

## Histone modifications in DNA damage response

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DNA damage is a relatively common event in eukaryotic cell and may lead to genetic mutation and even cancer. DNA damage induces cellular responses that enable the cell either to repair the damaged DNA or cope with the damage in an appropriate way. Histone proteins are also the fundamental building blocks of eukaryotic chromatin besides DNA, and many types of post-translational modifications often occur on tails of histones. Although the function of these modifications has remained elusive, there is ever-growing studies suggest that histone modifications play vital roles in several chromatin-based processes, such as DNA damage response. In this review, we will discuss the main histone modifications, and their functions in DNA damage response.

### DNA damage response, histone modifications, chromatin, DNA repair

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## INTRODUCTION

Eukaryotic cells are exposed to numbers of factors that cause DNA lesions and genomic integrity is continually challenged. Chemicals, UV radiation, ionizing radiation outside the cells and reactive oxygen species, S-adenosyl-methionine inside the cells are known to induce various DNA damage (Lord and Ashworth, 2012). DNA lesions interfere with DNA replication and transcription, and if they are not repaired correctly, they can have deleterious effects, such as mutations and even wider-scale genome aberrations (Polo and Jackson, 2011). DNA damage is closely associated with many human diseases, including cancer, neurodegenerative disorders, immune deficiencies, infertility and cardiovascular disease (Ciccia and Elledge, 2010; Jackson

and Bartek, 2009).

In order to preserve genomic integrity, DNA damage must be repaired precisely. Indeed, eukaryotic cells have developed several highly conserved DNA repair pathways to counteract different types of damage, including (i) Base excision repair. (ii) Nucleotide excision repair. (iii) Mismatch repair. (iv) Nonhomologous end-joining (NHEJ)-mediated DNA double-strand break repair. (v) Homologous recombination (HR)-mediated DNA double-strand break repair (Sancar et al., 2004; Williamson et al., 2012). Different repair pathways are mediated by different repair factors, contributing to a series of large networks safeguarding the genomic DNA (Sancar et al., 2004). It has been clear that histone modification is also a critical part of these networks, and the roles of histone modification in DNA damage response have been well demonstrated in recent studies (Miller and Jackson, 2012; Sulli et al., 2012).

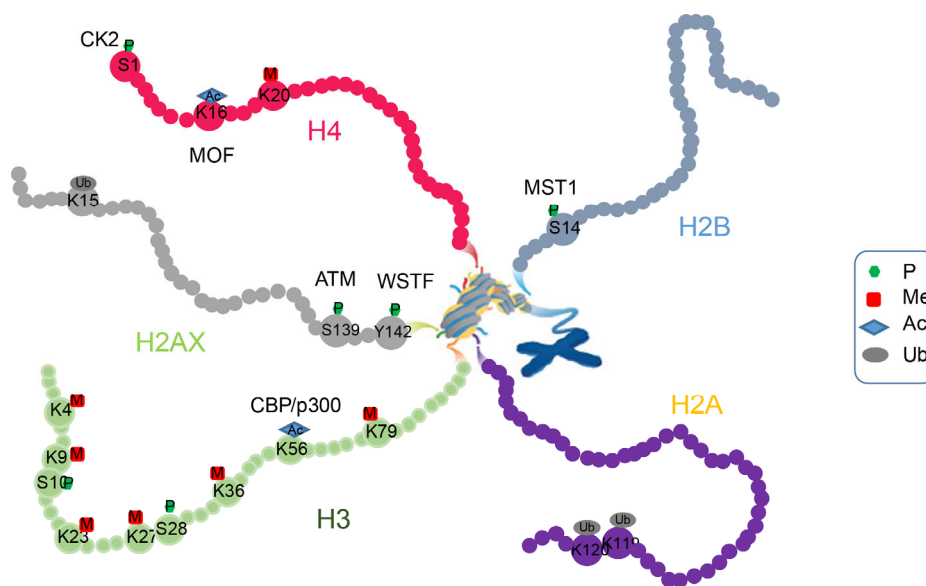
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In eukaryotes, chromatin is composed of repeating units of nucleosomes connected by linker DNA. The core component of nucleosome is histone octamer, which is formed by two copies each of histone H2A, H2B, H3, H4 (Margueron and Reinberg, 2010; Talbert and Henikoff, 2010), whereas histone H1 binds to the linker DNA between nucleosomes. Histones are easily catalyzed by histone modification enzymes to form different kinds of post-translational modifications, such as methylation, acetylation and phosphorylation (Campos and Reinberg, 2009; Kouzarides, 2007). Until now, more than a dozen types of histone modifications and hundreds of modification sites altogether have been found (Arnaudo and Garcia, 2013; Dai et al., 2014; Kouzarides, 2007; Tan et al., 2011) (Figure 1).

Histone modifications play critical roles in many biological processes by regulating chromatin structure and function. There are several ways for histone modifications to exert their functions. At first, it is well known that the DNA-histone interaction in the chromatin structure is mediated by the attraction between negatively charged DNA backbone and positively charged lysine and arginine residues of histones (Bannister and Kouzarides, 2011). Some modifications including lysine acetylation, lysine citrullination and Serine/Threonine phosphorylation, can change the charge of the amino acid residues of histones and thereby regulate the compaction of chromatin (Gyorgy et al., 2006; Strahl and Allis, 2000). In addition, histone modifications can serve as high-affinity binding sites or platforms for the proteins containing specific binding domains. For example, the PHD zinc-finger domains, chromodomains and Tudor domains bind specifically to the methylated lysines, while bromodomains recognize acetylated lysines with high affinity (Yun et al., 2011). Furthermore, there are intricate

crosstalks between different histone modifications, and one histone modification can regulate the activities of chromatin by influencing another histone mark. For example, the existence of some histone modifications can inhibit the generation of other modifications at the same amino acid residue, and modulating the formation of modifications at the residues nearby because of steric effect (Lee et al., 2010b; Suganuma and Workman, 2008). In general, histone modifications regulate chromatin activities in different ways.

