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The histone DNA repair code: H4K20me2 makes its mark

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Abstract

Chromatin is a highly compact structure that must be rapidly rearranged in order for DNA repair proteins to access sites of damage and facilitate timely and efficient repair. Chromatin plasticity is achieved through multiple processes, including the posttranslational modification of histone tails. In recent years, the impact of histone posttranslational modification on the DNA damage response has become increasingly well recognized, and chromatin plasticity has been firmly linked to efficient DNA repair. One particularly important histone posttranslational modification is the methylation of H4K20. Here we discuss the regulation and function of H4K20 methylation (H4K20me) in the DNA damage response: we describe the *writers*, *erasers*, and *readers* of this important chromatin mark, and discuss combinatorial histone posttranslational modifications that modulate H4K20me recognition. Finally, we discuss the central role of H4K20me in determining whether DNA double-strand breaks are repaired by the error-prone nonhomologous DNA end joining or error-free homologous recombination pathways.

Keywords

H4K20me2; DNA DSB repair; histone posttranslational modification; homologous recombination; nonhomologous DNA end joining

Introduction

Chromatin is a highly organized and condensed structure that allows billions of base pairs of DNA to be tightly packaged into the nuclei of eukaryotic cells. The basic subunit of chromatin is the nucleosome, an octamer of histones around which 146 bp of DNA is wrapped 1.7 times. Each nucleosome contains two copies each of histones H2A, H2B, H3, and H4. Histones are highly conserved amongst eukaryotes, emphasizing their importance (1). Chromatin cannot be a rigid and unchanging structure, however. It is highly dynamic in order to facilitate DNA replication, transcription, and repair. Chromatin plasticity is a necessity, as without it, DNA interacting proteins would not be able to access this tightly condensed structure. Chromatin plasticity is facilitated by nucleosome repositioning, histone exchange, and the post-translational modification (PTM) of histone tails. Nucleosome repositioning involves the physical sliding of nucleosomes along the DNA or their eviction.

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In histone exchange, histone variants are substituted for the canonical histones H2A, H2B, H3 or H4. For example, H2A can be substituted with the variant H2AX upon the formation of DNA double-strand breaks (DSBs) (2). Histone PTM is the addition of small molecules, such as acetyl-, methyl-, and phospho-groups, or small proteins, such as SUMO (small ubiquitin-like modifier) and ubiquitin to the tails of histones, which extend from the core nucleosome. These PTMs change chromatin structure in several ways, for example, by modulating the strength of histone-DNA interactions, and by facilitating the recruitment of chromatin *reader* proteins and/or chromatin remodeling complexes, which can lead to marked changes in chromatin structure and compaction. Single and combinatorial PTMs can have distinct signaling and cellular outcomes. Combinatorial marks add to the variability and complexity of chromatin recognition and plasticity (3,4). In this review, we will discuss one aspect of chromatin plasticity, namely histone PTM. Specifically, we will focus on the dimethylation of histone H4 lysine 20 in mammalian cell lineages and how this particular PTM has become increasingly recognized as a major determinant of DNA repair. For more comprehensive reviews of chromatin plasticity and DNA repair, please refer to the following excellent reviews (5–7).

DNA Double-Strand Break Repair

DNA damage can arise as a result of endogenous agents, such as reactive oxygen species, a byproduct of normal cellular processes, or by exogenous means, such as exposure to UV light. DNA damage must be repaired in an efficient and timely manner in order to continue normal cellular processes like replication and transcription. While there are many distinct types of DNA damage, here we will focus on DNA double-strand breaks (DSBs). DSBs arise upon cellular exposure to ionizing radiation and as a consequence of replication fork collapse. DSBs can also arise transiently during DNA repair processes, including nucleotide excision repair and interstrand crosslink repair (8). Upon DSB formation, free ends of broken DNA are recognized by the MRN (MRE11-RAD50-NBS1) complex, which recruits the ATM (ataxia telangiectasia mutated) kinase (9,10). ATM phosphorylates a histone variant called H2AX on serine 139, forming γ H2AX (11,12). γ H2AX was one of the first recognized histone PTMs, and has been extensively studied in relation to DSB repair (11). MDC1 (mediator of DNA damage checkpoint 1) recognizes γ H2AX *via* its BRCT (BRCA1 C-Terminus) domain (13). MDC1 subsequently recruits additional molecules of ATM *via* its FHA (forkhead-associated) domain; ATM phosphorylates additional H2AX molecules thereby amplifying the γ H2AX signal up to two megabases proximal to the DSB site (13–15) (Figure 1A). As one of the first steps in DSB repair, H2AX phosphorylation is widely used as a marker for DSB formation.

