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REVIEW

Histone Modifications and DNA Double-Strand Break Repair after Exposure to Ionizing Radiations

Clayton R. Hunt, Deepti Ramnarain, Nobuo Horikoshi, Puneeth Iyengar, Raj K. Pandita, Jerry W. Shay and Tej K. Pandita[1]

University of Texas Southwestern Medical Center, Dallas, Texas 75390

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Ionizing radiation exposure induces highly lethal DNA double-strand breaks (DSBs) in all phases of the cell cycle. After DSBs are detected by the cellular machinery, these breaks are repaired by either of two mechanisms: (1) nonhomologous end joining (NHEJ), which re-ligates the broken ends of the DNA and (2) homologous recombination (HR), that makes use of an undamaged identical DNA sequence as a template to maintain the fidelity of DNA repair. DNA DSB repair must occur within the context of the natural cellular DNA structure. Among the major factors influencing DNA organization are specific histone and nonhistone proteins that form chromatin. The overall chromatin structure regulates DNA damage responses since chromatin status can impede DNA damage site access by repair proteins. During the process of DNA DSB repair, several chromatin alterations are required to sense damage and facilitate accessibility of the repair machinery. The DNA DSB response is also facilitated by hierarchical signaling networks that orchestrate chromatin structural changes that may coordinate cell-cycle checkpoints involving multiple enzymatic activities to repair broken DNA ends. During DNA damage sensing and repair, histones undergo posttranslational modifications (PTMs) including phosphorylation, acetylation, methylation and ubiquitylation. Such histone modifications represent a histone code that directs the recruitment of proteins involved in DNA damage sensing and repair processes. In this review, we summarize histone modifications that occur during DNA DSB repair processes.

INTRODUCTION

Ionizing radiation (IR) induces many types of DNA damage but the most lethal lesions are DNA double-strand breaks (DSBs), the repair of which is critical for cell survival. One of the major challenges of radiotherapy related repair research is the lack of mechanistic and structural details about DNA damage repair as it occurs in the natural context of the cell, i.e., within chromatin. Chromatin, the physiological packaging structure of histones and DNA, is now gaining appreciation as a relevant regulator of multiple signaling pathways (*1, 2*). The spatial and temporal control of DSB repair may be critically dependent on histone modifiers and specific histone modifications (*3–6*) as many reports link chromatin structure to IR sensitivity (*7–20*). It has been suggested that the packing and accessibility of DNA in chromatin are major factors influencing IR sensitivity (14). Consistent with this proposition is that cells derived from individuals with ataxia-telangiectasia are radiation sensitive and have a higher rate of conversion of DNA DSBs into chromosome breaks postirradiation (*17, 18, 20*). Cells deficient for ATM have an increased frequency of chromosome aberrations that has been attributed to an altered chromatin status is observed at both the global chromatin level as well as specifically in telomeric chromatin (*16, 21–24*). It has also been reported that DNA damage renders chromatin more sensitive to micrococal nuclease digestion (*25*) and leads to chromatin decondensation at the local as well as global level (*26, 27*). Such chromatin structural alterations are an essential requirement for activation of the DNA damage response (DDR) and subsequent DSB repair. Recent studies have begun to reveal that eukaryotic cells orchestrate a complex array of responses to sense DNA damage (*28*) and these responses during DNA repair specifically involve chromatin structure alterations. This review will summarize the status of histone modifications in relationship to modulation of the DDR and DSB repair.

[1]Address for correspondence: Department of Radiation Oncology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX, 75390; e-mail: tej.pandita@utsouthwestern.edu.

Chromatin Structure

The basic repeating unit of chromatin, the nucleosome, consists of approximately 146 bp of DNA wound around an octamer of histone proteins that includes two molecules each of histones H3, H4, H2A and H2B (Fig. 1) (*29*). Histone-DNA interactions within the nucleosomes can be transiently broken by ATP-dependent nucleosome remodelers (*30*) and such histone-DNA interactions are regulated by enzymes through acetylation, methylation, phosphorylation and ubiquitination of specific amino acid residues (Fig. 2). Histone post-translational modifications usually function to recruit specific proteins to chromatin, the identity of which is determined by the type of post-translational modifications and the specific histone residue that is modified (*31*). Damaged DNA can be fully exposed by total removal of histones from DNA, a process mediated by histone chaperones (*32*). Thus histone modifications can influence DNA damage responses during signaling as well as during the opening and restoration of chromatin to its original native state. In addition to the post-translational modifications occurring after DNA damage, the initial state of histone modifications prior to DNA DSBs induction can also influence the DDR.