Histone modification is involved in a lot of physiological and pathological processes. Firstly, histone modification is closely related to tumorigenesis and cancer progression. For instance, H3K36 dimethylation is the transcriptional activator of *DUSP3* (dual-specificity phosphatase 3) gene, whose protein dephosphorylates ERK1/2 (extra cellular-regulated kinase 1/2) and downregulates ERK1/2 activity. Histone demethylase KDM2A (lysine-specific demethylase 2A), which is capable of erasing H3K36 dimethylation and repressing *DUSP3* expression, is frequently overexpressed in lung tumors, and promotes lung tumorigenesis and metastasis by enhancing ERK1/2 signaling (Wagner et al., 2013). In addition, histone modification plays a role in cell metabolism. It has been demonstrated that histone demethylase KDM3A directly regulates the metabolic gene *Ppara* and *Ucp1* expression, both of which are the important regulator of energy balance, by demethylating H3K9me1/2 (Tateishi et al., 2009). Moreover, histone modification is the modulator of stem cell division and differentiation. There is a large cohort of developmental gene promoters containing bivalent chromatin domain, which is enriched simultaneously with the transcriptionally active histone mark H3K4me3 and transcriptionally repressive histone mark



**Figure 1** Many histone modifications are involved in DNA damage response. The representative modification sites are shown here.

H3K27me<sub>3</sub>, and stem cell fate is controlled by the specific bivalent domain (Voigt et al., 2013). Furthermore, histone modification is also tightly correlated with DNA damage at every stage of cell response to DNA damage (Rossetto et al., 2010). In this review, we will summarize and discuss the most recent studies, and describe the functions of each histone modification in DNA damage response (Figure 1).

## PHOSPHORYLATION

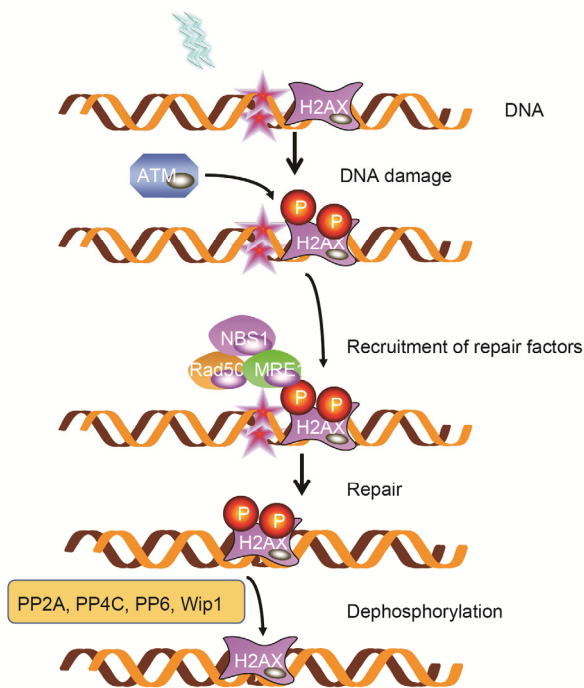
One of the first histone modification events correlated with DNA damage response is the phosphorylation of the H2A variant H2AX, and the phosphorylated H2AX is referred as  $\gamma$ H2AX. H2AX phosphorylation occurs within minutes after exposure to DNA damage, and the phosphorylation site is Serine 139 (S139) of the H2AX human variant or Serine 129 (S129) of yeast H2A in a unique conserved SQ motif in the C-terminal tail (Downs et al., 2000; Rogakou et al., 1998). It has been described that ATR (ataxia-telangiectasia mutated and Rad3 related) is responsible for  $\gamma$ H2AX foci formation in response to replication stress (Ward and Chen, 2001), while ATM (ataxia-telangiectasia mutated) and DNA-PK (DNA-dependent protein kinase) function redundantly to phosphorylate H2AX after exposure to ionizing radiation (Stiff et al., 2004) (Figure 2).  $\gamma$ H2AX spreads over a large region (over 1 Mb in human cells) around a DNA break site (Downs et al., 2004; Rogakou et al., 1999; Shroff et al., 2004; Unal et al., 2004). Therefore, it is easily detected by specific antibodies against  $\gamma$ H2AX in fluorescence microscopy and chromatin immunoprecipitation analysis, and has been commonly used as a biomarker of DNA damage nuclear foci (Bonner et al., 2008; Mah et al., 2010).  $\gamma$ H2AX distributes on both sides of a DNA break in an asymmetrical manner because of transcription state of the genes surrounding DNA damage site (Iacovoni et al., 2010). In addition, the pattern of  $\gamma$ H2AX is different on different chromatin regions, and  $\gamma$ H2AX foci formation in euchromatin is more efficient than that in heterochromatin in yeast and mammals (Kim et al., 2007).