DSBs are repaired by one of two ways: homologous recombination (HR) or non-homologous DNA end joining (NHEJ). HR is an error-free repair pathway that uses a homologous DNA sequence as a template to repair damaged DNA (16). HR is a cell cycle-dependent pathway, occurring primarily during S-phase due to the presence of homologous DNA in the sister chromatid. Briefly, upon γ H2AX phosphorylation, the MRN complex, CtIP (CtBP-interacting protein), EXO1 (exonuclease 1), and DNA2 (DNA replication/helicase protein 2) all promote 5'–3' DNA end resection, resulting in the generation of 3' single-stranded overhangs on each strand (ssDNA) (17–21). The ssDNA overhangs are first

coated by RPA (replication protein A) to protect against nucleolytic degradation. The major DNA strand recombinase, RAD51, is subsequently loaded onto ssDNA in a process facilitated by functional homologs of the yeast Rad52 epistasis group and the BRCA2 protein (22–24). RAD51 forms a nucleoprotein filament coating the ssDNA and a displacement loop (D-loop) is formed upon invasion of the ssDNA into the complementary sister chromatid duplex, referred to as the synaptic complex (22–26). New DNA is then synthesized using the sister chromatid as a template, and Holliday junctions (branched heteroduplex DNA intermediates comprising newly synthesized DNA on the invading strand and the template strand) are resolved, resulting in a duplicate of the sister chromatid (gene conversion), with no loss of genetic information (16) (Figure 1B).

Conversely, NHEJ is typically an error-prone pathway that simply ligates the free ends of broken DNA. NHEJ occurs in all phases of the cell cycle, and can result in catastrophic events such as deletions and translocations. In brief, again MRN is recruited to DSBs after damage. Additionally, 53BP1 (p53 Binding Protein 1) is recruited to DNA damage sites, and blocks HR proteins and end resection (27,28). Ku70 (Lupus Ku auto antigen p70) and Ku80 (Lupus Ku auto antigen p86) recognize the DSB ends and together with DNA-PKcs (DNA-dependent protein kinase catalytic subunit), Artemis, XRCC4 (X-ray repair cross-complementing protein 4), and LIG4 (DNA ligase 4), directly ligate the free ends of the DSB (29–33) (Figure 1C). Often, there is no specificity for which ends are ligated, which can result in translocations if ends from two previously noncontiguous DSBs are rejoined (34,35).

There are multiple factors that influence the decision to repair DSBs by HR or NHEJ, including cell cycle stage. Two proteins that play a major role in this decision are BRCA1 and 53BP1. While the MRN complex plays a key role in both HR and NHEJ, its binding partners determine whether BRCA1 or 53BP1 becomes loaded onto DSB ends (36). Chromatin factors that regulate the recruitment of BRCA1 and 53BP1 will be discussed in greater detail later in the review. Depletion of Brca1 results in increased NHEJ and decreased HR (37). Depletion of both Brca1 and 53bp1, however, restores normal levels of HR in mice (28). This, along with evidence that 53BP1 physically blocks end resection, indicates that BRCA1 may play a role in the removal of 53BP1 from DSBs, allowing HR to proceed (36).

