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Protein methylation and DNA repair

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Abstract

DNA is under constant attack from intracellular and external mutagens. Sites of DNA damage need to be pinpointed so that the DNA repair machinery can be mobilized to the proper location. The identification of damaged sites, recruitment of repair factors, and assembly of repair "factories" is orchestrated by posttranslational modifications (PTMs). These PTMs include phosphorylation, ubiquitination, sumoylation, acetylation, and methylation. Here we discuss recent data surrounding the roles of arginine and lysine methylation in DNA repair processes.

Keywords: Arginine methylation; Lysine methylation; Tudor domain; Chromo domain; P53; 53BP1; PHF20

1. Introduction

The amount of information encoded by the 20 amino acids that are incorporated into proteins by the ribosomes is enormous. After synthesis, many proteins are given a further level of complexity by posttranslational modifications (PTMs) on some of the incorporated amino acids. These modifications include phosphorylation, acetylation and methylation. The latter modification occurs predominantly on arginine, lysine and histidine residues, and is catalyzed by *S*-adenosylmethionine (AdoMet)-dependent enzymes that donate a methyl-group to the side-chain nitrogen atoms of these residues. The metabolic price of methylation is high. In the case of a reaction that is catalyzed by a kinase, the amount of metabolic energy expended is one ATP equivalent. In reactions where AdoMet serves as a methyl donor, the metabolic cost of the reaction is 12 ATP equivalents, which makes active methyl the most expensive metabolic compound on a per-carbon basis in the cell [1]. Thus, if a particular methylation event is not important to the cell, it will very likely not survive evolutionary pressure.

Amino acid side chain methylation of proteins was first reported in the mid-sixties [2–5]. With extraordinary foresight, it was proposed that protein methylation and acetylation may regulate transcription [2]. In these early studies, methylated derivatives of lysine and arginine residues were identified by incubating calf thymus nuclei with *S*-adenosyl-[^{14}C-*methyl*]-L-methionine, followed by acid hydrolysis of the labeled proteins, and elution from a cation-exchange column [4,5]. Further analysis of proteins in urine, and *in vitro* methylated brain and liver proteins clearly identified ω-N^G-monomethylarginine (MMA), ω-N^G,N^G-asymmetric dimethylarginine (aDMA) and ω-N^G,N'^G-symmetric dimethylarginine (sDMA) as methylarginine species (Fig. 1). Three forms of methylated lysine residues – monomethyllysine, dimethyllysine and trimethyllysine – were identified (Fig. 2) [6,7].

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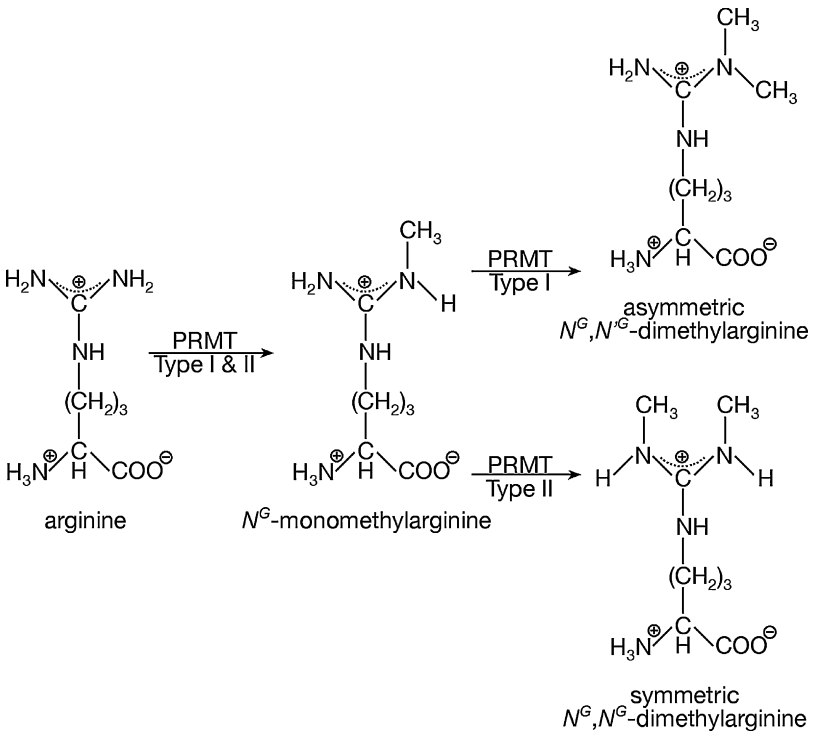

Fig. 1. The methylation of arginine residues. Arginine residues within different sequence motifs are methylated by different protein methyltransferases (PMT). The type I enzymes catalyze the formation of asymmetric N^G,N'^G-dimethylarginine residues and the type II enzyme catalyzes the formation of symmetric N^G,N'^G-dimethylarginine residues. N^G-monomethylarginine is an intermediate that is generated by both enzyme types.

