Published in final edited form as:

Prog Mol Biol Transl Sci. 2012 ; 110: 207–227. doi:10.1016/B978-0-12-387665-2.00008-0.

Overview for the Histone Codes for DNA Repair

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Abstract

DNA damage occurs continuously as a result of various factors—intracellular metabolism, replication, and exposure to genotoxic agents, such as ionizing radiation and chemotherapy. If left unrepaired, this damage could result in changes or mutations within the cell genomic material. There are a number of different pathways that the cell can utilize to repair these DNA breaks. However, it is of utmost interest to know how the DNA damage is signaled to the various DNA pathways. As DNA damage occurs within the chromatin, we postulate that modifications of histones are important for signaling the position of DNA damage, recruiting the DNA repair proteins to the site of damage, and creating an open structure such that the repair proteins can access the site of damage. We discuss the modifications that occur on the histones and the manner in which they relate to the type of damage that has occurred as well as the DNA repair pathways that are activated.

Cells are exposed to many agents that can cause DNA damage and in order to maintain genomic stability, this damage must be repaired. The signaling pathways are well described from ataxia telangiectasia mutated/ATM and Rad3 related (ATM/ATR) through Chk1 and Chk2, which signal the cell in which damage has occurred and needs to be repaired, but the signals that occur at the site of damage are only now becoming better understood. Histones are nuclear proteins that package and organize DNA into nucleosomes. There are five types of histones: H1, H2A, H2B, H3, and H4. Histone H1 is involved in the higher order structure of chromatin whereas the other four histone proteins associate with the DNA to form nucleosomes. Each nucleosome is composed of 146 bp of DNA and eight histone molecules, two copies each of histone H2A, H2B, H3, and H4. The DNA is wrapped around the histones. The N terminus of each histone contains a number of lysine (K) residues. These residues are positively charged and these positively charged residues can then interact with the negatively charged phosphates in DNA. When this positive charge is neutralized, say by acetylation, then the binding affinity between the histones and the DNA is reduced. This modification, acetylation, is important in the regulation of gene transcription.

Little is known about the role of histone modifications in relation to DNA repair. There are a number of potential modifications that histones could undergo, such as acetylation, phosphorylation, and ubiquitylation. We review the most recent studies, which suggest that DNA repair is influenced and affected by histone modifications. Each of the five DNA repair pathways is discussed.

I. Histone Modifications of Homologous Recombination Repair

Homologous recombination repair (HRR) is one of two primary pathways in the repair of DNA double-strand breaks (DSBs). Mechanistically, HRR utilizes a homologous template, such as a sister chromatid or homologous chromosome, to repair broken DNA. This process is generally considered error-free repair, though it may lead to loss of heterozygosity and further chromosomal instability. HRR functions primarily during the latter portions of the cell cycle including S and G2, likely because of the presence of homologous sister chromatids. A complete review of HRR is presented elsewhere in this text.

HRR plays important roles in multiple oncogenic processes. Its most infamous role was described over a decade ago in breast cancer. The breast cancer genes (BRCA) 1 and 2 have since been thoroughly implicated in HRR and account for a significant proportion of familial inherited breast cancers. Different portions of the HRR pathway have further been shown to be mutated in a variety of nonfamilial, somatic, sporadic breast cancers and this is the basis for the current Phase I, II, and III clinical trials with several molecules that inhibit a parallel pathway including, specifically, PARP-1.¹ HRR has been shown to be mutated in a variety of other cancers including leukemia, ovarian, pancreatic, colon, and uterine cancers. This pathway has also been shown to be involved in several genetically inherited diseases including ataxia telangiectasia, and Werner's, Bloom's, and Cockayne syndromes. All told, this process has a number of implications in a number of different pathological processes.¹

HRR is a complex process that must have significant access to DNA and involves the disruption of chromatin structure, at least temporarily. The first step in HRR is the processing of DNA ends to produce 3' DNA ends for RAD51 binding. Then, RAD51 induces the search for homology that must disrupt base pairing and, by proxy, the chromatin structure. Next, the synthesis portion of HRR requires significant access to DNA and will not be possible without chromatin access, similar to that seen during replication. Finally, after the entire process has occurred, there must be a process responsible for the reorganization of the chromatin structure to resolve the enzymatic sequence of events. It can be predicted that each of these steps has associated pathways to regulate the intricate nature of modulating chromatin. In the current literature, there are three basic types of regulation of chromatin described, including histone variants, histone modifications, and ATP-dependent chromatin remodeling. In this section, we describe these events in their basic temporal activation during HRR.