It is clear that  $\gamma$ H2AX is essential for DNA damage response. At first,  $\gamma$ H2AX is required for the error-free HR repair and genome stability, and H2AX deficient mice or cells show increased use of error-prone single-strand annealing, enhanced radiosensitivity and oncogenic translocations, resulting in increased rates of tumorigenesis and cancer development (Bassing et al., 2002; Bassing et al., 2003; Celeste et al., 2003a; Celeste et al., 2002; Xie et al., 2004). Additionally, although  $\gamma$ H2AX is dispensable for the constitution of the primary DNA damage signal and the initial recruitment of repair factors, it is critical for the accumulation and retention of Rad50, Rad51, BRCA1 (breast cancer 1), MDC1 (mediator of DNA damage checkpoint 1) in human cells and 53BP1 homolog in yeast in response to DNA damage (Celeste et al., 2003b; Javaheri et al., 2006;

Nakamura et al., 2004; Paull et al., 2000; Stucki et al., 2005). Moreover,  $\gamma$ H2AX does not affect chromatin organization in the initial stage of DNA damage recognition and signaling (Fink et al., 2007). However, it is necessary to open the chromatin structure to facilitate access for the repair factors around the damage sites after the initial signaling, and histone modifiers and ATP-dependent chromatin remodelers are required at the DNA breaks (Osley et al., 2007). In fact,  $\gamma$ H2AX plays a role in recruiting NuA4, INO80 chromatin remodeling complexes and promotes histone acetylation to render the chromatin environment surrounding the DNA breaks more accessible for the repair factors (Downs et al., 2004; Lee et al., 2010a; Morrison et al., 2004; van Attikum et al., 2004).

Because of the essential role in DNA damage response,  $\gamma$ H2AX must be tightly regulated. Indeed, many factors are capable of modulating  $\gamma$ H2AX. For example, ATM promotes  $\gamma$ H2AX formation to maximal distance and maintains  $\gamma$ H2AX densities, while MDC1 is required for  $\gamma$ H2AX formation at high densities near damage sites, but not for generation of  $\gamma$ H2AX over distal sequences (Savic et al., 2009). In addition, SWI/SNF chromatin remodeling complex facilitates  $\gamma$ H2AX induction and binds to  $\gamma$ H2AX-containing nucleosomes (Lee et al., 2010a; Park et al., 2006). Moreover, evidence from yeast models suggests that yINO80 is required for maintaining a high level of  $\gamma$ H2AX during DNA damage response, while ySWR1 functions antagonistically and plays a role in replacing  $\gamma$ H2AX with the H2AZ variant (Papamichos-Chronakis et al., 2006). Last but not least,  $\gamma$ H2AX has to be eliminated from chromatin after the repair process is completed. It can be achieved either by the function of SWR1 (Papamichos-Chronakis et al., 2006), or by  $\gamma$ H2AX dephosphorylation. Many phosphatases have the ability to erase  $\gamma$ H2AX, including PP2A, PP4C, PP6, Wip1 in human cells and yPph3 in yeast (Figure 2).  $\gamma$ H2AX dephosphorylation is important for DNA repair and efficient recovery from the DNA damage checkpoint (Chowdhury et al., 2005; Chowdhury et al., 2008; Douglas et al., 2010; Keogh et al., 2006; Macurek et al., 2010; Nakada et al., 2008). In general,  $\gamma$ H2AX is an important marker of DNA break sites and essential modulator of DNA damage response, and is also tightly regulated during the DNA repair process.

In addition to  $\gamma$ H2AX, there are some other phosphorylation events occurring on histone H2AX or other histones linked with DNA damage response. For instance, H2AX is also phosphorylated on its C-terminal tyrosine 142 (Y142) by the non-canonical tyrosine kinase WSTF (Williams-Beuren syndrome transcription factor), and dephosphorylated by tyrosine phosphatase EYA (Cook et al., 2009; Xiao et al., 2009). Y142 is constitutively phosphorylated under normal growth conditions, and becomes gradually dephosphorylated during the DNA damage response (Xiao et al., 2009). WSTF and/or Y142 phosphorylation not only



**Figure 2** The role of  $\gamma$ H2AX in DNA damage response. In response to DNA double-strand break, histone H2AX is quickly phosphorylated by ATM or other kinases, after which many repair factors are recruited to the DNA damage site to promote DNA repair. As long as the DNA repair process is completed,  $\gamma$ H2AX will be dephosphorylated by some phosphatases, such as PP2A, PP4C, PP6, Wip1.

regulate the recruitment of active ATM and MDC1, and the maintenance of  $\gamma$ H2AX upon DNA damage (Xiao et al., 2009), but also further determine repair/survival versus apoptotic responses to DNA damage (Cook et al., 2009). Additionally, phosphorylation of threonine 101 (T101) on H2AX is identified as a DNA damage responsive modification as well. T101 mutation increases  $\gamma$  radiation (IR) sensitivity of ES cells, but does not affect DNA repair efficiency (Xie et al., 2010). Moreover, histone H2B is phosphorylated on serine 14 (S14) by protein kinase MST1 (mammalian sterile twenty-like kinase 1) following DNA damage, and H2BS14ph foci appears at the late time points in a  $\gamma$ H2AX-dependent manner (Cheung et al., 2003; Fernandez-Capetillo et al., 2004). Furthermore, the N-terminal serine 1 (S1) of H4 in nucleosomes proximal to the break sites is phosphorylated by casein kinase 2 (CK2) at the end of the repair process. H4S1 phosphorylation inhibits H4 acetylation to stabilize the nucleosome during chromatin restoration, and promotes NHEJ-mediated double-strand break repair (Cheung et al., 2005; Utley et al., 2005). Finally, histone H3 phosphorylation, such as H3S10p and H3S28p, decreases upon DNA damage due to the activation of cell cycle checkpoint and thus reduced mitotic cells (Tjeertes et al., 2009).