Arginine methylation is a relatively common post-translational modification with about 2% of arginine residues asymmetrically dimethylated in rat liver nuclei [8]. Within the nuclear compartment, aDMA residues are further enriched in the heterogeneous nuclear ribonucleoprotein (hnRNP) fraction. About 12% of the arginine residues isolated from hnRNPs are asymmetrically dimethylated [8,9]. The tissue concentration of aDMA is always greater than sDMA and MMA [10,11]. The methylation of lysine residues has focused predominantly on the modification of histone tails—the histone code [12]. Research on arginine methylated residues has centered on non-histone proteins. However, recent work has identified a number of histone arginine residues that are methylated by CARM1, PRMT5 and PRMT1. How these histone modifications play into the histone code and epigenetics is under investigation [13]. Thus, both arginine and lysine methylation are abundant PTMs that impact protein function.

2. Arginine methylation and DNA repair

The methylation of arginine residues is catalyzed by the protein arginine methyltransferases (PRMTs) [14]. This family of enzymes contains at least nine members. The PRMTs predominantly, but not exclusively, methylate their substrates at sites that are arginine and glycine-rich (GAR motifs). These GAR motifs are methylated by PRMT1, PRMT3, PRMT5, PRMT6 and PRMT8 [14]. CARM1 (PRMT4) does not methylate

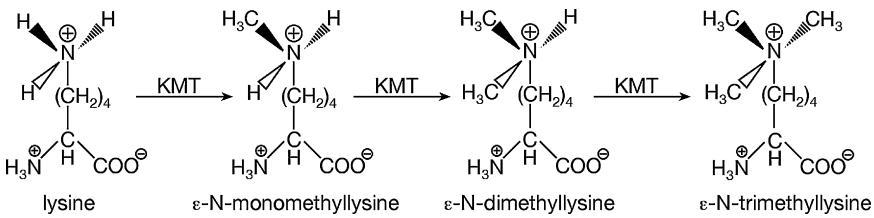

Fig. 2. The methylation of lysine residues. Lysine residues within different sequence motifs and different substrates are methylated by different SET domain containing protein methyltransferases (PMT). Lysine residues can be mono-, di-, and tri-methylated.

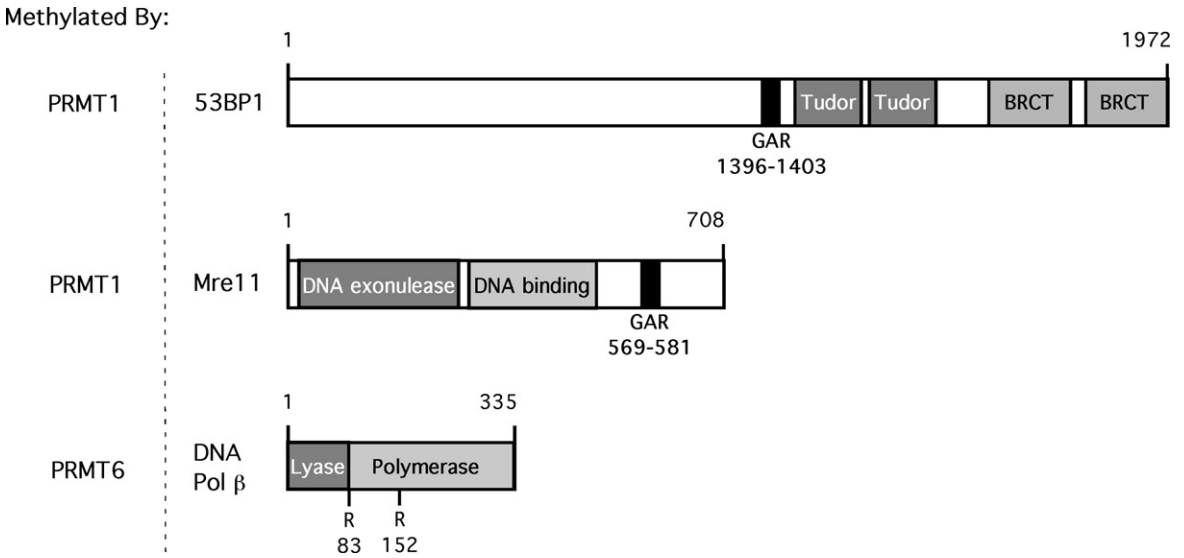

Fig. 3. Arginine methylated protein involved in the DNA damage response. 53BP1 and Mre11 are methylated in glycine- and arginine-rich regions (GAR motifs) by PRMT1. Pol-β is methylated by PRMT6, predominantly at two arginine residues (R83 and 152).