H4K20me2

KMT5A—Prior to H4K20 di- or tri- methylation, H4K20 must first be monomethylated (38–40). The enzyme responsible for H4K20 monomethylation is KMT5A (Aliases: SET8, SETD8, PR-SET7) (lysine methyltransferase 5A), a SET-domain (Su(var)3–9, Enhancer-of-zeste and Trithorax) containing methyltransferase (Table 1). KMT5A, similar to all methyltransferases, is referred to as a *writer* of chromatin marks. It has recently been shown that KMT5A prefers the entire nucleosome as its substrate, rather than individual H4 histones or peptides, and that it interacts with H2A and H2B in order to monomethylate H4K20 (41–44). Loss of Kmt5a in both fly and mouse results in embryonic lethality (45,46). Studies have shown that with knockout of Kmt5a, H4K20 di- and tri-methylation are down regulated (45). In HeLa cells, KMT5A knockdown results in reduced 53BP1 recruitment to

DSBs (47,48). Additionally, Kmt5a knockout embryonic stem cells and KMT5A depleted HeLa and U2OS cells display increased DSBs and γ H2AX formation, even in the absence of exposure to DNA damaging agents (45,49,50). This is likely an accumulation of spontaneous DNA damage throughout the cell cycle, that remains unrepaired due to lack of H4K20 methylation (45). KMT5A depleted U2OS cells have increased cell cycle checkpoint activation, decreased cell cycle progression, and accumulate in S-phase, also in the absence of DNA damage (50).

KMT5B/C—The H4K20me2 mark has been shown to be involved in DNA repair. This histone mark is found throughout the nucleus, however it has been reported to be enriched at sites of DNA damage (51). Globally, Kmt5b (Aliases: Suv4-20h1, SUV420H1) and Kmt5c (Aliases: Suv4-20h2, SUV420H2) are responsible for H4K20 di- and tri-methylation, respectively (52). KMT5B/C has been shown to catalyze dimethylation more efficiently than trimethylation *in vitro* (38,39,53). This suggests that additional proteins may be necessary for efficient H4K20 trimethylation, or that another HMT catalyzes this reaction (38,52). While *in vitro* studies show that SMYD3, a SET domain containing methyltransferase, is capable of recognizing H4K20me2 and catalyzing the addition of an additional methyl group, depletion of KMT5B/C results in complete lack of H4K20me3 (52,54). KMT5B and KMT5C are unique in that they have leucine and cysteine substitutions in the two conserved tyrosine residues that regulate substrate specificity in other SET domain containing methyltransferases. However, KMT5B and KMT5C still maintain the characteristic SET-domain structure (38,39). Like many methyltransferases, they require a SAM (S-adenosyl-L-methionine) cofactor to donate a methyl group to the substrate (38,39,52). These enzymes regulate H4K20 dimethylation, and knockdown of Kmt5b/c in fly and kmt5b/c^{-/-} double knockout MEFs results in decreased H4K20me2/3 (but not H4K20me1) (53). Cells depleted of KMT5B/C and kmt5b/c^{-/-} double knockout MEFs exhibit delayed 53BP1 foci formation, one of several proteins that binds H4K20me2 (53,55,56). The Kmt5b/c methyltransferases have also been linked to DNA repair and genome instability, and play a role in telomere length maintenance and the regulation of heterochromatin compaction. Kmt5b/c^{-/-} double knockout mice also experience perinatal lethality (55). Kmt5b/c^{-/-} null MEFs show decreased cell cycle progression, increased sensitivity to DNA damaging agents, altered chromatin structure and increased chromosomal aberrations, indicating that Kmt5b/c plays an important role in maintaining genome stability (Figure 2A).

MMSET—Recent studies have also implicated the MMSET (Multiple Myeloma SET Domain Containing Protein) HMT as a *writer* of H4K20 dimethylation. Specifically, MMSET has been shown to catalyze the dimethylation of H4K20 locally at sites of DSBs and to promote the recruitment of 53BP1. Accordingly, HeLa cells depleted of MMSET lack H4K20me2 enrichment at DSBs and are defective for 53BP1 foci formation (51,57). MMSET is known to bind to γ H2AX and MDC1 in order to localize to damage sites (51). However, multiple groups have since brought to light more evidence that KMT5B/C are primarily responsible for H4K20 dimethylation and 53BP1 foci formation (56,58). It is important to note that the majority of KMT5B/C studies have been performed in mouse and fly models, while the MMSET studies were performed in transformed cells. Further studies are required to clearly define the contributions of each HMT to DSB repair. It remains

possible that MMSET and KMT5B/C catalyze H4K20 dimethylation under different cellular conditions or in a tissue-specific context. KMT5B/C is likely to be responsible for *writing* the bulk of genome-wide H4K20me2 while MMSET may be responsible for localized enrichment of H4K20me2 at DSBs under specific conditions.