Role of Pre-Existing Histone Modifications in DDR

Histone modifications have been shown to play a primary role in DDR by facilitating repair protein access to DNA breaks (*20, 33–35*). Several studies indicate that pre-existing histone modifications play an important role in DDR. For example, repair proteins (53BP1, *Schizosaccharomyces pombe* Crb2 [SpCrb2] and *Saccharomyces cerevisiae* Rad9 [ScRad9]) require methylated histone H3 Lys79 (H3K79) (*36*) or methylated histone H4 Lys20 (H4K20) and/or CBP/p300-mediated acetylation of histone H3 lysine 56 (H3K56) (*36–40*) for focus formation at DNA-damage sites. All three of these histone modifications are normally present on chromatin and do not change in response to IR-induced DNA damage. Most histone acetylation modifications do not change appreciably after genotoxic stress, however, some studies have indicated that histone H3 acetylated at K9 (H3K9ac) and H3K56ac are rapidly deacetylated in response to DNA damage in human cells (*41*). Whether preexisting modifications can influence DNA damage responses at the level of signaling, chromatin relaxation or opening and recruitment of repair proteins, and the importance of restoring chromatin to the original native state has just begun to be recognized. In human cells, histone modifications such as H2AX S139 (phosphorylation), H4K16 (acetylation), H3K79 and H4K20 (methylation) have been linked with damage signaling; H3K9, H4K16 and H2A(X) are linked with chromatin opening and modifications like H4S1 and H2B S14 (phosphorylation), H2AX S139 (dephosphorylation), H3 K14, K23, K56 and H4 K5, K8, K12, K16, K91 (acetylation), H3/H4 Ks (deactylation), and H2A K119

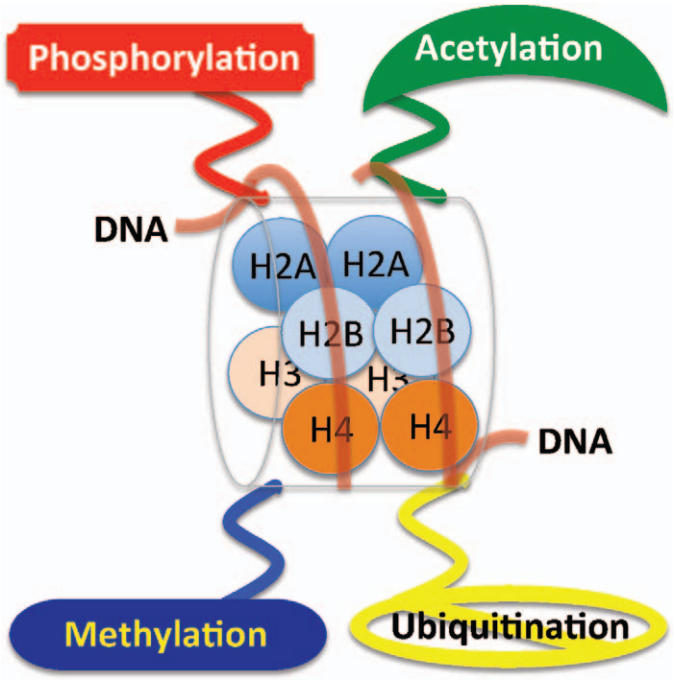

FIG. 1. Structure of eukaryotic nucleosome consisting of DNA wound in sequence around four histone protein cores with covalent modifications of phosphorylation, acetylation, methylation and ubiquitination.