GAR motifs and possesses no obvious consensus sites, leading to slower progression in substrate identification as compared to PRMT1 substrates. Methylated proteins have been identified by serendipity [15], using candidate approaches [16–18], using screens with recombinant enzymes and radio-labeled AdoMet [19], and methyl-specific antibody detection [20]. PRMT substrates range from RNA binding proteins to histone acetyltransferases and how methylation regulates their biological function is an active research area. In addition, how PRMT's themselves are regulated is essential to understand. Importantly, no arginine demethylase has been reported suggesting arginine methylation is a PTM that remains for the life of the protein substrate. Arginine deiminases have been described that act on unmodified or mono-methylated arginine, but these enzymes are not classic demethylases as they convert arginine to citrulline and in this indirect fashion prevent methylation [21]. It may play out that arginine methylation is needed for the normal function of proteins rather than a transient signal transduction modification such as kinase–phosphatase interplay. However, identification of arginine demethylases or methyl-arginine dependent protein degradation would completely change this notion. DNA repair in the context of replication fidelity is a cellular process continuously occurring, while DNA damage caused by exogenous agents are less frequent albeit still common. Both repair processes are crucial for maintaining the genome and preventing cancer initiation and promotion. As more PRMT substrates are identified, DNA repair proteins are emerging as a group that is modified by methylation. This section will review arginine methylation of Mre11, 53BP1, and DNA polymerase β (Fig. 3), and the bearing that this methylation has on DNA repair.

2.1. *Mre11 methylation*

In higher eukaryotes DNA double strand breaks, induced by ionizing radiation or during replication, are repaired through homologous recombination repair (HRR) or non-homologous end joining (NHEJ). The mammalian Mre11/Rad50/NBS1 (MRN) complex (Mrell/Rad50/Xrs2 in yeast) plays a critical role in HRR and possibly contributes to break processing during NHEJ. Mre11 contains a nuclease domain which displays ATP dependent $3' \rightarrow 5'$ exonuclease activity, enhanced by the presence of ATPase containing Rad50. The nuclease activity of Mre11/Rad50 can also cleave DNA bound proteins, and may be important for clearing secondary structures for $5' \rightarrow 3'$ resection by recruited nucleases to form the invading $3'$ overhangs required in HRR. [22,23]. A more recently identified role of the MRN complex is activation of ataxia-telangiectasia mutated (ATM) kinase. The ATPase activity of Rad50 which stimulate DNA unwinding is required for ATM activation [24]. Specifically, the C-terminal domain of NBS1 binds ATM leading to its autophosphorylation, dimer dissociation and activation [25].

In addition to the nuclease domain, Mre11 contains a DNA binding domain and a glycine-arginine rich (GAR) motif. The first inkling that the GAR motif of Mre11 was methylated was obtained from proteomic studies carried

out in the Richard laboratory [20]. They developed arginine methyl-specific antibodies, and used these antibodies to isolate protein complexes from HeLa cell extracts that harbored arginine methylated components. One of these complexes, purified using the aDMA-specific ASYM25 antibody, was the Mre11/Rad50/NBS1 complex. Mre11 is the only protein in this complex that harbors a GAR motif and follow up studies have shown that this region of Mre11 was indeed methylated by PRMT1 *in vitro* [26]. Importantly, it was also shown that Mre11 is hypomethylated in *Prmt1*$^{-/-}$ mouse embryonic stem cells, which provides genetic evidence that PRMT1 is the enzyme that modifies Mre11 *in vivo*.

Further investigation showed that the GAR motif regulates the $3' \rightarrow 5'$ exonuclease activity of Mre11 on dsDNA [26]. The arginines within the GAR motif were mutated to alanine or lysine, and assayed for nuclease activity. R/A mutations resulted in complete loss of nuclease activity, while R/K mutations showed limited activity. Mre11 was hypomethylated in HeLa cells by treating with PRMT1 shRNA. DNA synthesis, normally stalled by damage, continued in PRMT1 shRNA treated cells, suggesting that methylation of Mre11 is important for activating the intra-S-phase checkpoint after DNA damage. Importantly, exogenous MRN complex containing methylated Mre11 significantly reduced DNA synthesis in the PRMT1 shRNA treated cells, partially rescuing the checkpoint defect. The mechanism connecting checkpoint activation and Mre11 methylation may have to do with foci formation around DSBs. Mre11 associates with PRMT1 in PML nuclear bodies, and it is thought that Mre11 is recruited to DSBs from this reservoir. In cells treated with a global methyltransferase inhibitor (MTA) followed by the DNA damaging agent etoposide, few γH2AX foci formed and Mre11 was not recruited to DSBs, implying that methylation regulates MRN relocalization to DSBs [27]. Structure analysis of the MRN complex suggests that Mre11, Rad50, and Nbs1, when in complex, form the functional DNA binding module [23]. Also, Mre11 and Rad50 work together to form the functional nuclease. The literature implies that MRN complex (with MDC1) is one of the first 'sensors' of double strand breaks [28]. The possible absence of MRN at damage sites would prevent ATM association with Nbs1, therefore decreasing activation of ATM. Activated ATM phosphorylates and therefore activates targets involved in intra-S-phase checkpoint response including Chk1 and Chk2, as well at H2AX [22]. Once phosphorylated, γH2AX helps retain proteins such as 53BP1 and MDC1 involved in DNA repair at the break sites [29]. These findings suggest that Mre11 methylation is important for MRN 'sensing' DSB's, activating ATM leading to downstream intra-S-phase checkpoint activation and repair protein retention at γH2AX foci. At this point it is unclear how arginine methylation of the GAR motif regulates $3' \rightarrow 5'$ exonuclease activity of Mre11, but there are at lease two possibilities. First, GAR motif methylation may alter the structure of Mre11, and this in turn could activate the enzymatic activity. Second, Mre11 methylation may generate a docking site for a protein that regulates its activity and localization.