## METHYLATION

The second most common histone modification linked with DNA damage response is histone methylation. Histone methylation occurs at specific sites on H3 and H4 such as H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20. It is performed by histone methyltransferases and reversed by histone demethylases, indicating that it is dynamically regulated in eukaryotic cells. Histone methyltransferases refer to a family of proteins containing the catalytic SET domain except the H3K79 methyltransferase DOT1L, while two families of histone demethylases have been reported, including flavin adenine dinucleotide (FAD)-dependent histone demethylases and JmjC domain-containing histone demethylases. Many histone methyltransferases/demethylases, as well as their targeted histone methylations, are involved in DNA damage response.

H3K9 methylation is critical for genome stability and DNA damage response. At first, H3K9me3 is an essential histone marker of heterochromatin (Grewal and Jia, 2007). H3K9 methyltransferase SUV39H1 is required for the maintenance of heterochromatic state, and its methylation by SET7/9 impairs its enzymatic activity resulting in decreased H3K9me3 and heterochromatin instability (Peng and Karpen, 2009; Wang et al., 2013). Next, H3K9 methylation is important for the cellular response to DNA damage, but the role of H3K9 methylation is different at the early-stage and late-stage of DNA damage response. DNA damage immediately induces the loading of a protein complex containing KAP-1, HP1, SUV39H1 onto the DNA double-strand breaks, and therefore upregulates the level of H3K9me3 nearby. With the assistance of HP1, H3K9me3 can spread over tens of kilobases away from the damage sites to form a large repressive heterochromatin domain (Ayrapetov et al., 2014). Subsequently, HP1 is released from chromatin, and histone acetyltransferase Tip60 binds to H3K9me3 via its chromodomain. The enrichment of Tip60 activates its acetyltransferase activity, and stimulates the subsequent acetylation and activation of ATM at the damage sites (Ayoub et al., 2008; Ayrapetov et al., 2014; Sun et al., 2010; Sun et al., 2009). Therefore, H3K9 methylation and heterochromatin formation is required for ATM activation, and ATM-mediated DNA damage signaling such as  $\gamma$ H2AX localization at the early phase of DNA damage response (Sasaki et al., 2014). However, because the chromatin adjacent to the damaged DNA needs to be open in order to increase the accessibility of repair proteins, H3K9 methylation has to be reversed to promote the repair process. It has been demonstrated that DNA damage induces the degradation of G9a/GLP, which results in decreased H3K9me2 at the promoters of *IL-6* and *IL-8* in senescent cells (Takahashi et al., 2012). In addition, H3K9me2/3 demethylases KDM4B and KDM4D are recruited to the DNA damage sites mediated by PARP1, and are responsible for H3K9 demethylation (Khoury-Haddad et al., 2014; Young

et al., 2013). However, a recent study reveals that H3K9me2 is required for BARD1 and BRCA1 retention at sites of DNA damage, thus promoting HR repair in S phase of cell cycle (Wu et al., 2015). It indicates that H3K9me3 is removed, but H3K9me2 is reserved at damaged DNA.

Similar to H3K9 methylation, H3K36 methylation is an important histone marker for recruiting repair factors as well. H3K36 methyltransferase Metnase promotes DNA integration by opening chromatin and facilitating joining of DNA ends (Lee et al., 2005). More importantly, H3K36me2 is induced in response to DNA double-strand break, and Metnase itself and its phosphorylation by Chk1 (checkpoint kinase 1) are responsible for the increase of H3K36me2, which is a platform for recruiting Ku70 to enhance the activity of NHEJ (Fnu et al., 2011; Hromas et al., 2012). Additionally, our group discovered that the phosphorylation of H3K36 demethylase KDM2A mediated by ATM is also required for H3K36me2 induction, and H3K36me2 is capable of favoring MRE11 complex localization through the direct interaction between H3K36me2 and NBS1 (Cao et al., 2015). Compared to H3K36me2, it seems that H3K36me3 exerts more complicated functions in DNA damage response although it is not induced by DNA damage. At first, Set2-dependent H3K36me3 is essential for the loading of mismatch recognition protein hMuts $\alpha$  via direct interaction with the hMSH6 PWWP domain, and is required for DNA mismatch repair to ensure the fidelity of DNA replication (Li et al., 2013). However, the role of H3K36me3 in cellular response to DNA double-strand break is controversial at present. One study in fission yeast indicates that H3K36me3 is cell cycle regulated, peaking in G1 when NHEJ occurs and decreasing in S and G2/M phases, and H3K36me3 reduces chromatin accessibility, impairs DNA end resection and HR (Pai et al., 2014). Other studies show that Set2-dependent H3K36me3 is critical for appropriate resection and HR through recruiting repair factors CtIP (retinoblastoma binding protein 8), RPA (replication protein A1) and Rad51 (Jha and Strahl, 2014; Pfister et al., 2014). In the end, H3K36 demethylase Rph1 functions as a transcriptional repressor and inhibits the expression of DNA repair enzyme gene *PHR1* and other stress-response genes by H3K36 demethylation, while DNA damage and environmental stress induce Rph1 phosphorylation and dissociation from chromatin to facilitate gene expression (Liang et al., 2011; Liang et al., 2013), indicating that H3K36 methylation is essential for DNA repair.