(mono-ubiquitination) have been associated with chromatin restoration (*42*). The following is an example of how a pre-existing histone modification impacts DNA damage responses and DSB repair after irradiation. The amino-terminal tail of histone H4 is a well-described target for posttranslational modification, including acetylation (*4, 19, 82*). Transcriptionally active gene regions have histones that are post-transcriptionally modified and these regions are thought to have preferential DNA DSB repair. In support of this, studies in yeast of HO endonuclease induced DSBs generated at the ADH1 and MAT-loci have shown that DNA repair is coupled to transcription. Faster DSB repair at the highly active ADH1 locus compared to the nearly silent MAT locus suggests preferential DSB repair at active genes *in vivo* (*43*).

Direct evidence linking histone modification and transcription comes from the examination of males absent on the first (*MOF*) gene function, which is a highly conserved histone acetyl-transferase that specifically acetylates histone H4 at K16 (*44*). Acetylation of H4K16 is prevalent in *Drosophila* on the hyperactive male *Drosophila* polytene X chromosomes (*45*), where it contributes to transcriptional upregulation (*46*) although in yeast, H4K16ac does not correlate with active genes (*47*). However, all K5, K8 and K12 acetylation marks on histone H4 are linked with enhanced transcription (*48*). Among the 4 histone H4 acetylation sites, the H4K16ac modification poses a structural constraint on the formation of higher-order chromatin (*49*). Characterization of histone H4K16 acety-

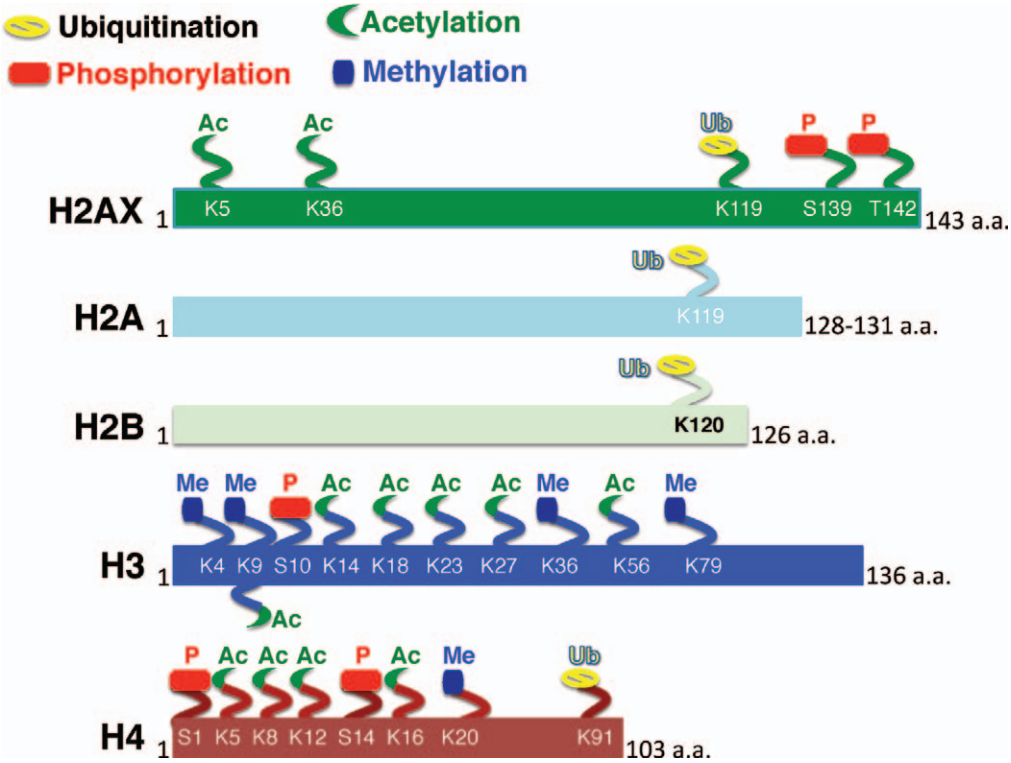

FIG. 2. Major types of histone modification of specific histone residues linked with the DNA damage response (DDR) and DSB repair.