2.2. 53BP1 methylation

Like Mre11 and the MRN complex, 53BP1 is also a critical protein involved in double strand break repair. Mice deficient in 53BP1 are viable, however, they display defects in *B* and *T* lymphocyte maturation and are hypersensitive to ionizing radiation [30,31]. Both these phenotypes are explained through 53BP1′s emerging role as a DSB sensor, mediator, and repair protein. Although the exact place in the repair pathway is still uncertain, 53BP1 is present at IR induced foci along with γH2AX and MDC1. Genetic studies show that γH2AX is required for 53BP1 accumulation at DSB foci, but not for its initial recruitment [32]. In addition, 53BP1 and MDC1-MRN complex work in parallel to influence autoactivation of ATM.

53BP1 contains two C-terminal BRCT domains and a kinetochore binding domain (KBD). The exact role of the BRCT domains is unclear, but similar BRCT domains of BRCA1 were shown to bind p(S/T)Q peptides *in vitro* and ATM/ATR phosphorylated substrates *in vivo* [33]. In the same study, however, the BRCT domains of 53BP1 did not bind p(S/T)Q peptides. One can speculate that the BRCT domains may function to weakly bind phosphorylated ATM substrates in order to retain foci formation until repair is completed. The KBD region containing the two tudor domains is sufficient for 53BP1 foci formation after IR, as assayed with HA-tagged deletion constructs in MCF7 cells [31]. 53BP1 has both DNA and chromatin binding abilities. *In vitro* dsDNA and ssDNA binding assays show that 53BP1 contains two independent DNA binding motifs; one containing the GAR motif, and the other containing and requiring the tandem tudor domains [31]. The chromatin binding ability of 53BP1 lies with the tudors, which bind methylated lysine residues on H3 (K79) and H4 (K20) [34,35]. DSB's may result in more relaxed chromatin structure exposing these methyl-lysine residues and thus facilitating 53BP1 recruitment.

Like Mre11 and other GAR motif containing proteins, 53BP1 is methylated by PRMT1 *in vivo* [36,37]. Methylation of 53BP1 does not increase after DNA damage and transfected 53BP1 with a mutated GAR

motif that no longer functions as a methyl-acceptor, showed undisrupted foci formation [37]. The biological relevance of methylation was investigated through analyzing the DNA binding abilities of wild type and R/A mutants. Recombinant 53BP1 with a wild type GAR sequence, or various arginine to alanine mutants of 53BP1, were incubated with ssDNA or dsDNA and subjected to an EMSA. The triple R/A mutant constructs showed severely reduced DNA binding abilities compared to the wild type peptides. However, this assay does not say for certain if the DNA binding ability was influenced by the lack of arginine methylation *per se*, or lack of arginine. Interestingly, another group showed bacterial expressed KBD (which was not methylated) bound dsDNA and ssDNA while R/K mutations reduced the binding, implying that the structural importance of arginine is greater than its presence as a methylation substrate [36]. Regardless, the GAR motif and tandem tudors are both required for recruitment of 53BP1 to sites of DNA damage [37].

2.3. DNA polymerase β methylation

Base excision repair (BER) is critical for the removal of oxidized DNA (insult of reactive oxygen species), alkylated DNA (from exogenous agents or metabolites such as *S*-adenosyl methionine), and unnatural DNA bases [38]. The most common BER mechanism occurs as follows: (i) removal of the damaged/wrong base by DNA glycosylase, (ii) cleavage 5′ to the remaining deoxyribose backbone by apurinic/apyrimidinic (AP) endonuclease, (iii) DNA synthesis to replace the nucleotide which contained the damaged base, (iv) removal of the original deoxyribose *via* lyase creating a 5′phosphate, and (v) DNA ligation of the gap. DNA polymerase β (Pol-β) is the polymerase of step (iii) and lyase of step (iv), and is thus a critical player in BER. Indeed, Pol-β null mice are embryonic lethal and MEFs derived from these knockouts are hypersensitive to DNA alkylating agents [38].