Although H3K79 methylation is also a pre-existing histone modification and not induced by DNA damage (Huyen et al., 2004), it plays an extensive role in DNA damage response and the maintenance of genome stability. The best characterized function of H3K79 methylation is recruiting 53BP1 to DNA break sites. Dot1L-dependent H3K79 methylation is required for the localization of 53BP1 around damaged DNA and 53BP1 tandem Tudor domain binds to

methylated H3K79 directly, resulting in the induction of 53BP1 phosphorylation and checkpoint activation (Giannattasio et al., 2005; Huyen et al., 2004; Wakeman et al., 2012; Wysocki et al., 2005). In addition, H3K79 methylation mediated by Dot1L promotes nucleotide excision repair, and H3K79R mutation increases the binding of histone deacetylase complex to eliminate histone acetylation and reduce DNA lesion accessibility to repair enzymes (Chaudhuri et al., 2009; Tatum and Li, 2011). Moreover, Dot1L and H3K79me3 contribute to favorable sister chromatid exchange during HR and facilitate HR repair (Conde et al., 2009; Rossodivita et al., 2014). Furthermore, there is crosstalk between H3K79 methylation and other histone methylations. For example, H3K79 methylation is important for the maintenance of heterochromatic mark H3K9 methylation and H4K20 methylation at centromeres and telomeres, and loss of H3K79 methylation results in heterochromatin instability (Jones et al., 2008). Lastly, it has been described that H3K79 methylation is critical for Mek1 autophosphorylation and activation, and thereby modulates the meiotic checkpoint response (Ontoso et al., 2013).

Another histone modification involved in 53BP1 localization is H4K20 methylation. H4K20 methyltransferase MMSET (Wolf-Hirschhorn syndrome candidate 1) regulates the induction of H4K20 methylation on histones around double-strand breaks, which in turn facilitates 53BP1 recruitment (Hajdu et al., 2011; Pei et al., 2011). In addition to MMSET, another two H4K20 methyltransferases Set8 and Set9 are also responsible for H4K20 methylation and the recruitment of 53BP1 (Dulev et al., 2014; Greeson et al., 2008; Oda et al., 2010; Sanders et al., 2004; Yan et al., 2009). Similar to H3K79 methylation, H4K20 methylation recruits 53BP1 via its tandem Tudor domain as well (Botuyan et al., 2006). Interestingly, histone demethylase KDM4A also contains a tandem Tudor domain, and it competes with 53BP1 for binding to methylated H4K20. In order to favor 53BP1 localization, KDM4A is degraded in a RNF8- and RNF168-dependent manner in response to DNA damage (Mallette et al., 2012). Actually, H4K20 methylation not only promotes 53BP1 localization, but also serves to modulate genome stability. H4K20me1/2/3 is required for maintenance of proper high order chromatin structure, and loss of H4K20 methylation impairs genomic integrity and may induce tumorigenesis (Oda et al., 2009; Sakaguchi and Steward, 2007; Schotta et al., 2008). In fact, global H4K20 methylation is much lower in bladder cancer samples than in normal tissues and has been proposed as a potential prognosis biomarker in bladder cancer (Schneider et al., 2011).

Other histone methylations such as H3K27 methylation and H3K4 methylation are also involved in DNA damage response. DNA double-strand break initiates the recruitment of polycomb group proteins EZH2, SUZ12, CBX8, which constitute a repressive chromatin structure at the sites of DNA damage via H3K27 methylation to block transcription

and facilitate DNA repair (Campbell et al., 2013; Chou et al., 2010; O'Hagan et al., 2008). As for H3K4 methylation, it seems to be reversed upon DNA damage. At first, LSD1 (lysine-specific demethylase 1), as well as its *Caenorhabditis elegans* ortholog Spr-5, is enriched on double-strand break sites and responsible for H3K4me1/2 demethylation, resulting in increased recruitment of repair factors (Mosammaparast et al., 2013; Nottke et al., 2011). In addition, KDM5A and KDM5B, the H3K4me2/3 demethylases, are recruited to damaged DNA to induce loss of H3K4me2/3, and required for efficient DNA repair (Li et al., 2014; Seiler et al., 2011). However, the role of H3K4 methylation in DNA damage response is disputable, because it has been reported that the yeast Set1p methyltransferase as well as its substrate H3K4me3 become detectable on a newly created double-strand break in budding yeast cells, and this enrichment of Set1p and H3K4me3 are important for DNA repair by NHEJ (Faucher and Wellinger, 2010). Moreover, the role of H3K4 methylation in nucleotide excision repair is interesting, and H3K4R mutation exerts different impacts on nucleotide excision repair on different genomic loci (Chaudhuri et al., 2009). In our previous studies, we also described the critical role of an H3K4 methyltransferase SET7/9 in DNA damage response and oxidative stress, but through methylating non-histone proteins (Shen et al., 2015; Wang et al., 2013). At last, H3K23 trimethylation (H3K23me3), a novel characterized histone modification, is reported to blocks DNA damage in pericentric heterochromatin during meiosis in *Tetrahymena* (Papazyan et al., 2014).