lation function in mammalian cells by generating mutations has been difficult but both chemical and genetic approaches have validated its role in the DNA damage response where it potentially serves as a platform structure to generate proper signaling for DDR (*50*). MOF, as well as H4K16 acetylation, localizes on the X chromosome of male *Drosophila*, which has almost a twofold greater transcription level when compared to the X chromosomes of female *Drosophila*. Depletion of MOF in human cells reduced H4K16ac levels and such cells have reduced levels of ATM activation (*51*) as well as defective appearance of postirradiation γ-H2AX foci (*50*). Additional studies using either the deacetylase inhibitor trichostatin A or deletion of the SirT2 gene have also confirmed that H4K16ac levels are critical for a timely DDR. Furthermore, Sharma *et al*. (*50*) observed a relationship between H4K16ac levels and DNA damage responses in differentiated HL60 cells where reduced H4K16ac levels and a decreased frequency of γ-H2AX foci per cell following irradiation are observed, supporting the concept that the initial levels of H4K16ac are critical for sensing the DNA damage.

In addition to histone acetylation, chromatin contains other histone modifications that could impact DDR. The best studied histone modifications that occur post IR exposure or after induction of DNA DSBs are listed in Fig. 2 and Table 1. Histone modifications have been detected on serine/threonine residues as phosphorylation, on lysine as acetylation, methylation or ubiquitination modifications, and on arginine as methylation. Except for ubiquitination, these modifications alter histone/DNA electrostatic interactions and ultimately change chromatin dynamics and function by altering access of cellular factors involved in DDR. How histone modifications (Fig. 3) occur and influence repair of DSBs are summarized in the next section.

Histone Phosphorylation

One of the first histone modification events linked with the DNA damage responses is H2A phosphorylation (*52*), specifically the H2A variant H2AX (phosphorylated H2AX is referred as γ-H2AX), which occurs within minutes after exposure to ionizing radiation. H2AX phosphorylation is carried out mostly by ATM or ATR and is thought to modify higher order chromatin structure at the DNA damage site. Phosphorylation of H2AX is critical for signaling/repair protein recruitment to DNA damage sites since IR induced formation of γ-H2AX foci is rapid, precedes repair factor assembly into repairosome foci and is required for subsequent foci formation by 53BP1, NBS1, BRCA1 and MDC1 [reviewed in ref. (*53*)] (Fig. 3). Loss of H2AX increases genomic instability, possibly because γ-H2AX physically interacts with NBS1, 53BP1 and MDC1, further supporting the critical role of γ-H2AX in DDR [reviewed in ref. (*53*)]. H2AX phosphorylation occurs in all phases of the cell cycle, consistent with IR-induced ATM activation occurring in all phases of the cell cycle (*54*). Thus H2AX phosphorylation is required for both DNA DSB