Cell cycle regulation—Studies have shown that about 80% of histone H4 is dimethylated on lysine 20 at any given time (59). Recently, it has been shown that H4K20me2 is most abundant during G1 phase, and is diluted 2-fold during S phase as new, unmodified histones are deposited onto nascent DNA. H4K20me2 levels are subsequently restored during G2 phase upon expression of KMT5A. Decreased levels of H4K20me2 during S phase are highly likely to impact DNA repair pathway choice. Consistently, restoration of H4K20me2 levels during G2 phase coincides with 53BP1 nuclear foci formation (60).

H4K20me2 Binding Proteins

53BP1—Several proteins are known to bind to H4K20me2. Proteins that bind to chromatin marks are referred to as chromatin *readers*. 53BP1 is a major *reader* of H4K20me2 (61). 53BP1 is a large protein and is known to promote NHEJ. More specifically, the balance between 53BP1 and BRCA1 dictates whether NHEJ or HR occur during S and G2 phases, and evidence shows that 53BP1 must be removed from DSBs in order for HR to proceed. This is facilitated by the chromatin remodeler SMARCA1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1) (see H2AK127ub section) (62). 53BP1 first recognizes γ H2AX via its BRCT domains, and then subsequently binds to H4K20me2 using its tandem Tudor domains, which are comprised of β -sheet folds (63–67). The binding affinities of the 53BP1 tandem Tudor domains for H4K20me2 and H4K20me1 are 19.7 and 52.9 μ M, respectively. They show an affinity of >1 mM for unmodified and trimethylated H4K20. Disruption of the 53BP1 tandem Tudor domains results in loss of H4K20me2 binding and loss of 53BP1 foci formation (61). 53BP1 also contains a UDR (ubiquitination-dependent recruitment motif), which mediates binding to H2AK15ub (see below) (68). Importantly, disruption of the 53BP1 UDR also results in loss of 53BP1 foci formation and impaired NHEJ (68) (Figure 2A).

JMJD2A—JMJD2A (Jumonji domain-containing protein 2A) is also a H4K20me2 *reader*, and similar to 53BP1, binds to H4K20me2 *via* its tandem Tudor domains. The function of JMJD2A binding to H4K20me2 remains unclear. JMJD2A is a histone demethylase with specificity for H3K9me2/3 and H3K36me2/3 (69). Proteins that remove chromatin marks are referred to as chromatin *erasers*. Upon DNA damage, JMJD2A is ubiquitinated by RNF8 (RING finger protein 8) and RNF168 (RING finger protein 168), and then degraded in a VCP (Valosin-containing protein) dependent manner. Removal of JMJD2A from H4K20me2 results in the subsequent recruitment of 53BP1 (70). These findings indicate that DNA damage not only leads to *de novo* H4K20me2 synthesis but the unmasking of this mark as well. The Tudor domains of JMJD2A bind to H4K20me2 with a K_D of 2.0 μ M, which represents an ~10-fold increased affinity over 53BP1, at least *in vitro*. Additionally, overexpression of JMJD2A abrogates 53BP1 foci formation, while knockdown of JMJD2A rescues 53BP1 foci formation in RNF8- and RNF168-depleted cells (70). These data suggest

that JMJD2A may outcompete 53BP1 for H4K20me2 occupancy and must be removed in order for 53BP1 to bind. This same study demonstrated that the catalytic JmjC (Jumonji C) domain of JMJD2A is not required for blocking 53BP1 recruitment, indicating that 53BP1 recruitment is not dependent on JMJD2A demethylase activity (70) (Figure 2B).