Histone structure has been well described elsewhere, but in short, there are the canonical replication inserted histones: H2A, H2B, H3, and H4. Histone H2A has two variants that have been associated with DNA repair: H2AX and H2AZ. The classical event in DNA DSB repair is phosphorylation of histone H2AX, a variant of histone 2A, on Serine 139 (termed γ -H2AX) in mammals. This event has been shown to be mediated by Mec1 and Tel 1 in *Saccharomyces cerevisiae* or ATM, ATR, and DNA PKcs in mammalian cells. γ -H2AX phosphorylation results from the production of DSBs by extrinsic or intrinsic factors such as ionizing radiation (IR), chemotherapeutic drugs, or spontaneous cell damage as a result of replication fork arrest or oxidative damage, and is involved in the recognition of DNA DSBs. In mammalian cells, H2AX represents 2–25% of the H2A variants in the overall

chromatin depending on cell type. Yeast cells that express unmodifiable H2AX are hypersensitive to treatment with drugs such as miracle mineral supplement (MMS) or phleomycin, and mammalian stem cells lacking γ -H2AX are sensitive to IR and prone to genomic instability.²

The exact function of γ -H2AX is still not completely understood, though several important roles have been well described. γ -H2AX is not necessary for the initial steps in repair, but is involved in localizing and concentrating repair factors at the site of the DSB including chromatin modifiers such as the histone acetyl transferases (HAT) NuA4, which is implicated in the relaxation of chromatin surrounding a break. Further, γ -H2AX is an important binding site for MDC1, an adapter protein that serves as a landing bay of sorts for a plethora of proteins including BRCA1, the MRN (Mre11/Rad50/Nbs1) complex, and 53BP1.³ Interestingly, an AA substitution for glutamic acid, mimicking phosphorylation, does not induce localization of repair factors indicative that γ -H2AX is only one step in a more complicated process.⁴

No text on γ -H2AX in the context of chromatin would be complete without a discussion on γ -H2AX's role in the relaxation of heterochromatin. It is still up for debate whether the phosphorylation of H2AX induces chromatin relaxation, or whether relaxed chromatin is more likely to be marked by phosphorylation. The first model is that the phosphorylation event following damage recruits proteins that relax heterochromatin in preparation for repair. This is supported by the general finding that γ -H2AX foci are rarely seen in heterochromatin, indicating that the structure is relaxed rapidly after phosphorylation has occurred, as has been reported around a DSB. Second, it could be that heterochromatin is structurally less likely to be marked. As heterochromatin is more compacted, it yields less access to kinases, less phosphorylation, and further, is less likely to be damaged by oxidative damage produced during IR or by chemotherapy.⁵

In either of the current models, γ -H2AX has become a marker for the presence and/or location of DSBs within the cell. It is a commonly used antibody for determining the presence of DSBs using multiple laboratory techniques including ChIP, Western blotting, and immunofluorescence. It has been established that there are near 1 to 1 ratios (0.7–0.9 depending on cell type) of DNA DSBs to γ -H2AX nuclear foci in mammalian cells, which is especially useful in localizing other factors to sites of DNA DSBs. Of note, γ -H2AX is phosphorylated in most DNA DSBs including those produced during DNA replication and those frank DSBs that will be repaired via nonhomologous end-joining (NHEJ), which is discussed separately in this chapter.

The other H2A variant, H2AZ, is less well defined in DNA repair. In transcription, H2AZ is inserted in most RNA polymerase II promoters to allow easier eviction. Its role in repair is thought to be similar to this. It has been shown in yeast to be inserted by SWR1 around DSBs depending on sumoylation at K126 and K133 in the C-terminal tail, and this relocalization is important for further steps in the HRR process including RAD51 function.⁶ This process likely enhances chromatin eviction in preparation for HRR and has been shown to occur during the initial steps in the process to influence access to chromatin.

The initial steps in the DSB pathway involve Tip60 (Tat-interacting protein, or NuA4 in yeast), MRN, and ATM, which have been more recently elucidated to shed light on the events leading to γ -H2AX production.⁷ The current model begins with undamaged, normal chromatin compacted and stabilized by the heterochromatin protein 1 (HP1) and KRAB-associated protein (KAP-1). These proteins sit on heterochromatin and maintain the condensed state. HP1 is an adapter molecule that is primarily responsible for maintaining heterochromatin. KAP-1 was shown to actively regulate the decondensation of chromatin surrounding DSBs when deactivated, likely by phosphorylation. When a DSB occurs, the DNA is structurally opened and the MRN complex recognizes this occurrence by an unknown mechanism. MRN binds the free DNA ends. Then this action unmasks trimethylated H3 at K9 (H3K9me3) in the vicinity of the DSB. In undamaged cells, Tip60 and ATM form a stable complex and Tip60 is capable of recognizing H3K9me3 via its chromodomain, which allows its recruitment to the DSB, along with ATM. Tip60 then acetylates ATM, which activates the preliminary kinase functions, and allows autophosphorylation and the production of γ -H2AX, which begins a cascade that results in megabases of γ -H2AX production around the DSB. In the same time frame as ATM is activated, Tip60 begins to hyperacetylate H3 and H4 at multiple residues, resulting in further chromatin relaxation⁸ (Fig. 1).