## ACETYLATION

Histone acetylation has also been extensively studied in the context of DNA damage response modulation. It is well-known that histone acetylation can influence chromatin structure (Shogren-Knaak et al., 2006; Turner et al., 1992). On the one hand, acetylation neutralizes the positively charged lysine residues, resulting in diminished interaction between DNA backbone and histones to facilitate chromatin decondensation and enhance the accessibility of nucleosomal DNA (Kouzarides, 2000; Shahbazian and Grunstein, 2007). On the other hand, it is capable of recruiting chromatin remodelling complex such as SWI/SNF complex to modulate the chromatin structure (Lee et al., 2010a). Histone acetylation is dynamically regulated by histone acetyltransferases and histone deacetylases. Histone acetyltransferases are responsible for transferring an acetyl group from acetyl-coenzyme A to histone lysine residues, while histone deacetylases are able to remove the acetyl group of histones (Grunstein, 1997). Compared to histone methyltransferases/demethylases, histone acetyltransferases/deacetylases don't exhibit rigid site-specificity. For example, p300/CBP acetylate all four nucleosomal core histones equally well, and they exhibit multisite acetylation pattern in different

histones (Roth et al., 2001).

Many histone acetyltransferases are involved in DNA damage response. At first, it is described that histone acetyltransferase MOF and its substrate H4K16 acetylation is required for IR-induced ATM activation (Gupta et al., 2005; Smith et al., 2005), ATM-dependent phosphorylation of DNA-PKcs (Sharma et al., 2010) and MDC1 recruitment (Li et al., 2010), and MOF depletion greatly decreased DNA double-strand break repair by both NHEJ and HR (Sharma et al., 2010). Interestingly, a recent study reveals that the proteasome activator PA200 in mice specifically recognizes acetylated H4K16 via its bromodomain-like regions, and targets the core histones for acetylation-mediated degradation by proteasomes in response to DNA double-strand break, thus relaxing the chromatin and promoting DNA repair (Qian et al., 2013). In addition, human CBP/p300 and yeast Rtt109 are responsible for H3K56 acetylation *in vivo* and is required for DNA replication and genome stability (Das et al., 2009; Han et al., 2007). It is reported that H3K56 acetylation are reduced initially in response to DNA damage, followed by full renewal of an acetylated state, and H3K56Ac is colocalized with other proteins involved in DNA damage signaling pathways such as phospho-ATM, CHK2, and p53 at the sites of DNA repair (Battu et al., 2011; Tjeertes et al., 2009; Vempati et al., 2010). During the S phase of cell cycle, H3K56Ac is on the newly synthesized histone H3 that is incorporated into chromosomes (Masumoto et al., 2005), and it drives chromatin reassembly and checkpoint recovery after DNA repair with the assistance of histone chaperone Asf1 (Chen et al., 2008; Driscoll et al., 2007). H3K56Ac abrogation results in sensitivity to genotoxic agents that cause DNA strand breaks, genome instability and decreased sister chromatid recombination in the S phase (Munoz-Galvan et al., 2013; Wurtele et al., 2012). However, cells proceed into G2 phase after DNA replication fork damage repair is completed, and H3K56 acetylation largely disappears in G2 phase (Masumoto et al., 2005). In addition to p300/CBP, the acetyltransferase GCN5 in human cells is also able to acetylate H3K56 (Tjeertes et al., 2009), but GCN5 is responsible for H3K9 acetylation as well, thus stimulating the recruitment of repair factors in the nucleotide excision repair pathway (Guo et al., 2011). Moreover, the acetyltransferase Tip60 also plays a role in DNA damage response (Ikura et al., 2000). Human Tip60 or its yeast homolog Esa1 is recruited to DNA double-strand breaks *in vivo* by  $\gamma$ H2AX (Bird et al., 2002; Downs et al., 2004), and it induces acetylation of histones surrounding DNA damage sites, thus resulting in chromatin relaxation and loading of repair proteins (Murr et al., 2006). Besides chromatin relaxation, Tip60-mediated acetylation of phospho-H2Av,  $\gamma$ H2AX homolog in *Drosophila melanogaster*, induces the exchange of phospho-H2Av with unmodified H2Av (Kusch et al., 2004), while Tip60-dependent H4K16 acetylation diminishes 53BP1 binding to H4K20me2 and promotes HR repair (Tang et al., 2013). Tip60 depletion

impairs homologous recombination and rendered cells sensitive to cisplatin (House et al., 2014; Miyamoto et al., 2008; Tang et al., 2013). Furthermore, similar to human CBP/p300 or yeast Rtt109, histone acetyltransferase 1 in yeast and human cells is also required for the incorporation of acetylated H3 at sites of double-strand breaks, and facilitates subsequent recruitment of RAD51 to promote efficient homologous recombination (Qin and Parthun, 2002; Yang et al., 2013).