L3MBTL1—L3MBTL1 (Lethal (3) malignant brain tumor-like protein 1) binds to H4K20me1 and H4K20me2 *via* its triple MBT (malignant brain tumor) domains in order to condense chromatin and repress transcription (71). Upon exposure to ionizing radiation, one study found as much as a 40% decrease in L3MBTL1 signal at DSBs. It was found that RNF8 and RNF168 ligase activity was indispensable for this reduction (72). While L3MBTL1 was shown to be ubiquitinated, it has yet to be determined if it is a substrate of RNF8 and RNF168. Acs et al. also found that L3MBTL1 is degraded *via* VCP, and that L3MBTL1 ubiquitination and VCP ATPase catalytic activity are both required for the removal of L3MBTL1 from DSBs. Finally they showed that VCP is required for 53BP1 foci formation, and that RNF8 and RNF168 are required for VCP activity. The authors suggest that L3MBTL1 must be removed *via* VCP from H4K20me2 in order for 53BP1 to bind (72) (Figure 2B). Further studies into the relationship between 53BP1, L3MBTL1, and H4K20me2 are required to determine if 53BP1 binding to H4K20me2 is dependent on removal of L3MBTL1.

MBTD1—MBTD1 (malignant brain tumor domain-containing protein 1) binds to H4K20me2 *via* its four-MBT repeat domain (73,74). MBTD1 was recently identified as a component of the NuA4 chromatin-remodeling complex, which contains the histone acetyltransferase TIP60 (60 kDa Tat-interactive protein). Depletion of MBTD1 using siRNA results in persistent γ H2AX foci formation following exposure to DNA damaging agents, indicating that MBTD1 is required for the timely repair of DNA damage. Additionally, depletion of MBTD1 leads to compromised HR and increased NHEJ. *In vitro* studies showed that MBTD1 can outcompete 53BP1 for H4K20me2 binding (75). *In vivo*, depletion of MBTD1 leads to persistent 53BP1 foci formation, attributed to inefficient removal of 53BP1 after damage. This group also found that TIP60 is responsible for acetylating H2AK15 (see H2AK15ac section), which also impacts 53BP1 binding to H4K20me2, discussed in more detail below (75) (Figure 3A).

FANCD2—Our lab recently discovered that FANCD2 (Fanconi Anemia group D2) binds to H4K20me2 *via* a methyl-binding domain. FANCD2 association with H4K20me2 increases in the presence of DNA damaging agents. FANCD2 is part of the Fanconi Anemia/Breast Cancer (FA/BRCA) DNA repair pathway, which removes interstrand crosslinks (ICLs) and promotes HR. Upon ICL damage, FANCD2 is monoubiquitinated and associates with chromatin and recruits HR repair proteins (76–78). FANCD2 has been shown to associate with the MRN complex *in vivo* and *in vitro*, and loss of any of the MRN complex members results in loss in FANCD2 foci formation (79,80). FANCD2 also relies on the presence of BRCA1 for foci formation (77,81,82). FANCD2 is required for CtIP localization to DSBs, and its reduction results in reduced end resection and single strand DNA formation (83,84). FANCD2 monoubiquitination is also required for TIP60 recruitment, which acetylates H4K16 (see H4K16ac section) (85,86) (Figure 3B). Finally, FANCD2 harbors a CUE

(coupling of ubiquitin to endoplasmic reticulum degradation) domain, which binds to a currently unknown ubiquitinated substrate. Mutation of this domain results in loss of FANCD2 chromatin localization, and increased cellular sensitivity to ICL-inducing agents (87). Disruption of the methyl-binding domain results in loss of FANCD2 foci formation, and increased 53BP1 chromatin and H4K20me2 association. FANCD2 promotes HR, and indeed, disruption of the methyl-binding domain leads to increased NHEJ markers and chromosomal aberrations associated with loss of HR and repair *via* NHEJ. We speculate that FANCD2 may compete with 53BP1 for H4K20me2 binding sites in order to promote HR and restrict NHEJ. As previously mentioned, 53BP1 is a bivalent reader of histones. It is possible that FANCD2 is also recruited to histones in a bivalent manner, through recognition of H4K20me2 *via* its MBD and an ubiquitinated histone *via* its CUE domain. This may explain how FANCD2 can be recruited to specific damage sites when H4K20me2 is quite an abundant mark.