At this point in the activation cascade, γ -H2AX has been activated and repair factors are beginning to be recruited. This recruitment is facilitated by a further decondensation of chromatin, which results in a number of chromatin modifications becoming exposed. Dot1 trimethylates H3 at K79 and is important in the recruitment of 53BP1, which activates checkpoint factors simultaneously to HRR.^{9,10} This modification is considered constitutive, but becomes unmasked during chromatin remodeling and functions as a binding site for 53BP1. Another methylation event that is constitutive, but important in HRR is H4K20me. This modification is also unmasked during DNA repair and results in the recruitment of multiple factors by their Tudor domains that recognize H4K20me, which appears to be species specific.¹¹

There are a number of acetylation events that occur on H3 and H4 that have been associated with the early events in HRR. These include H3 lysines (K9, 14, 18, 23, and 27) and H4 lysines (K5, 8, 12, and 16). Multiple HATs have been implicated in this process including GCN5, NuA4, and HAT1.^{12,13} These modifications increase near the DSB and are thought to be involved in loosening the chromatin structure for proper repair. Interestingly, when the four acetylated K residues on H4 are all mutated, DSB repair is abolished overall, but their individual functions are not well established. H3 acetylation has been most associated with HRR.¹⁴ GCN5, an H3 HAT, has been well established in the eviction of chromosomes during transcription and has more recently been implicated in HRR, leading to the INO80-mediated chromosome eviction.^{15,16} Specifically, H3 K14 and K23 appear to be the most critical events and are stimulated by phosphorylation of H3 S10.¹⁷ Mutation in these residues also confers sensitivity to MMS, a chemotherapy which produces lesions primarily repaired by HRR. Further, the competing pathway, NHEJ, protein DNA PKcs has been reported to phosphorylate GCN5 in human cells and inactivate its HAT domain,¹⁸ indicating that GCN5 plays a pro-HRR role in DSB repair. GCN5 also interacts with and coregulates BRCA1 depending on its HAT activity, which further establishes it in the HRR pathway.¹⁹

Protein ubiquitylation is an important process in cell biology rivaling the significance of phosphorylation events. Ubiquitin requires a chain of events to occur including several steps mediated by classes of proteins labeled E1, E2, and E3 ligases. In short, ubiquitin is prepared by E1, ligated by E2, and specificity is mediated by E3. There are more than 500 E3 ubiquitinating enzymes and multiple mono- and polyubiquitin chain products. With the complexity that has been described for this pathway, it has inevitably found a role in chromatin modification that affects HRR. Not only is H2AX modified by phosphorylation, it is also modified by ubiquitin dependent on the RNF8 (E3) and UBC13 (E2) proteins, and this modification is present in colocalization with γ -H2AX.²⁰ This modification occurs downstream of γ -H2AX and is dependent on the binding of MDC1 to γ -H2AX and the activity of p400, an SWI/SNF ATPase chromatin-remodeling enzyme.⁷ MDC1 then recruits RNF8, which ubiquitylates H2AX. In the best described model, this signals a transition from early to later recruitment of repair factors. The BRCA1 complex was previously reported to be dependent on γ -H2AX it is now known to fall later in the pathway and be dependent upon ubiquitylation of H2AX as well. Once BRCA1 is recruited, it is further responsible for the maintenance of H2AX ubiquitylation by means of its own E3 ligase function.²¹ This pathway then remains intact to continue to recruit and maintain the damage response.^{22,23}

As mentioned earlier, not only are histone variants and modifications important in the process of HRR, but histone remodeling also plays a central role in allowing access to DNA. There are multiple enzymes that play a role in histone remodeling in HRR. These include INO80, SWI/SNF, and SWR1. Each of these proteins is recruited to the break in a DSB-dependent manner and function to allow HRR to occur.

The ATPase motifs of the chromatin-remodeling proteins are in the SWI/SNF family. Within this family are several subdivisions including the INO80 family, which is present from yeast to mammals and includes INO80 and SWR1 in *S. cerevisiae* and INO80, Snf2-related CBP activator protein, and p400 in mammals. This family of proteins has been most described in transcription, but also plays a significant role in DSB repair and HRR.²⁴

Both INO80 and SWR1 contain an Arp4 subunit that directly interacts with γ -H2AX and is required for recruitment to the DSB.^{25,26} Both INO80 and SWR1 are required for HRR. SWR1 functions in concert with the HAT NuA4 (Tip60 in mammals) to introduce H2AZ into the chromatin around the break,²⁷ and when H2AZ is deleted, less single-stranded DNA is produced, though the kinetics of HRR are not significantly altered.¹⁵ This process allows chromatin eviction to occur more readily and promotes proper processing of DNA to yield access for HRR.