Histone deacetylation is also important for DNA repair and cell cycle progression. At first, loss of histone deacetylase Hdac3 in MEFs causes DNA damage and S phase checkpoint activation, indicating that Hdac3 is essential for genome stability during DNA replication (Bhaskara et al., 2008). In addition, in *Saccharomyces cerevisiae*, Sin3p-mediated H4K16 deacetylation is required for 53BP1 binding to methylated H4K20, thus inducing efficient NHEJ repair (Hsiao and Mizzen, 2013; Jazayeri et al., 2004). Except for NHEJ, HR also triggers localized histone deacetylation by histone deacetylases Rpd3, Sir2, and Hst1 at DNA double-strand breaks, and the ability to modulate histone acetylation during HR is essential for cell viability (Tamburini and Tyler, 2005). Moreover, many histone deacetylases are responsible for the removal of H3K56Ac, including HDAC1, HDAC2, hSIRT1, hSIRT2, hSIRT3, yHst1, yHst2, yHst3 and yHst4p, and sustained H3K56 hyperacetylation impedes the completion of DNA repair and increases cell sensitivity to DNA-damaging agents (Celic et al., 2006; Das et al., 2009; Maas et al., 2006; Miller et al., 2010; Vempati et al., 2010). In general, histone acetylation is dynamically regulated during DNA repair, and the balance between histone acetyltransferases and histone deacetylases is critical for the repair process and genome integrity.

## UBIQUITINATION

Ubiquitination is a complicated process, in which the conserved 76-residue polypeptide ubiquitin is covalently conjugated to the  $\epsilon$ -amino group of a substrate lysine residue (Komander, 2009). It requires the sequential actions of three enzymes: an E1 activating enzyme that forms a thiol ester with the carboxyl group of G76, an E2 conjugating enzyme that transiently carries the activated ubiquitin molecule as a thiol ester and an E3 ligase that transfers the activated ubiquitin from the E2 to the substrate (or ubiquitin) lysine residue (Pickart, 2001). Ubiquitination regulates many critical cellular functions, mainly by ubiquitin-dependent degradation of substrates, while histone ubiquitination regulates a broad range of DNA related processes (Bennett and Harper, 2008; Cao and Yan, 2012). For example, histone H2B ubiquitylation interferes with chromatin compaction and leads to an open and biochemically accessible fiber conformation (Fierz et al., 2011). However, another study showed that H2A ubiquitylation prevents RNA polymerase II elongation-dependent chromatin decondensation, and induces

transcriptional silencing at regions distal to DSBs (double-strand breaks) (Shanbhag et al., 2010). Nevertheless, histone ubiquitylation is capable of modulating chromatin structure, transcription and DNA damage response. The indispensable role of histone ubiquitylation in DNA damage response has been well-recognized nowadays (Messick and Greenberg, 2009).

The first important ubiquitination event in DNA damage response is RNF8/RNF168-mediated histone ubiquitination. Upon DNA double-strand break or UV radiation, MDC1 recruits RNF8 through phosphodependent interactions between the RNF8 forkhead-associated domain and motifs in MDC1 that are phosphorylated by ATM (Kolas et al., 2007; Mailand et al., 2007; Marteijn et al., 2009). The E3 ligase activity of RNF8 is required for the formation of lysine 63-linked ubiquitin chains at damage sites to induce the recruitment of another E3 ligase RNF168, by its ubiquitin binding domains (Doil et al., 2009; Stewart et al., 2009). RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes (Campbell et al., 2012; Doil et al., 2009; Wang and Elledge, 2007; Wu et al., 2009). MDC1-mediated and RNF8/RNF168-excuted ubiquitination occurs at K15 on H2A/H2AX, and ubK15 is directly recognized by 53BP1 (Fradet-Turcotte et al., 2013; Mattioli et al., 2012). RNF8 or RNF168 deficient cells display impaired cellular responses to DNA damage, a defective G2/M checkpoint and increased radiosensitivity (Doil et al., 2009; Huen et al., 2007). In addition to K63 linked ubiquitination, RNF168 promotes noncanonical K27 linked ubiquitination *in vivo* and *in vitro*, and this specific ubiquitination is also required for the proper activation of the DNA damage response (Gatti et al., 2015). Additionally, the polycomb repressive complex 1, which contains Bmi1, Ring1, and Ring2, is required for H2A/H2AX ubiquitination (Bergink et al., 2006; Cao et al., 2005). Although the role of Ring1-mediated H2A ubiquitination in DNA damage response is not clear, Bmi1 and Ring2 are recruited to sites of DNA damage where they contribute to the monoubiquitylation of H2A/H2AX at Lys119/Lys120 (Ginjala et al., 2011; Pan et al., 2011). Bmi1/Ring2-dependent H2A/H2AX ubiquitination is critical for the localization of 53BP1, BRCA1 and other repair factors (Facchino et al., 2010; Ismail et al., 2010). Consequently, loss of Bim1 or Ring2 leads to impaired repair of double-strand break, G2/M cell cycle arrest and increased cellular sensitivity to irradiation or genotoxic agents (Chagraoui et al., 2011; Ginjala et al., 2011). However, the relationship between H2A/H2AX ubiquitination and  $\gamma$ H2AX is not clear. Moreover, some other ubiquitin E3 ligases, such as B-lymphoma and BAL-associated protein (BBAP) (Yan et al., 2009), RNF20-RNF40 heterodimer (Moyal et al., 2011; Nakamura et al., 2011), Cul4-DDB-Roc1 (Wang et al., 2006) and checkpoint with forkhead-associated (FHA) and RING finger domain protein (CHFR) (Liu et al., 2013), have been shown to be involved in his-

tone ubiquitination in response to DNA damage as well.