TABLE 1

Histone	Residue	Modifier	Role in DDR	References
Acetylation				
H2AX	K5	TIP60	Helps in K119ub of H2AX and removal of γ-H2AX	(*104*, *105*)
H2AX	K36	CBP/p300	Recruits Ku during NHEJ	(*88*)
H3	K9, K14, K23, K27	GCN5, CBP/p300	Recruits SW1/SNF, promotes spreading of γ-H2AX domain	(*76*)
H3	K18	GCN5, CBP/p300	Regulates Ku protein recruitment during DSB repair	(*75*)
H3	K56	GCN5, CBP/p300 RTT109	Depletes after IR to promote NHEJ, enriches K56ac after HR repair	(*106*)
H4	K5, K8, K12, K16	MOF, TIP60, TRRAP, CBP/p300	Recruits DDR/repair proteins and SW1/SNF nucleosome remodeling complex	(*50*, *51*, *67*)
Phosphorylation				
H2AX	S139	ATM, ATR, DNA-PKcs	Recruits and accumulates DDR proteins (MDC1) to the repair lesion and promotes histone acetylation.	(*52*, *64*, *99*, *107*, *108*)
H2AX	T142	WSTF	Recruits activated ATM and MDC1.	(*109*, *110*)
H4	S1	CK2	Role in DDR	(*60*, *61*)
H4	S14	CK2	Promotes NHEJ by 53BP1 recruitment and methylation of V(D) J recombination via RAG complex	(*111*)
Methylation				
H3	K4me3	SET1	Stimulates V(D) J recombination via RAG complex	(*112*, *113*)
H3	K9me3	Suv3-9H1/Suv3-9H2	Interacts with HP1β, phosphorylates damage induced HP1β and activates TIP60	(*114*, *115*)
H3	K36me2	Metnase/SETMAR	Accumulates NBS1 and Ku to stimulate NHEJ	(*116*, *117*)
H3	K79me3	DOT1	Recruits RAD9 in budding yeast	(*118*)
H4	K20me2	Suv420H1/Suv420H2, MMSET	Recruits DDR and repair proteins	(*39*, *101*, *119*)
Ubiquitination				
H2AX	K119ub/ K119ub2, K119poly-ub	RNF8, RNF168, TIP60-UBC13	Recruits DDR proteins to the repair lesion	(*84*, *120*, *121*)
H2A	K119ub/ K119ub2, K119poly-ub	RNF8, RNF168	Accumulates BRAC1 and 53BP1 for DNA repair	(*105*)
H2B	K120ub	RNF20-RNF40	Recruits XRCC4 and Ku for NHEJ and BRCA1 and RAD51 FOR HR	(*111*)
H4	K91ub	BBAP	Induces H4K20me modification and recruits 53BP1 to promote NHEJ	(*93*)

repair pathways e.g., NHEJ and HR (Fig. 3). For example, phosphorylation of H2AX facilitates damage site recruitment of the DDR component MDC1, during HR, which binds to γ-H2AX via its BRCT domain (*55*). γ-H2AX-recruited MDC1 is phosphorylated by ATM and also by casein kinase 2 (*56, 57*). Furthermore, binding of MDC1 to γ-H2AX is modulated by MOF dependent H4K16 acetylation (*58*). As shown in Fig. 3, H2AX phosphorylation facilitates the recruitment of SW1/SNF and RSC remodeling complexes and several other repair proteins including RAD16, CSB/RAD26, RAD5 and RAD54 that belong to the SWI2/SNF2 family of helicases involved in DNA repair (*4*). As summarized in Table 1, H2B phosphorylation at Ser14 and N-terminal phosphorylation of H4 also occur as part of the DDR (*59*). Both of these histones are abundant, present in close proximity to double-strand breaks (*60, 61*), and are modified by sterile 20 kinase (Mst1) (*62*) and casein kinase 2 (*60, 61*). Most of phosphorylation events occurring on histone H2AX and H2A have been linked with the sensing and opening of the DNA damage sites while H4 modification has been linked with chromatin condensation.

Histone Acetylation

The second most common histone modification linked with both transcription and DNA damage repair is histone acetylation. Acetylation neutralizes positively charged lysine residues, thus altering chromatin fiber intra- and internucleosomal interactions to facilitate chromatin decondensation and enhance access to nucleosomal DNA (*63, 64*). The major acetylated histone residues, the modifying