Histone Modifications that Impact Binding to H4K20me2

H2AK15ub—As previously mentioned, 53BP1 not only binds to H4K20me2 *via* its tandem Tudor domains, but also binds to H2AK15ub *via* its UDR. Disruption of the UDR results in loss of 53BP1 foci formation, indicating that both H4K20me2 and H2AK15ub are necessary for efficient 53BP1 recruitment (61,68). H2AK15 ubiquitination is catalyzed by RNF8 and RNF168, which are both required for 53BP1 accumulation at DNA damage sites (88–91). Following γ H2AX and MDC1 foci formation, RNF8 recognizes MDC1 *via* its FHA domain, and then polyubiquitinates histone H1, a histone found within linker DNA between nucleosomes (88,92). RNF168 recognizes the K63-linked polyubiquitination of H1, and subsequently monoubiquitinates H2AK15 (89–92). Loss of RNF168 results in abrogation of 53BP1 foci formation. Recognition of both H2AK15ub and H4K20me2 by 53BP1 is necessary for efficient NHEJ. Interestingly, FAAP20, (Fanconi anemia core complex-associated protein 20) which promotes FANCD2 nuclear foci formation and HR, binds to ubiquitin chains and requires RNF8 activity for its chromatin localization (93). However, the ubiquitinated substrate to which FAAP20 binds remains unknown (93,94). Finally, as previously touched upon, FANCD2 contains a CUE ubiquitin-binding domain and the ubiquitinated substrate to which it binds has yet to be determined (87). An intriguing possibility is that FANCD2 and 53BP1 may compete for bivalent recognition of both H4K20me2 and H2AK15ub.

H2AK15ac—H2A can be acetylated on lysine 15 (H2AK15ac) as well. The switch between H2AK15 ubiquitination and acetylation may also influence DNA repair pathway choice. Additionally, nucleosomes can be combinatorially modified, so that H2AK15ac co-occur on nucleosomes containing H4K20me2. The NuA4/TIP60 complex acetylates H2AK15, precluding its ubiquitination, thereby preventing 53BP1 chromatin binding. As previously mentioned, MBTD1 recognizes H4K20me2 as part of the NuA4/TIP60 complex. Analysis of MBTD1 CRISPR/Cas9 knockout clones shows a modest reduction in H2AK15 acetylation, and MBTD1 overexpression slightly increases H2AK15ac levels, however the requirement of MBTD1 for H2AK15ac needs to be more closely examined. H2AK15ac appears most predominantly in G2/M phase, potentially overlapping with HR in early G2, and continuing to evict 53BP1 throughout mitosis (75). In general, histone acetylation is

downregulated after DNA damage, however H2AK15 acetylation was shown to increase after DNA damage, indicating a specific important role of H2AK15 acetylation in DNA damage repair (75). While the authors suggest that this mark promotes HR, our understanding of the function and regulation of H2AK15ac and MBTD1 in HR is in its infancy.

H2AK127ub—As mentioned above, upon damage recognition, MRN complex recruitment, and H2AX phosphorylation, CtIP is recruited to DSBs during HR to promote DNA end resection. In BRCA1-deficient cells, 53BP1 blocks end resection, and NHEJ takes place. However, in BRCA1-proficient cells, HR is favored during S-phase (36). BRCA1 recruits its heterodimeric binding partner, BARD1 (BRCA1-associated RING-domain protein), and unknown E2 ubiquitin-conjugating enzyme(s), and then acts as an E3 ubiquitin ligase to catalyze H2AK127 ubiquitination (95). SMARCAD1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1), a member of the chromatin remodeling family of SWI/SNF proteins, then localizes to sites of damage. SMARCAD1 contains a CUE ubiquitin-binding domain, and its chromatin recruitment is dependent on both its CUE domain and BARD1 expression. It was shown that the SMARCAD1 CUE domain binds to H2A-ub fusion proteins *in vitro*, which suggests that SMARCAD1 is recruited to chromatin *via* its CUE domain binding to H2AK127ub. However this remains to be clearly established. Upon SMARCAD1 recruitment, chromatin remodeling complexes reposition and evict nucleosomes, ultimately evicting 53BP1 from DNA damage sites. MRN and CtIP then promote end resection, and HR moves forward (62). This evidence suggests that SMARCAD1 binds to H2AK127ub in order to reposition 53BP1, supporting previous work that shows that BRCA1 is involved in 53BP1 repositioning (Figure 4). Nevertheless, much remains to be determined about the dynamics, regulation and molecular function of this particular chromatin mark.