In the last few years, the role of INO80 and chromatin eviction has become convoluted. It is clear that INO80 plays a major role in chromatin dynamics around a DSB because when the Arp8 subunit of INO80 is deleted, significantly less ssDNA is observed at the ends of the DSB.¹⁵ Several studies have indicated that INO80 is responsible for histone eviction surrounding the DSB during DNA processing.^{16,28} This is further corroborated by data indicating that the RSC complex also plays a role in chromatin remodeling surrounding the DSB.²⁹ On the other hand, some data indicate that H2B is not lost during DNA processing, even at the point at which RAD51 is bound to ssDNA.³⁰ An interesting point is that

transcriptional histone eviction leads to five- to tenfold decreases in ChIP signal whereas in DSB repair, only two- to fourfold decreases are seen.³¹ Taking all of this into account, it is clear that chromatin is significantly remodeled around a DSB due to INO80, but the amount of true histone eviction that occurs is still cloudy.

Once HRR has been activated, there is a defined set of enzymatic reactions that yields the final product. Within the process of homology search including strand invasion, RAD51 is capable of the reaction *in vitro* without any histone remodelers even in the presence of histones³² and no histone modifications have been associated. *In vitro*, the yeast SWI/SNF chromatin-remodeling complex enhances the process specifically in heterochromatin.²⁹ This indicates that although RAD51 is capable of performing the essential steps in the HRR mechanism, there are steps in certain situations that require further chromatin processing to be efficient in the nuclear environment. Otherwise, the chromatin regulation associated with basic processes of HRR is still unclear to date.

Finally, the resolution of HRR requires that the chromatin structure be restored. This process is still being elucidated. The most well-described portion of resolution is the dephosphorylation of γ -H2AX and this is consistent in both HRR and NHEJ. This process is mediated by protein phosphatase 2A (PP2A) in mammalian cells and phosphatase Pph3 in yeast or PP4C in mammals.³³ Inactivation of these phosphatases leads to sustained γ -H2AX and inefficient repair. It is clear that these factors are responsible for the resolution of γ -H2AX, but the activation of this process remains undescribed.

The removal of the acetylation events on H3 and H4 described earlier requires multiple histone deacetylases (HDACs), which are activated in the latter portion of the repair process and facilitate the condensation of chromatin back into its predamage configuration. The specific pathways are reviewed by Huertas *et al.*¹¹

The chromatin methylation events H3K79me and H4K20me are a result of the opening of the chromatin structure. It is reasonable to assume that this process is reversed by reformation of the original chromatin structure and these methylations being reburied within the tertiary structure. It is also possible that there are other factors that facilitate the binding and reformation of HP1 and KAP-1 structures associated with H3K9me3, but these have yet to be described.

In the resolution of HRR, chromatin must also be replaced in its original configuration. This is accomplished by the process of chromatin assembly, though it is somewhat controversial because, as already described, it is not completely clear whether the chromatin is truly evicted or not. In the case where chromatin is evicted, it must be reestablished.

Biochemically, two factors have been implicated in this process: chromatin assembly factor 1 (CAF-1) and antisilencing factor 1 (Asf1). CAF-1 is present at DSBs and is likely responsible for the insertion of H3.³⁴ Further, CAF-1 and Asf1 interact and it is proposed that Asf1 is recruited to sites of DNA damage in this fashion. Asf1 also stimulates acetylation of free H3 on K56 via the HAT Rtt109 and this modified H3 inserted into the DNA flanking the repaired DSB is the signal for completion of repair.³⁵

Overall, HRR is a complicated process that requires significant alterations to the chromatin structure to be efficient. In review, this includes insertion of variants of H2; modifications to all of the core histones and their variants including the production of phosphorylation, acetylation, and ubiquitylation, and the recognition of methylation; and the structural remodeling of chromatin in both the initiation and completion of HRR.

II. Histone Modifications of NHEJ

NHEJ is an error-prone pathway for repairing DNA DSBs. As with homologous recombination (HR), the first modification that occurs after DNA damage is the phosphorylation on histone H2Ax on Ser139, resulting in γ -H2Ax. This modified histone recruits the Ku70/80 complex to the DNA ends where it binds to the ends and protects the DNA ends from being degraded as well as acting as a mark of the DSB. The MRN complex is then brought into the DSB. Together these two complexes then recruit the DNA ligase IV/XRCC4 complex to religate the ends and repair the break. There are two main barriers for efficient and effective DNA repair by NHEJ: rapid recruitment of DNA repair proteins to the site of the DSB; and ease of access to this damage. Chromatin packed with nucleosomes forms a barrier to these DNA repair proteins so certain structural issues have to be overcome to allow access for the DNA repair proteins.

Many studies have been carried out on the function of γ -H2Ax and its role for marking a DSB and recruiting DNA repair proteins but there are other histone modifications that have been identified that may be involved in the NHEJ repair pathway. These modifications include methylation (me), acetylation (ac), phosphorylation (p), and ubiquitination (ub) of histones H2A, H2B, H3, and H4. These histone modifications have been postulated to have two functions: they can either mark the site of a DNA break to help recruit the DNA repair proteins or they can be involved in changing and opening the chromatin structure so that the DNA repair proteins can gain access to the DSB. As will be discussed, histone modifications do have one or both functions in NHEJ.