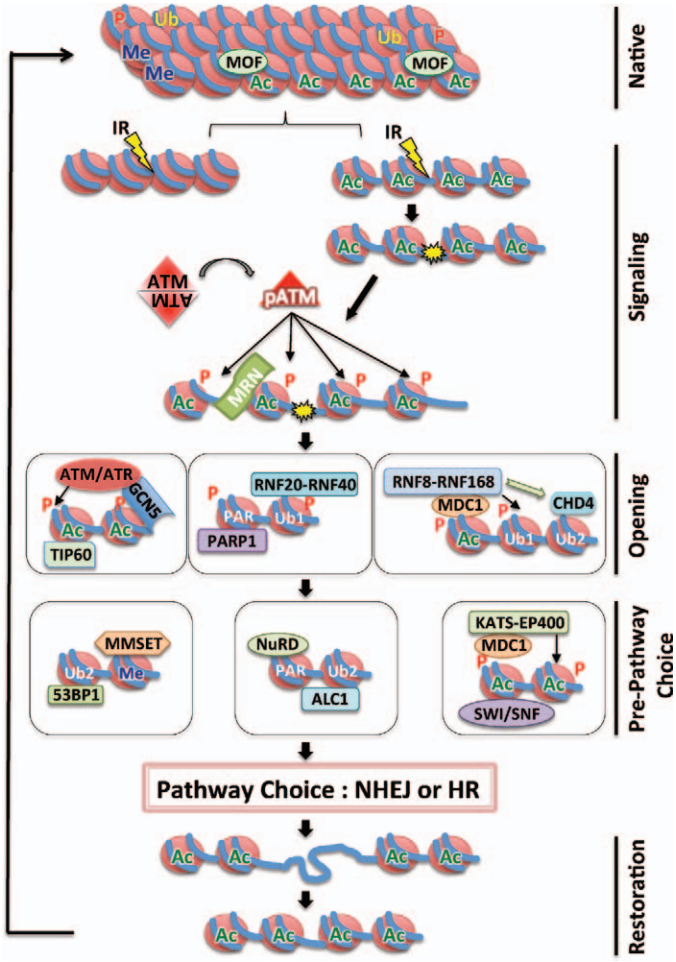

FIG. 3. Role of histone modifications in response to ionizing radiation induced DSB sites. As described in the text, in response to IR DNA DSB induction, the histone modifications facilitate the relaxation of chromatin structure that triggers ATM kinase activation that phosphorylates H2AX enabling recruitment of MDC1 and other proteins. Histones (red) are surrounded by DNA (blue). Subsequent histone modifications, and the associated modifiers involved in sensing the damage and restoration of the native structure are also shown.

enzymes and the function of the histone modification are summarized in Table 1 and Fig. 3. Acetylation is a dynamic histone marker regulated by the balance between histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs and HDACs transfer an acetyl moiety from acetyl-coenzyme A to the ε-amino group of lysine and remove the acetyl-group, respectively (*65*). Histone acetyl transferases are of two types, type-A acetylate histones in the nucleus whereas type-B enzymes acetylate cytoplasmic histones. HATs of type-A are responsible for chromatin dynamics in the nucleus and belong to GNAT superfamily and MYST family. Individual well-characterized HATs are MOF, TIP60, GCN5, KATS-EP400, SW1/SNF and p300/CBP. Histone H3 and H4 acetylation increases interactions with transcriptional machinery components containing bromodomains, H3 lysine 9 methylation allows heterochromatin protein 1 (HP1) binding via the chromodomain to chromatin, thereby blocking DNA binding of the transcription machinery. Acetylation at the H3 and H4 N termini and at H3K56 plays a critical role in DNA metabolism involving DNA replication, genomic stability and in the binding of the chromatin assembly factor (CAF1)-PCNA complex (*66*). The N-terminal tail of histone H4 is acetylated at K5, K8, K12 and K16, and H4K16ac is observed on the *Drosophila* male X chromosome (*45*) in relationship to control of gene dosage compensation and has also been implicated in the chromatin structure responsible for interaction of other proteins (*49*). The HAT responsible for histone H4 acetylation at K16 is MOF (*50, 51, 67, 68*) and acetylation at H4K16 has been implicated in the proper compaction of chromatin 30-nm fibers (*49*). Interestingly, MOF depletion also influences ATM functions (*51*) like delayed appearance of IR-induced γ-H2AX foci (*50*), thus providing more direct evidence for the role of histone modifications in the activation and function of DDR components. Since histone H4K16 acetylation levels correlate with ATM activation, HDAC inhibitor treatment results in global ATM activation even in the absence of DNA damage (*69*), supporting the argument that the histone modifications direct regulatory pathways sensing and processing DNA damage for repair. The current understanding of histone acetylation is still rudimentary and the field would be advanced if the acetylated residues could be mutated for analysis of their functional role in DDR.