Histone ubiquitination is tightly regulated during the cellular response to DNA damage. Firstly, some deubiquitinases are involved in the removal of histone ubiquitination. For instance, the deubiquitinating enzyme OTUB2 suppresses RNF8-mediated Lys 63-linked ubiquitin chain formation in a deubiquitinating activity-dependent manner. Depletion of OTUB2 enhances RNF8-mediated ubiquitination in an early phase of the DNA damage response, favors the accelerated accumulation of 53BP1 and RAP80 at DSBs, thus promoting DSB repair by NHEJ (Kato et al., 2014). Therefore, OTUB2 fine-tunes the speed of RNF8-mediated ubiquitination so that the appropriate DNA repair pathway is chosen. In addition to OTUB2, other deubiquitinases, such as usp44 (Mosbech et al., 2013), Dub3 (Delgado-Diaz et al., 2014) and BAP1 (Yu et al., 2014), are responsible for the H2A/H2AX deubiquitination as well. They might act in concert with the ubiquitin E3 ligases, such as RNF8/RNF168 and the PRC1 complex, to promote the dynamic ubiquitination/deubiquitination of histones at DNA damage sites. Next, it has been demonstrated that RNF169, an E3 ubiquitin ligase paralogous to RNF168, accumulates at DNA damage foci through direct recognition of RNF168-mediated histone ubiquitylation. Therefore, RNF169 functionally competes with 53BP1 for association with ubiquitinated histones, and impairs the 53BP1 recruitment at sites of DNA damage, resulting in stimulated HR and restrained NHEJ (Chen et al., 2012; Poulsen et al., 2012). Moreover, p400 SWI/SNF ATPase and HERC2 are required for RNF8/RNF168-mediated ubiquitination, either by destabilization of nucleosomes or by facilitating the assembly of the ubiquitin-conjugating enzyme Ubc13 with RNF8 (Bekker-Jensen et al., 2010; Xu et al., 2010).

## OTHERS

Many other histone modifications are reported to influence DNA damage response. At first, the ubiquitin-like protein NEDD8 accumulates at DNA damage sites in an E3 ligase RNF111-dependent manner, and H4 is polyubiquitinated at the N-terminal lysine residues. H4 neddylation can be recognized by RNF168, and loss of H4 neddylation impairs the localization of RNF168 and its downstream functional partners, such as 53BP1 and BRCA1, thus affecting the process of DNA repair (Ma et al., 2013). In addition, the histone variant H2A.Z, and its SUMO modification, is required for DNA resection, single DSB-induced checkpoint activation, and DSB anchoring to the nuclear periphery (Kalocsay et al., 2009). Moreover, it has been demonstrated in many studies that histones are covalently modified by mono(ADP)-ribose in response to DNA single-strand break (Adamietz and Rudolph, 1984; Bohm et al., 1997; Kreimeyer et al., 1984). TbSIR2RP1, a SIR2-related protein from the protozoan parasite *Trypanosoma brucei*, has been shown to catalyze the mono(ADP)-ribosylation of histones, partic-

ularly H2A and H2B, and treatment of trypanosomal nuclei with a DNA alkylating agent results in a significant increase in the level of histone H2A/H2B ADP-ribosylation. Consequently, depletion of TbSIR2RP1 decreased the cellular resistance to DNA damage (Garcia-Salcedo et al., 2003). However, histone ADP-ribosylation occurs only upon DNA single-strand break, and its role in double-strand break repair is not clear now.

## CROSSTALKS AMONG HISTONE MODIFICATIONS

There are various crosstalks among different modifications. At first, some modifications such as  $\gamma$ H2AX can serve as a platform for other modification factors, and promote the formation of some other modifications. In addition, some modifications can cooperate with each other to promote DNA repair. For example, both H3K36me2 and H3K36me3 can promote HR, while both H3K79 methylation and H4K20 methylation are capable of recruiting 53BP1. In the future, with an increasing understanding of the process of DNA damage response, much more crosstalks will be discovered.

## CONCLUSION AND FUTURE DIRECTIONS

Histones are the major protein components of chromatin and are subject to many posttranslational modifications, especially on their N-terminals. These modifications may constitute a "histone code" to modulate many cellular processes, such as DNA damage response. DNA damage response is a complicated process, in which many factors are involved, in order to repair the damaged DNA and maintain the genome stability. Although the kinetics of DNA damage response has been extensively studied, the exact order of histone modifications and other repair factors remains imprecise. It seems that many histone modifications regulate each other's formation and accumulation, and the intricate crosstalk among these modifications renders the study of the specific function of a single histone modification more difficult. However, it is clear that the timing of histone modifications is critical for the chromatin dynamics in the DNA damage response and efficient DNA repair. Therefore, it is still essential to clarify the exact role of each histone modification in DNA damage response in future studies. In addition, a lot of new histone modifications have been described recent years, and the functions of these modifications in DNA damage response are not clear. It is interesting to identify new histone modifications that are involved in DNA damage response in the future.

Considering the importance of histone modifications in DNA damage response, they may serve as targets for small molecules to interfere DNA repair to increase the radiosens-



sitivity or chemosensitivity of cancer cells. Actually, HDAC inhibitors have already been shown to alter tumor radiosensitivity through the modulation of histone acetylation and have been used in cancer treatment (Camphausen and Tofilon, 2007). In the future, it is of great value to develop drugs targeting histone methylation, ubiquitination and other histone modifications. Based on the basic research, it is promising to cure cancer via combination therapy, which contributes to DNA damage and histone modification interference.

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