A recent study identified dimethylation of lysine 36 on histone H3 (H3K36me2) as being a mark of DNA damage and subsequent recruitment of NHEJ proteins.³⁶ *In vitro* studies have identified Metnase, a DNA repair protein with a histone methylase SET domain, as being the methylase for this histone H3 residue.³⁷ Using a model system that allows the generation of a single DSB in a cell to study NHEJ, it was shown that Metnase could indeed dimethylate H3K36 *in vivo* at/around a DSB, and that the presence of this histone modification both recruited and stabilized DNA repair proteins at the DSB. Moreover, the levels of H3K36me2 were proportional to the efficiency of the repair. Therefore, this suggests that H3K36me2 is in a category with phosphorylated H2Ax and ubiquitylated H2A in that they are all DNA damage-induced modifications that lead to recruitment of DNA repair proteins and can then improve the efficiency of DNA repair.^{38–42}

Many histone modifications have been identified as being related to or marking sites of active transcription. One such modification is H3K4me3. This modification is carried out by Set1p methyltransferase and is found in newly created DSBs.⁴³ The recruitment was mediated by the RSC chromatin-remodeling complex, and monoubiquitylation of histone

H2B (H2BK123ub) was also required for the methylation of H3K4. When H3K4me3 was not present, there was a decrease in NHEJ activity, suggesting that H3K4me3 is modulating chromatin at the site of DNA damage.

In addition to methylation and phosphorylation, acetylation of histones at sites of DNA damage can also assist in the repair process. Acetylation by HATs is an important and critical chromatin modification in DNA repair. Histones H3 and H4 are acetylated on their N-terminal lysines (K5, K8, K12, K16) after DNA damage and these modifications are essential for NHEJ.⁴⁴ In addition, NuA4-Tip60 HAT acetylates histone H4 at DSBs and improves the repair of the DSBs.^{13,25,44} Chromatin structure and nucleosomes create a barrier to DNA repair proteins and acetylation of the histones facilitates the relaxation of chromatin and nucleosome repositioning. The SWI/SNF2 superfamily of remodeling complexes (INO80, SWR1, SWI/SNF, and RSC) is also involved in this relaxation and repositioning. INO80, SWR1, and RSC complexes are also necessary for Ku70/80 to be recruited to the DSB.^{26,45} The RSC complex localizes to the DSB and also interacts with and recruits Mre11; then ATPase remodeling occurs which opens the chromatin and allows NHEJ proteins to gain access to the DSB.⁴⁶ CBP and p300 acetylate histones H3 (K18) and H4 (K5, K8, K12, K16) after recruitment to the DSB, and then cooperate with the SWI/SNF complex to recruit Ku70/80.⁴⁷ These studies together suggest that histone acetylation at a DSB is important for the recruitment of NHEJ proteins mainly via chromatin relaxation and nucleosome repositioning while at the same time being important for the recruitment of the early-response NHEJ proteins.

In many studies, a single modification or type of modification appears to be important for enhancing NHEJ repair of DSBs. However, some studies suggest that there may be a combination of modifications in a specific order on a single histone protein. In yeast, acetylation of histone H4 on K16 (H4K16ac) is important for NHEJ.⁴⁸ This modification is removed by the Sin3p/Rdp3p HDAC complex. Histone H4 is also phosphorylated on serine residue 1 in response to DNA damage and this is important for NHEJ and inhibits the acetylation of H4 by NuA4.^{49,50} These studies indicate that there are a series of coordinated histone modifications that occur for DNA repair. H4K16ac by NuA4 relaxes the chromatin structure on the region surrounding the DSB; then this is removed by Sin3p/Rdp3p, which is then followed by the H4Ser1 phosphorylation that inhibits the reacetylation of H4K16 by NuA4.⁵¹

γ -H2Ax is phosphorylated after DNA damage. It can be phosphorylated by ATM and by DNA-dependent protein kinase (DNA PKcs), a protein important in NHEJ. It has been shown that DNA PKcs can phosphorylate H2Ax within the nucleosome environment. Studies are conflicting as to whether this phosphorylation is influenced by histone acetylation.^{52,53} Phosphorylated H2Ax does not affect nucleosome conformation but it does affect nucleosome stability as well as impairing histone H1 binding.⁵³

The ubiquitylation of histones can also regulate DNA damage responses. The pattern appears to be primarily monoubiquitylation rather than polyubiquitylation. It appears that ubiquitylation of specific histone residues is required prior to methylation on other histone residues.^{43,54} H2BK123ub is also required for H3K36 and H3K79 methylation.

There are multiple histone modifications that can occur to aid in this DNA repair pathway. From the studies, it appears that there is cross talk between different modifications, and even a specific order of modifications that is followed. Much more work needs to be done to further define these modifications in mammalian systems and to understand the way they relate to enhancement or inhibition of the NHEJ DNA repair pathway.