Histones H3 and H4 are reportedly acetylated on the N-terminal lysines during repair by the NHEJ pathway [reviewed in ref. (*3*)]. At DSBs, histone H4 is acetylated by NuA4-TIP60 and this modification has been linked with improved DSB repair (*70, 71*). Similarly, pre-existing H4K16ac, prior to IR exposure, influences repair as cells with decreased levels of H4K16ac have a reduced efficiency of DNA DSB repair by NHEJ (50). For recruitment of Ku70/80 at DNA DSB sites, the necessary histone modifications are carried out by the INO80, SWR1 and RSC complexes (*72, 73*). At DSBs, the RSC complex recruits Mre11, which is followed by ATPase-dependent remodeling to facilitate access to the site by proteins required for NHEJ mediated repair (*74*). Histone H3 and H4 acetylation by CBP and p300 cooperate with the SW1/SNF complex to facilitate recruitment of NHEJ proteins such as Ku70/80 (*75*). Thus histone acetylation occurs during the initial stages of NHEJ to facilitate the chromatin opening and subsequent access of repair proteins to the DNA DSB sites. During HR mediated repair, a number of acetylation events occur on histones H3 and H4 with the proteins implicated in the modification being GCN5, NuA4 and HAT1 (*71, 76*). GCN5 also participates in pathway choice for DSB repair as DNA-PKcs phosphorylates GCN5 to inactivate its HAT domain. In addition, GCN5 also interacts with BRCA1 through a mechanism that is dependent upon its HAT activity implicating a role in HR repair of DNA DSBs (*77*).

Histone Ubiquitination

Similar to highly conserved DNA damage repair process, ubiquitylation is also a tightly regulated process involving the enzymatic activity of E1, E2 and E3 (*78, 79*). Ubiquitylation is among the more unique forms of post-translational modification in that a single ubiquitin monomer can be polyubiquitylated through one of seven lysines or through the amino terminus to create polyubiquitin chains. Ubiquitination conjugates a 76 amino acid protein, ubiquitin, to the lysine ε amino group of specific proteins as a result of which ubiquitin molecule acts to regulate protein function and stability by altering the activity of its target in a variety of ways, e.g., changing its localization or enzymatic activity to targeting it for degradation. For example, ubiquitination of H2A at K119 by the E3 ubiquitin-protein ligase RNF2 or RING2 is associated with transcriptional repression via subsequent binding of the polycomb repressive complex (*80, 81*) (Fig. 3) and is stimulated by RING finger domain containing proteins such as BMI-1 and RINGIA (*82*). In addition, BMI-1, RINGIA and RINGIB are also involved in DSB-associated H2A ubiquitination (*83*). Ubiquitination of nuclear histones occurs after irradiation by RNF8 and RNF168, which catalyze formation of lysine 63 linked polyubiquitination chains on histones H2A and H2AX (*84, 85*). As shown in Fig. 3, RNF8 is rapidly recruited to the sites of DNA damage in an MDC1-dependent manner through its functional FHA domain and RNF8 is required to recruit subsequent repair factors (*86, 87*). Although polyubiquitination generally results in protein degradation, RNF8 catalyzed ubiquitin modification does not because RNF8/UBC 13-mediated polyubiquitin synthesis produces a lysine-63 linkage, rather than the lysine-48 canonical signal for protein degradation. While RNF8 mediated ubiquitination has a role in maintaining genomic integrity, the role of postdamage monoubiquitylation in chromatin reassembly remains unclear (*88*). Another important ubiquitination event during DDR is performed by BRCA1 E3 ligase activity, which promotes BRCA2 recruitment that in turn promotes RAD51 recruitment during DNA strand resection in HR repair (*89*). BRCA1 is localized to DSBs through an interaction with a repair protein complex containing RAP80, a protein that contains tandem ubiquitin interaction motifs (*90–93*). The domains of ubiquitin interaction motifs recognize K63-linked ubiquitin over K48-linked structures, suggesting that BRCA1 modification would be functioning in nondegradative ubiquitin roles (*92*). Recent studies have begun to reveal that specific ubiquitination markers are linked with specific DSB repair pathways. The UBC13 E3 ligase seems to be required for homologous recombination because cells lacking UBC13 are defective in break resection as determined by RPA recruitment to DNA DSBs (*94*). These observations suggest a DSB-associated ubiquitin requirement during the initial stages of repair. The major histone sites of ubiquitination (*95*), the enzymes required and the role of the modification in the DNA damage response are summarized in Table 1 and Fig. 3.