H4K16ac—In addition to catalyzing H2AK15 acetylation, TIP60 also catalyzes the acetylation of H4K16 (96). In the absence of FANCD2, or more specifically FANCD2 monoubiquitination, TIP60 nuclear foci formation and overall levels of H4K16 acetylation are markedly reduced (86). *In vitro* binding experiments show that acetylation of K16 of a H4 peptide dimethylated at K20 results in decreased 53BP1 binding (56). *In vivo*, acetylation of H4K16 blocks 53BP1 recognition and chromatin recruitment (56,86,97). How H4K16 acetylation affects other H4K20me2 binding proteins such as MBTD1 and FANCD2 remains to be determined.

Conclusions and Future Perspectives

H4K20me2 joins a growing list of histone PTMs that play a major role in the coordination of DNA repair processes. Until recently, γ H2AX was one of the few posttranslationally modified histones with a well-characterized role in the DNA damage response. However, the importance of chromatin plasticity and, in particular, histone PTMs for the orchestration of DNA repair has become increasingly well recognized. Many questions on the function and regulation of H4K20 methylation remain: For example, are the H4K20me *writers* and *erasers* differentially regulated in different tissue types? Do the different H4K20 methylation states have a role in the orchestration of loci-specific repair? In addition to H4K20

methylation, H3K9 and H3K27 methylation have also recently been shown to play key roles in the DNA damage response. Deciphering how the combinatorial modification of these marks and others coordinately contribute to DNA repair will be a considerable molecular challenge. While much remains to be answered, it is clear that the recognized roles for histone PTMs in the DNA damage response will continue to expand.

As many of the *writers*, *erasers*, and *readers* of histone PTMs are druggable targets, a greater understanding of their homeostasis is highly likely to lead to the development of more targeted and effective combination cancer chemotherapy regimens. For example, the EZH2 (enhancer of zeste homolog 2) HMT is frequently deregulated in hematopoietic malignancies, including follicular and diffuse large B cell lymphomas(98,99). Currently, there are several active phase I and II clinical trials evaluating the efficacy of the EZH2 inhibitor tazemetostat (EPZ-6438) for the treatment of B cell lymphomas and advanced solid tumors. As the *KMT5A*, *KMT5B*, and *KMT5C* genes are frequently amplified in neuroendocrine prostate cancer, pancreatic cancer, and metastatic breast cancer, inhibitors of these HMTs may also represent novel candidate chemotherapeutic agents.

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Implications

In this review article, we discuss the regulation and function of H4K20me2 in DNA DSB repair: we describe the *writers*, *erasers*, and *readers* of this important chromatin mark, combinatorial modifications that modulate its recognition, and its profound impact on DSB repair pathway choice.

Figure 1. Recognition of the double strand break (DSB), homologous recombination, and nonhomologous DNA end joining.

Upon DSB formation, MRE11, RAD50, and NBS1 recognize the free naked ends of DNA, and recruit ATM. ATM phosphorylates H2AX to form γ H2AX. MDC1 then recognizes γ H2AX, and recruits subsequent molecules of ATM, which in turn phosphorylate additional H2AX. This cascade reaches outward from the broken ends up to 2 megabases of DNA (A). After recognition of DNA damage and phosphorylation of H2AX, BRCA1, CtIP, and EXO1 are all recruited to DSBs, where they promote end resection. Exonuclease activity results in 3' ssDNA overhangs. RAD51 coats the ssDNA, and scans the sister chromatid for a

homologous sequence. BRCA2, RAD51, and its associated proteins invade the sister chromatid, forming the displacement loop. New DNA is synthesized off of the sister chromatid, and holliday junctions are resolved, repairing the broken DNA in an error free manner (B). In the absence of BRCA1, 53BP1 is recruited to DSB sites. It blocks CtIP, and therefore end resection. Ku70/Ku80 are then recruited to these sites, where they signal Artemis and LIG4 localization. The broken ends are then ligated together to repair the break (C).