III. Histone Modifications of Nucleotide Excision Repair

The nucleotide excision repair (NER) pathway acts to remove DNA double-helix distorting lesions, including cyclobutane pyrimidine dimers and 6-4 photoproducts, produced by ultraviolet (UV) light, as well as several kinds of bulky adducts induced by chemical agents such as cisplatin and 4-nitroquinoline oxide, which interfere with base pairing and block DNA duplication and transcription. NER is divided into two subpathways: global genomic NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER acts mainly on damage in nontranscribed regions of DNA, whereas TC-NER acts on damage in actively transcribed DNA. The first step of the NER reaction, the recognition of the DNA lesion, differs considerably between the GG-NER and TC-NER subpathways. In GG-NER, the xeroderma pigmentosa group C (XPC)–hHR23B complex (human homolog of yeast RAD 23) is responsible for the crucial damage sensing and repair recruitment step. This step also regulates the rate at which NER is carried out, while in TC-NER, the first signal for repair activity seems to be the blockage of transcription elongation by RNA polymerase II in front of DNA lesions.⁵⁵ The NER machinery is thought to be recruited to the stalled RNA polymerase II by the TC-NER-specific “coupling” factors CSA and CSB.⁵⁶ The subsequent steps in GG-NER and TC-NER are similar to each other and to those in NER in prokaryotes. XPB and XPD, which are subunits of transcription factor TFIIH, have helicase activity and unwind the DNA at the sites of damage. XPG protein has a structure-specific endonuclease activity, which makes an incision 3′ to the damaged DNA. Subsequently, the XPF–ERCC1 complex makes the 5′ incision during the NER. The dual incision leads to the removal of an ssDNA with a single-strand gap of 25–30 nucleotides. The resulting gap in DNA is filled by DNA polymerase δ or ϵ by copying the undamaged strand. Proliferating cell nuclear antigen (PCNA) assists the DNA polymerase in the reaction, and replication protein A (RPA) protects the other DNA strand from degradation during NER. Finally, DNA ligase seals the nicks to finish NER.

Histone acetylation is an important event in NER. Several investigators have shown that treatment of human cells with sodium butyrate (an inhibitor of HDACs), stimulates the initial rate of NER *in vivo* and appears to correlate with an increase in the highest acetylated form of histone H4.^{57–59} These authors have also shown that DNA repair synthesis occurring early after UV irradiation in mammalian cells is significantly enhanced in hyperacetylated mononucleosomes and seems not to result from increased UV damage in hyperacetylated chromatin.^{57–59}

A study has shown that the TATA-box-binding protein-free TAF-containing complex (TFTC), a Gcn5-containing HAT complex and a transcriptional coactivator, is involved in the NER pathway.⁶⁰ TFTC has also been previously reported to acetylate histone H3 both *in vitro* and *in vivo*.⁶¹ These authors demonstrated that TFTC shares strong homology with a

subunit of UV-damaged DNA-binding factor DDB1, which is recruited to UV-induced DNA lesions *in vivo*.⁶⁰ TFIIH binds preferentially to naked UV-damaged DNA and to nucleosomes assembled on UV-damaged DNA *in vitro*. Moreover, TFIIH preferentially acetylates nucleosomes assembled on UV-damaged DNA templates *in vitro*.⁶⁰ A more recent study also demonstrated that UV irradiation leads to a dramatic increase in H3 acetylation *in vivo*.⁶² As TFIIH acts as coactivator in transcription, it might be associated mainly with TC-NER.

There have also been studies showing that other transcription factors take part in the acetylation process of histones as well, thereby leading to NER. It was recently discovered that the E2F1 transcription factor accumulates at sites of UV-induced DNA damage and directly stimulates NER through a nontranscriptional mechanism by associating with the GCN5 acetyltransferase in response to UV radiation and thus recruiting GCN5 to sites of damage. UV radiation induces the acetylation of histone H3 lysine 9 (H3K9) and this requires both GCN5 and E2F1.⁶³ Moreover, as previously observed for E2F1, knockdown of GCN5 results in impaired recruitment of NER factors to sites of damage and inefficient DNA repair. These findings demonstrate a direct role for GCN5 and E2F1 in NER involving H3K9 acetylation and increased accessibility to the NER machinery.⁶³

Histone methylation occurs on lysine and arginine side chains but does not alter the charge on histone protein as done by acetylation and phosphorylation. Not much research has been done to find the role of histone methylation in NER in humans, but one study has shown that lysine methylation in histones is required for efficient NER in *S. cerevisiae*.⁶⁴

Although phosphorylation is important for some histones and DNA repair pathways, there are still no studies which clearly prove some role of histone phosphorylation on NER. One study showed that histone H2A phosphorylation controls Crb2 (a cell cycle checkpoint protein) recruitment at DNA breaks, maintains checkpoint arrest, and influences DNA repair in fission yeast.⁶⁵ Another study on human primary fibroblasts proposed that histone H2AX phosphorylation occurs after UV-induced NER starts operating.⁶⁶

As described for other DNA repair pathways, monoubiquitylation of histones happens in NER. UV-induced monoubiquitylation of H2A is dependent on functional NER and occurs after incision of the damaged strand.⁶⁷ However, although this modification has been shown to occur at UV, it still has to be seen how this modification affects the process of NER.