Histone Methylation

More than forty years ago, histone methylation on lysine and arginine (*96–99*) was discovered (*100*), but, in contrast to acetylation, histone methylation does not alter the charge of arginine and lysine residues. Thus methylation does not directly modulate the nucleosomal interactions required for chromatin restructuring. Histone methylation is crucial for proper programming of the genome during development but the recent identification of histone demethylases such as LSD1/AOF2, JMJD1, JMJD2 and JHDM1 has also indicated methylation is reversible and also provides a rationale for a role in DNA DSB repair. Specific methylation DNA sites linked to the DNA damage response are summarized in Table 1. Methylations at multiple sites on H3 and H4 have been reported (mono- di- and trimethyl groups per residue) including K4, K9, K27, K36, K79 and R2, R8, R17, R26 for H3 and K20 and R3 for H4. The source of the methyl group required by histone methyl transferases is S-adenosyl-methionine. SET domain containing proteins (Dm Su(var)3–9), Enhancer of zeste (E(z)) and trithorax (trx) methylate lysine, while methylation of arginine is performed by coactivator arginine methyltransferase (CARM1) and arginine methyl transferase (PRMT1). In contrast to histone acetylated site detection by bromodomain containing proteins, methylated sites are detected by proteins containing a chromodomain motif. In mammals, dimethylation of histone H4 lysine 20 (H4K20me2), is mediated by the histone methyltransferase MMSET (also known as NSD2 or WHSC1) (*101*), which does not seem to increase globally after DNA damage, however, it is critical for recruitment of 53BP1 at DSBs (*101*) and increases in vicinity to DSBs. Interestingly, MMSET depletion significantly decreases H4K20 methylation at DSBs as well as 53BP1 accumulation at DSBs. It has been shown that MMSET recruitment to DSBs requires γ-H2AX-MDC1 and involves the interaction between the MDC1 BRCT domain and phosphorylated Ser102 of MMSET (*101*). These observations suggest a pathway involving γ-H2AX-MDC1-MMSET regulates the induction of H4K20 methylation on histones around DSBs, which, then facilitates 53BP1 recruitment. Pre-existing chromatin modifications at the histone level can directly affect the initial DNA damage response and subsequent pathway choice for NHEJ and HR. Modifications such as H4K16ac or H4K20me2, regulated by MOF and MMSET respectively, are epigenetic markers that could act as a histone code since the absence of appropriate markers results in the loss of a DDR during DSB repair. Metnase, a methylase which dimethylates histone H3 residue K36 (H3K36me2) in the DNA DSB region, and the levels of H3K36me2 have been found to correspond positively to DSB repair efficiency [reviewed in ref. (3)]. Another modification, H3K4me3, carried by Set1p

methyltransferase has been found at DSBs and the absence of this modification has been linked to defective DNA DSB repair (*102*).

Conclusion and Future Directions

The major protein components of chromatin are histones, which are subject to many types of posttranslational modifications especially on their flexible N-terminal tails. Based on the presence of specific interactions between histone modifications and proteins involved in cellular metabolism, these modifications may constitute a ''histone code'' and could be used to manage epigenetic information that determines the selection of DSB repair pathway choice. The pre-existing histone code for DNA damage responses may be critical for generating the damage identification signal and such a code could have implications for the recruitment of repairosome factors specific to particular pathways. In the future it will be important to establish the relative impact of epigenetic changes in the context of specific DNA sequences and their role in DNA DSB repair. The modulation of histone acetylation with HDAC inhibitors has already been shown to alter the response to IR exposure resulting in some instances to radioprotection while in other situations radiosensitization (*103*). This indicates that small molecule modulation of histone post-translational modifications is a potential route to enhance radiotherapy responses. As with other areas of basic research, a detailed mechanistic understanding of chromatin modifications (e.g., the histone code) that regulate gene expression and the DDR will help to develop effective patient specific radiotherapies based on their own tumor epigenetic phenotype.

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