The polymerase domain of Pol-β is comprised of three subdomains including a dsDNA binding domain, nucleotidyl transferase domain and dNTP selection domain [38]. The lyase domain of Pol-β resides at the N-terminus. Recently, El-Andaloussi et al. discovered association of Pol-β lyase domain with protein arginine methyltransferase 6 (PRMT6) [39]. *In vitro* and *in vivo* methylation experiments revealed that Pol-β is indeed a substrate for PRMT6. The *in vitro* experiments were performed with GST-PRMT6 in the presence of radiolabeled *S*-adenosyl methionine. Mutational analysis showed that R83 and R152 are the predominant, although not exclusive, targets for methylation. Importantly, both these sites are exposed on the surface of the Pol-β X-ray structure, thus allowing easy access for PRMT6 [40,41]. *In vivo* methylation assays utilizing transfected HA-tagged Pol-β and myc-tagged PRMT6 demonstrated an increased in Pol-β methylation when cells were treated with methyl methanesulfonate (MMS) alkylating agent. The biological relevance of Pol-β methylation was investigated by comparing cell recovery after MMS treatment with wild type or R83/152K mutant Pol-β. *Pol-β*$^{-/-}$ cells were virally transduced with WT Pol-β, R83/152 K mutant Pol-β, or a control vector followed by MMS treatment. Cell viability 72 h after treatment revealed that WT Pol-β increased cell viability 70% over the control vector while the mutant only increased viability 55% over control, a significant reduction. Furthermore, DNA fragmentation repair post MMS treatment was significantly less in cells infected R83/152 K Pol-β compared to those with WT Pol-β (COMET assay analysis) [39]. Therefore, the arginine residues methylated by PRMT6 increase Pol-β′s activity in DNA damage repair.

Short-patch BER is the addition of one nucleotide by Pol-β, while in long-patch BER Pol-β adds up to 12 nucleotides. Some reports give credit to Pol(δ/ε) as the ‘long-patch’ BER polymerases [42]. It should be noted that Pol-β is error-prone, possesses no proofreading ability and works best on one nucleotide gaps [38]. No difference between the WT Pol-β and R83/152 K Pol-β was observed in an *in vitro* short-patch BER assay. Pol-β primer extension and processivity analysis further investigated the biological role of methylation. Extension of a 17/73-mer primer/template proceeds the best with methylated WT Pol-β, while the ‘methylated’ double mutant showed marked reduction in extension past 60 nucleotides. Thus methylation stimulated DNA polymerase activity of Pol-β. Importantly, the extension pattern between the WT and the double mutant Pol-β looked similar when PRMT6 was not added, implying that the R/K mutations effect Pol-β′s activity due to the reduction of PRMT6 methylation and not structural changes. In addition, the same primer extension assay but with DNA template as a trap showed that methylated WT Pol-β possessed better processivity than the double mutant [39]. Because methylation improves Pol-β′s elongation ability and processivity, one can speculate that hypermethylated Pol-β may work in other pathways where longer stretches of DNA are added. It would be interesting to see if methylation also improves Pol-β′s ‘error proneness’.

PRMTs methylate proteins involved in the DNA repair process thereby altering their enzymatic activity

(in the case of Mre11 and Pol-β), and perhaps influence the DNA binding affinity of 53BP1. As yet, there is no evidence that levels of arginine methylation on histone tails, in the vicinity of DNA damage, are altered.

3. Lysine methylation and DNA repair

Studies on lysine methylation have focused on histone proteins. There are a number of lysine residues on the N-terminal tails of histones H3 and H4 that are methylated. Lysine methyltransferases (KMTs) encompass a growing family of enzymes that harbor a catalytic SET (initially identified in *S*u(var), *E*nhancer of Zeste & *T*rithorax) domain. The one exception to this is Dot1, which has lysine methyltransferase activity, but does not carry a SET domain. In vertebrates, over 50 proteins contain SET domains [43]. As mentioned earlier, these enzymes have the ability to mono-, di, or tri-methylate a specific site on the histone tail, and each enzyme usually controls only one degree of methylation. The methylated histone tails recruit proteins that carry chromo, tudor, WD40, MBT or PHD domains, and the degree of methylation at a definite residue is critical for most of these interactions [35,44–46]. This section will review lysine methylation of the histone tail with a special emphasis on the H3K79me2 and H4K20me2 marks, and the two tudor domain-containing proteins that can "read" these marks—53BP1 and PHF20. In addition, one of the few identified non-histone proteins that is a KMT substrate is p53, and we will discuss the importance of this methylation event.