IV. Histone Modifications of Base Excision Repair

Base excision repair (BER) is the primary DNA repair pathway that corrects base lesions that arise due to oxidation, alkylation, deamination, and depurination/depyrimidination damage. BER facilitates the repair of damaged DNA via two general pathways: short and long patch. The short-patch BER pathway leads to a repair tract of a single nucleotide. Alternatively, the long-patch BER pathway produces a repair tract of at least two nucleotides. The BER pathway is initiated by one of many DNA glycosylases, which recognize and catalyze the removal of damaged bases. The completion of the BER pathway is accomplished by the coordinated action of at least three additional enzymes. These downstream enzymes carry out strand incision, gap filling, and ligation.

There are only a few studies published about covalent histone modification in BER pathways. Though several repair factors involved in BER pathways, such as Fen1, DNA-polymerase- β , and TDG, are *in vitro* and *in vivo* substrates for phosphorylation and acetylation,^{68–72} phosphorylation, methylation, or acetylation of histones in BER pathways has not been reported so far.

However, for BER, there are many studies that indicate that histones are covalently modified by mono(ADP)-ribose in response to DNA damage. It has been shown that when cells were exposed to damage by $\cdot\text{OH}$ radicals or methylating/alkylating agents, total covalent mono(ADP ribosyl)ation of histones increased by factors of 5–12, while the levels of histone H1-linked mono (ADP-ribose) groups were elevated even more than 30-fold.^{73–75} Initial reports suggested that histone H1 is covalently mono(ADP ribosyl)ated at E2, E14, E116, or R34, and histone H2B at E2.^{76–79} In addition, some other studies have shown that mono(ADP ribosyl)ation occurs also at glutamic acid residues of H2A and at arginine residues of H3 and H4.⁸⁰ Interestingly, butyrate exposure elevated basal levels of histone mono(ADP ribosyl)ation on H4 but reduced subsequent mono(ADP ribosyl)ation of histones initiated by DNA damage,⁸¹ suggesting an antagonistic cross talk of histone mono(ADP ribosyl)ation and histone acetylation in BER pathways. Still, no nuclear mono(ADP ribosyl) transferase responsible for these modifications has been identified so far in higher eukaryotic cells. Because of this missing link, many authors, especially in the poly(ADP ribose)polymerase field, are quoting these modifications as poly (ADP ribosyl)ation. Interestingly, a recent study showed evidence that SIR2-like proteins (a family of β -nicotinamide adenine dinucleotide (NAD)-dependent HDACs) may function as mono(ADP-ribose) transferases in these processes.⁸² SIR2-like proteins have been shown to take part in a wide range of cellular events, including chromosome silencing, chromosome segregation, DNA repair, DNA recombination, and the determination of life span.⁸³ TbSIR2RP1, a SIR2-related protein from the protozoan parasite *Trypanosoma brucei*, has been shown to catalyze mono(ADP ribosyl)ation of histones, particularly H2A and H2B, *in vitro*.⁸² Under- or overexpression of TbSIR2RP1 decreased or increased cellular resistance to oxidizing DNA damage, respectively.⁸² Remarkably, treatment of trypanosomal nuclei with a DNA-alkylating agent resulted in a significant increase in the level of histone mono(ADP ribosyl)ation, in particular H2A and H2B, and a concomitant increase in chromatin sensitivity to micrococcal nuclease.⁸² Both of these responses correlated with the level of TbSIR2RP1 expression. Moreover, these studies supported previous evidence for a link between deacetylation and mono (ADP ribosyl)ation.

V. DNA Mismatch Repair and Histone Modifications

DNA mismatch repair (MMR) is a highly conserved DNA repair system, which is essential to all organisms. It has an important role in maintaining genomic instability. MMR targets mismatches that arise during replication and homologous recombination (HR), as well as repairing mismatches that occur in DNA following treatment with alkylating agents.^{83,84} In terms of disease, a loss of the MMR pathway is associated with an increase in genomic instability and has been linked to hereditary nonpolyposis colorectal cancer.^{85,86}

MMR has been well characterized in bacteria, yeast, and mammalian cells. In mammalian cells, the MMR pathway involves two heterodimers called MutS and MutL. MutS heterodimers contain MSH2 complexed with either MSH6 or MSH3. MutL heterodimers contain MLH1 complexed with either PMS2 or MLH3. In addition to these two complexes, a number of other proteins are required including RFC, EXO1, RPA, and PCNA. MutS heterodimers are responsible for “sensing” or recognizing the mismatch followed by recruitment of the MutL heterodimer to the chromatin.⁸⁷ The two heterodimers then bind ATP and undergo a conformational change, resulting in a protein that can more freely diffuse along the DNA in either direction. This suggests that the MutS–MutL complex may be functioning as a “sliding clamp” on the DNA. The MutS–MutL complex diffuses across the region containing the DNA mismatch and meets either RPA at the 5′ terminus or PCNA at the 3′ terminus. After this, it loads and activates EXO1 to degrade the faulty strand until the mismatch is removed. Finally, DNA polymerase δ fills the gap and DNA ligase I completes the process.