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Figure 2. 53BP1 recruitment to DNA DSB breaks.

Upon damage, KMT5B/C methylate H4K20me1, forming H4K20me2. 53BP1 recognizes H4K20me2 *via* its tandem Tudor domains. Additionally, RNF8 and RNF168 facilitate the ubiquitination of H2AK15. 53BP1 recognizes H2AK15ub *via* its UDR domain. 53BP1 is a bivalent reader of modified histones (A). Prior to damage, existing molecules of H4K20me2 are occupied by L3MBTL1 (B, left) and JMJD2A (B, right), which recognize H4K20me2 *via* their MBT and Tudor domains, respectively. Upon DNA damage, RNF168 polyubiquitinates L3MBTL1 and JMJD2A, and VCP facilitates its removal and subsequent

degradation by the proteasome. This leaves free H4K20me2, which can be recognized by 53BP1.

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Figure 3. TIP60 recruitment to H4K20me2 and H2AK15 acetylation.

Upon damage, KMT5B/C again catalyze H4K20 dimethylation. MBTD1 recognizes H4K20me2 *via* its MBT domains. As part of the NuA4/TIP60 complex, it recruits TIP60, which acetylates H2AK15. This acetylation blocks H2AK15 ubiquitination, and subsequent recognition by 53BP1 (A). After damage recognition, and H4K20 dimethylation by KMT5B/C, FANCD2 reads H4K20me2 *via* its MBD. TIP60 is recruited to FANCD2, where it acetylates H4K16. This blocks 53BP1 recognition of H4K20me2 and promotes HR (B).

Figure 4. BRCA1-BARD1 and H2AK127 ubiquitination.

Upon damage, BRCA1-BARD1 are recruited to DSB sites. Together they form an E3-ubiquitin ligase, and monoubiquitinate H2AK127. SMARCAD1, which is part of the SWI/SNF chromatin remodeling complex, recognizes H2AK127ub *via* its CUE ubiquitin binding domain. SWI/SNF remodels the chromatin, and evicts/blocks 53BP1 from H4K20me2 sites, promoting HR.

Table 1.
H4K20me2 binding proteins.

Overview of H4K20-binding proteins and their roles in DNA damage repair.

Protein	Binding Partner	Role	Comments
KMT5A	H4K20	Writer	Methylates H4K20 to form H4K20me1 ⁴³
KMT5B/C	H4K20me	Writer	Methylates H4K20me1 to form H4K20me2/3 ^{52,53}
MMSET	H4K20me	Writer	Methylates H4K20me1 to form H4K20me2 ⁵¹
53BP1	H4K20me2	Reader	Blocks CtIP and inhibits end resection; promotes NHEJ ^{61,67}
	H2AK15ub		
JMJD2A	H4K20me2	Reader	Specific H4K20me2 binding function unknown; removed by VCP after DSB ⁷⁰
L3MBTL1	H4K20me1/2	Reader	Binds to repress transcription; removed by VCP after DSB ⁷¹
MBTD1	H4K20me2	Reader	Part of NuA4/TIP60 complex, which acetylates H2AK15; promotes HR ⁷⁵
FANCD2	H4K20me2	Reader	Recruits ICL repair proteins and TIP60; promotes HR ^{78,89}
RNF8 and RNF 168	H2AK15	Writer	Monoubiquitinates H2AK15 ⁹⁰
	JMJD2A		Polyubiquitinates to signal removal of JMJD2A and L3MBTL1 ^{70,71}
	L3MBTL1		
BRCA1 and BARD1	H2AK127	Writer	Ubiquitinates H2AK127; read by SMARCD1 and evicts 53BP1 ⁶²
TIP60	H4K16	Writer	Acetylates H4K16, blocking 53BP1 binding to H4K20me2 ^{56,96,97}