, was required for efficient BER [105]. In this study, the ALC1 gene was disrupted in mammalian TK6 cells, and the resulting ALC1-/- cells showed a delay in the repair of BER single strand break intermediates following exposure to MMS and H_2O_2. Since ALC1-/- cells also exhibited compromised chromatin relaxation following exposure to H_2O_2, it was proposed that ALC1 likely functions as a chromatin-remodeling enzyme and BER factor. It has also been demonstrated that depletion of the RSC chromatin remodeler subunit Sth1 in yeast leads to reduced BER of 7-methylguanine in positioned nucleosomes [106].

The facilitates chromatin transcription (FACT) complex has also been recently implicated in BER [107]. FACT is a known histone chaperone that has been extensively studied in the context of replication and transcription and is thought to be involved with transcription-coupled NER and DSB repair, and it was recently shown that it may enhance glycosylase excision activity. It was discovered that upon oxidative stress, FACT is released from transcription related complexes and associates with repair proteins and SWI-SNF chromatin remodelers. It was shown to rapidly colocalize to the site of oxidized damage in HeLa cells with the OGG1 glycosylase, indicating that it may assist in the repair of oxidative DNA damage.

In addition to external factors, one study showed that thymine DNA glycosylase (TDG) itself can alter chromatin structure directly through its interactions with DNA [108]. It was demonstrated that TDG induces chromatin decompaction of individual chromatin fibers and promotes the condensation of nucleosome arrays into higher orders of structure upon binding. These results suggest that enzymes within the BER pathway may also be involved in controlling chromatin organization.

Future Outlooks and Perspectives

Given that BER must occur within the highly complex and still undefined chromatin environment, understanding how BER functions in the context of higher order chromatin structures remains unclear and largely uncharted. As PTMs have been implicated in DNA accessibility in transcription, recombination, and other repair pathways including NER and DSB repair, it is likely that histone modifications play an important role in promoting DNA accessibility to BER enzymes through signaling chromatin remodelers or changing the DNA-histone and histone-histone interactions.

The work that has been done brings us closer to understanding how BER overcomes the chromatin barrier and highlights the importance of studying BER in higher orders of chromatin structure. Looking forward, new chemical biology techniques and advances in structural biology will be paramount to further defining the BER landscape. Given that BER must occur within a variety of structural and chemical chromatin states, future studies aimed at elucidating BER in higher-order chromatin structures *in vitro* and *in vivo* will provide important insights into the elusive and seemingly intertwined relationships between histone PTMs and variants, chromatin remodelers, chromatin structure, and other nuclear co-factors and the BER pathway *in vivo*.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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