MMR is strongly associated with replication, a process that induces significant chromatin remodeling and has a number of associated histone modification events.^{88–90} However, it is not yet fully defined as to whether MMR actually requires any specific chromatin remodeling or any histone modifications. Most biochemical studies have been carried out on naked DNA substrates so the effect of chromatin on this process is not well studied.⁹¹ However, studies using the yeast genome have demonstrated that MMR has different efficiencies in different regions, which is suggestive of chromosome structure being an influence on MMR.⁹²

DNA modifications resulting from treatment with alkylating agents are known to activate MMR, but there are subtle differences depending on the dosage. High doses lead to a G2 cell cycle arrest after one round of replication and PCNA is no longer present in the chromatin-bound MutS–MutL complex, but with low doses, the same effects are seen only after the second round of replication.⁸⁷ This latter effect might be a method of maintaining the MMR complexes on the chromatin longer such that other proteins can be recruited and activated.

Although aberrant DNA methylation has been shown to be responsible for the silencing of MMR genes, studies on hypoxia and stem cells have suggested that epigenetic chromatin inactivation is responsible for silencing the MMR genes MLH1 and MSH6.⁹³ Chromatin immunoprecipitation identified hypoacetylated/hypermethylated histone H3 lysine 9 (H3K9me3) as being present, which was associated with impaired SP1 binding to the promoters of these genes. Treatment with HDAC inhibitors increased histone H3K9 acetylation, which in turn increased SP1 activity on the promoters, and hence increased expression of MLH1 and MSH6. Therefore, a deregulated MMR pathway may result in genomic instability in stem cells, thus leading to their malignant transformation into cancer stem cells.

The MMR pathway operates within the nucleosomal environment but the impact of nucleosomal organization on MMR or vice versa is not understood. Nucleosomes are highly stable protein–DNA complexes that sterically block other DNA-binding proteins from

accessing the chromatin. This could be a hindrance to the diffusion of the MutS–MutL heterodimers. A recent study has shown that the MMR complexes can cause disassembly or disruption of the nucleosome.⁹⁴ Acetylation of histone H3 lysine 56 within the nucleosomes both reduces their affinity for DNA and enhances their disruption by the diffusing MMR complexes. This disruption is passive as it requires only binding but not hydrolysis of ATP. Therefore, the MMR complex carries out two functions: it specifically recognizes mismatched DNA; and it creates a nucleosome-free environment to allow other DNA repair proteins access when the MMR sliding clamp performs passive DNA lesion-dependent chromatin remodeling.

While histone modifications have not been fully elucidated for the efficacy or activities of MMR, recent studies do suggest that chromatin/histone modifications are important in the MMR response to DNA damage.

As seen from this overview, there are multiple modifications that can occur on all histones, including phosphorylations, acetylations, methylations, and ubiquitylations. These modifications are common to all the DNA repair pathways. However, ribosylation appears to occur only for the DNA BER pathway. For the base excision and the mismatch repair pathways, there are few studies showing a link between histone modifications and the function of those pathways, but recent studies suggest that there is some role for histone modifications and their activity, so more studies should be carried out. Histone modifications appear to have many functions. They can mark the site of damage and recruit specific DNA repair proteins to the damage. They can also change the conformation of the histone such that it results in the opening of the chromatin around the DSB to allow better access for the DNA repair proteins. There can also be cross talk between some histone modifications to regulate the repair process, from opening the chromatin to allow repair to occur to closing the chromatin again once the DSB is fully repaired. There are also modifications that occur that activate different repair pathways. The repair pathway that becomes activated may therefore be a combination of the histone modification and the type of damage that has happened—DSB versus an interstrand cross-link versus a mismatched base. Further studies should be able to determine which histone modifications are important and how cross talk between them affects the function of these DNA repair pathways.

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Fig. 1.

(1) DNA bound to chromatin tightly compacted and maintained by Hp1 and KAP-1. (2) DSB occurs and MRN is recruited to DSB displacing Hp1 and KAP-1, revealing H3K9me3. (3) Tip60 and ATM are recruited to H3K9me3. Tip60 acetylates and activates ATM. ATM induces γ -H2AX and autophosphorylates. (4) MDC1 is recruited to γ -H2AX, ATM is activated and extends γ -H2AX, Tip60 acetylates H3/4. (5) MDC1 expands and binds to γ -H2AX which recruits a plethora of repair factors in conjunction with acetylated and methylated histones. Multiple steps are then ready to proceed, including HRR end resection, chromatin remodeling and the rest of the HRR mechanism.