3.1. Stabilization of p53

Transcription factor p53 is extensively studied, justly so due to its crucial activities in tumor suppression. Approximately 50% of human tumors have mutated p53. Transcriptional targets of activated p53 include apoptotic factor BAX and G1/S checkpoint effecter p21, just to name a few. p53 is subjected to many posttranslational modifications that regulate its function and stability including phosphorylation, ubiquitination, sumoylation, and acetylation [47,48]. The E3 ubiquitin ligase, Mdm2, binds p53 targeting it for proteosomal degradation through ubiquitination of carboxy terminal lysine residues. C-terminal lysine residues can also be acetylated, shown to block ubiquitination and thereby protecting p53 from degradation [48]. Phosphorylation of p53 and MDM2 also plays a role in destabilizing their association. In 2004, Chuikov et al. found that K372 of p53 is mono-methylated by Set7/9 lysine methyltransferase [49]. Set7/9 was originally identified as a histone modifying enzyme which methylates lysine 4 on H3 *in vitro* [50,51].

The specific residue methylated by Set7/9 was identified through *in vitro* methylation on a series of deletion constructs. GST-p53 could not be *in vitro* methylated by Suv39H1, PR-Set7, or PRMT1. In addition, Set7/9 could not methylate other proteins in the same assay suggesting a specific enzyme-substrate interaction. The lysine targeted by Set7/9 is in the C-terminal regulatory domain of p53. Stable transfection of wild type or inactive Set7/9 revealed that p53 is methylated *in vivo* on the same residue. Interestingly, the amount of p53 methylated by endogenous Set7/9 increased after cells were treated with a DNA damaging agent. In addition, methylated p53 only immunoprecipitated from the nuclear fraction (using a methyl-specific antibody) while with a general anti-p53 antibody immunoprecipitated p53 equally from the nuclear and cytoplasmic fractions. Unlike arginine methylation, there are enzymes which demethylate lysines. LSD1 was the first identified demethylase that converts H3K4me2 to H3K4 and requires FAD as a co-factor [52]. Following this discovery, lysine demthylation by JHDM1 and JMJD2A proteins came to light. The JmjC domain of these proteins is the catalytic domain, requiring Fe(ii) and alpha-ketoglutarate [53,54]. A possible demethylase activity justifies lysine methylation as a means to transiently regulate protein function, which for p53 leads to transcriptional regulation. Indeed, Chuikov et al reported that stable overexpression of Set7/9 increased methyl-p53 levels and subsequently p53 target gene levels such as p21, BAX, and MDM2. siRNA knockdown of Set7/9 lead to reduced levels of p53, suggesting that methyl-p53 is nuclear and stabilized leading to increased gene transcription. Finally, apoptosis levels in cells treated with adriamycin was quantitated using Annexin V-flourescein isothiocyanate staining. U2OS cells stably transfected with wild-type Set7/9 showed increased Annexin *V* staining while those with mutant Set7/9 or wild type Set7/9 in Saos-2 cells (p53 null), did not show an increase. These data show that p53 methylation is an important PTM that regulates it stability and cellular localization. The effects methylation has on p53 may be mediated by interacting proteins that recognize methylated motifs. Candidate interacting proteins would harbor proteins domains that belong to the "Royal Family" [55].

3.2. 53BP1 recognizes a dimethyllysine-mark on histones

53BP1 is an arginine methylated protein and has been introduced in Section 2.2. The tudor domains of 53BP1

have the capacity to bind the H3K79me2 mark [34], and this study demonstrated for the first time that members of the tudor domain family do not only have methylarginine binding potential. The H3K79 methyl-mark is deposited by the DOT1L enzyme, which is an evolutionarily conserved methyltransferase [56]. Deletion mutants of Dot1 in yeast and histone H3K79 mutants are sensitive to X-ray but not UV radiation [57], and loss of Dot1 prevents activation of the yeast 53BP1 ortholog Rad9 [58]. These yeast studies indicate a key role for the H3K79 methyl-mark in DNA damage signaling. Complementary studies in mammalian cells have shown that siRNA knock down of DOT1L impairs, but does not inhibit, the recruitment of 53BP1 to DSBs *in vivo* [34]. Importantly, the H3K79me2 mark is not elevated at sites DSBs. This finding spawned the proposal that DSBs induce local changes in higher-order chromatin structure, and this in turn exposes the H3K79me2 mark and allows tudor-mediated 53BP1 docking. The Huyen et al. study only investigated the ability of the 53BP1 tudor domains to bind the H3K79me2 and H3K27me2 marks. When looking at the expanded histone code it was found that H4K20me2 is also strongly recognized by the tandem tudor domains of 53BP1 [35]. It is not clear whether this mark is elevated in response to DNA damage.

The importance of H4K20 methylation for the DNA damage checkpoint was only recently realized through work in *S. pombe* [59]. The SET domain protein, Set9, mono-, di-, and tri-methylates H4K20 in fission yeast. Loss of Set9 activity does not impair gene expression or heterochromatin function, two processes normally associated with histone lysine methylation. However, phenotypic analysis of *set9*Δ cells identified a defective DNA damage response. *set9*Δ cells are hypersensitive to DNA damage induced by ultraviolet light and ionizing radiation, and are defective in checkpoint mediated cell cycle arrest [59]. Genetic experiments have placed Set9 in the Crb2/Chk1 pathway. Importantly, Crb2 is the fission yeast ortholog of the mammalian DNA repair molecule, 53BP1. Both proteins contain two tudor domains and a BRCT domain [60]. The tudor domains of 53BP1 bind H3K79me2 [34] and H4K20me2 [35], and are necessary for localizing 53BP1 to sites of DNA double stranded breaks (DSB) [31]. Like 53BP1, Crb2 also forms foci at sites of DSB [61]. These foci are not formed after DNA damage induced by ionizing radiation in *set9*Δ cells [59]. Thus, the methyllysine mark at H4K20 is critical for normal DSB repair in *S. pombe*, and possibly also in higher eukaryotes.

The tudor domain containing protein, 53BP1, and its orthologs likely play a vital role in relaying the early DNA damage signal from the histone tails to the DNA repair machinery.

3.3. PHF20 and the MOF complex

Using a protein domain array approach it was found that the tudor domains of 53BP1 not only binds H3K79me2, as reported by Huyen et al. [34], but also interact with di-methyl marks on H3K4, H3K9, and H4K20 [35]. The tudor domain of PHF20 also binds these same marks, suggesting that 53BP1 and PHF20 may be functionally related. Surface plasmon resonance (SPR) was used to evaluate the relative affinity of the different histone methyl-marks to the tudor domains of 53BP1 and PHF20, making it clear that both these proteins bind most strongly to the H4K20me2 mark [35].

PHF20 is a scaffolding protein with MBT, PhD and tudor domains, an AT hook and a Zn finger. It was recently linked indirectly to the DNA damage response through its identification in the MLL1/hMOF complex [62]. The histone acetyltransferase, hMOF, interacts with ATM and that hMOF knockdown results in reduced ATM autophosphorylation at Ser 1981 [63]. A second report in the same vein has shown that knockdown of hMOF leads to the accumulation of cells in the G2 and M phases of the cell cycle, and these cells have an impaired repair response to ionizing radiation [64]. hMOF is responsible for histone H4K16 acetylation [62,64]. In addition, after ionizing radiation (IR) induced DSBs there is an accumulation of the H4K16ac mark, in a hMOF-dependent fashion [63]. This mark is located close to the H4K20me2 mark that is recognized by the tudor domains of both 53BP1 and PHF20. It is possible that H4K16 hyperacetylation could lead to chromatin reorganization, allowing accessibility of the H4K20 methyl-marks for binding of repair proteins to sites of DSBs. Alternatively, these two modifications could function in concert to increase the affinity of tudor domain binding.

Global changes in histone modification patterns in cancer cells have been used as a predictor of clinical outcome [65]. It has been reported that loss of H4K16 acetylation and H4K20me3 is a common feature of human tumors [66]. When the relative abundance of H4 posttranslational modifications were assessed by mass spectrometry, it was found that normal lymphocytes had two-fold more H4K16ac/K20me2, and four-fold more H4K16ac/K20me3, than a cancer cell line [66]. These trends were shown for normal tissues and primary tumors as well. The role of H4K16 acetylation and H4K20 methylation in tumorgenesis is unclear. But these two modifications are linked and their loss serves as a common characteristic of human tumors.

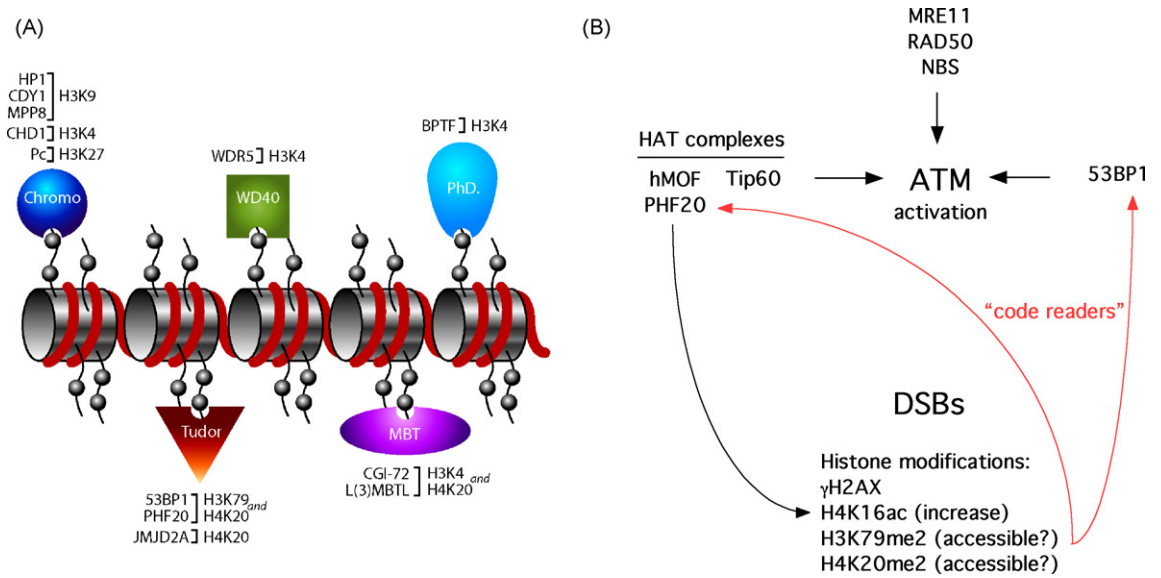

Fig. 4. Protein domains that "read" the histone code. (A) Methyllysine binding proteins contain one of five domains that mediate this interaction: the chromodomain, the WD40-repeat domain, the tudor domain, the MBT domain, or the PhD domain. The proteins that harbor each particular domain are listed along with the site of methylation that is bound by that domain. Some domains recognize more than one site. Most domains are also able to discriminate the degree of methylation at a site. For example, MBTs can bind mono- and di-methyl marks but not tri-methyl marks. The tudor domains of PHF20 and 53BP1 selectively bind di-methyl marks, and the tudor domain of JMJD2 prefers tri-methyl marks. Thus, the specificity of binding not only lies within the primary sequence surrounding a methylated site, but also within the degree of methylation at each site. (B) The tudor domain containing proteins 53BP1 and PHF20 read the histone code associated with DNA damage. Although the downstream signaling pathways activated by ATM are well characterized, the mechanism by which DBSs are detected and how this leads to the activation of ATM is less clear. The MRN complex, the Tip60 and hMOF HAT complexes, and 53BP1 all play a role in activating ATM. The remodeling of chromatin structure and changes in the histone code at sites of DNA damage may be early signals in the DSB response. At least two HAT complexes are involved in this process, but they seem to have different functions. Tip60 binds directly to ATM, and is involved in its acetylation and subsequent activation. hMOF/PHF20 is responsible for the acetylation of H4K16 in response to DNA damage. How this acetylation impacts PHF20 and 53BP1 binding to histone tail is still unknown.

The list of enzymes that methylate H4K20 include Suv4-20h1, Suv4-20h2, PR-SET7, and NSD1. It has been shown that Suv4-20h proteins are nucleosomal enzymes that preferentially direct H4K20 di- and tri-methylation [43], and the PR-SET7 (SET8) enzyme is a monomethyltransferase [67–69]. NSD1 has been demonstrated to possess dimethyltransferase activity at H4K20 [70], although it unclear if this enzyme also has the ability to mono- and/or tri-methylate this site. Mutations in NSD1 cause Sotos syndrome [71]. This is an autosomal dominant overgrowth syndrome, and individuals with this syndrome are predisposed to cancer [72,73]. The molecular mechanism by which mutations in NDS1 causes Sotos syndrome, and why these patients are prone to various malignancies, is unclear [74]. Because of the ability of NSD1 to generate a H4K20me2 mark, Sotos syndrome patients could have an attenuated DNA damage checkpoint similar to that seen in *S. pombe set9*Δ cells [59].

It is possible that the presence of PHF20 within the MLL1/hMOF complex facilitates recruitment of this complex to sites of DSBs, and the MLL1/hMOF acetyltransferase and methyltransferase activities lead to an open/active chromatin structure at these sites of DNA damage. PHF20 can therefore aid in the spreading of an open chromatin structure (Fig. 4), helping make these regions accessible to DNA damage repair molecules.

4. Concluding remarks

In this review we have focused on arginine and lysine methylation events and the enzymes that mediate this methylation. We should not overlook the emerging field of lysine demethylation. These are two families of dimethylates; the amine oxidases [52] and the Jumonji C (JmjC) domain demethylases [53,75]. These enzymes have the ability to demethylate by degrees. Thus, an enzyme that could convert H4K20me3 to H4K20me2 could well play a central role in the DNA damage response, as it would be responsible for generating docking sites for 53BP1 and PHF20. Protein methylation is a PTM that affects proteins at the site of DNA damage

(the histones) and proteins that respond to that damage (Pol-β, 53BP1, PHF20 and MRE11). It is clear that protein methylation plays important roles at multiple nodes of the DNA damage response.

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