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Epigenetic Modifications in Double Strand Break DNA Damage Signaling and Repair

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Abstract

Factors involved in the cellular response to double strand break (DSB) DNA damage have been identified as potential therapeutic targets that would greatly sensitize cancer cells to radiotherapy and genotoxic chemotherapy. They could disable the repair machinery and/or reinstate normal cell cycle checkpoint leading to growth arrest, senescence and apoptosis. It is now clear that a major aspect of the DNA damage response occurs through specific interactions with chromatin structure and its modulation. It implicates highly dynamic post-translational modifications of histones that are critical for DNA damage recognition/signaling, repair of the lesion and release of cell cycle arrest. Therefore, drugs that target the enzymes responsible for these modifications or the protein modules reading them have very high therapeutic potential. This review presents the current state of knowledge on the different chromatin modifications and their roles in each step of eukaryotic DSB DNA damage response.

1/ Introduction

In order to preserve its genomic integrity, the eukaryotic cell needs to be protected against agents that cause DNA damage. Indeed, cellular DNA is continuously exposed to exogenous (such as chemicals, UV-radiation, ionizing radiation) as well as endogenous (reactive oxygen species, alkylating agents such as S-adenosylmethionine etc...) insults known to induce various DNA lesions (1). To counteract these injuries, the cell has developed highly conserved DNA-damage responses (DDRs) that activate different repair pathways specifically adapted to the type of damage. These include (i) base-excision repair, (ii) nucleotide-excision repair, (iii) mismatch repair, and (iv) double-strand break repair (DSB) which is the most deleterious form of DNA damage since it can lead to loss of genetic material (2). DSB are mainly repaired by homologous recombination (HR) and non-homologous end-joining (NHEJ). HR uses the undamaged homologous chromosome or sister chromatid as a template to copy the missing information at the break. In contrast, NHEJ consists in the direct ligation of the two broken ends, which can produce short deletions.

In eukaryotic cells, the DNA-damage repair occurs in the context of chromatin. The chromatin is a DNA-protein structure that exists as a repetition of the basic unit called the nucleosome. A nucleosome is formed by an octamer of histones, containing two copies of each H2A, H2B, H3 and H4, wrapped with 146bp of DNA. The chromatin is a dynamic structure that regulates DNA accessibility during essential nuclear events such as

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replication, transcription, recombination and DNA-damage repair. Modulation of chromatin compaction can be regulated by different processes: introduction of histone variants into the nucleosome which confers different bio-physical features; post-translational histone modifications mainly occurring on histone tails protruding from the nucleosome; ATP-dependant chromatin remodeling complexes that have the ability to disrupt, evict or slide the entire nucleosome on the chromatin fiber; and histone chaperones which assist in nucleosome assembly/disassembly (3).

After DNA damage induction, the chromatin needs to be in an “open” state in order to allow the repair factors to access to the DNA molecule. This DDR process requires multiple steps including the initial signaling of the break, the access to the DNA for efficient repair, and the restoration of the chromatin to its initial state. In this review, we describe these steps of DDR involved in the DSB repair. Drugs that target the chromatin modifiers or readers implicated DNA damage response have very high therapeutic potential (see (4-6) and accompanying Focus reviews (7-10)).

2/ Recognition and Signaling of DNA-damage: Key role of γ -H2AX

When DNA damage occurs in the cell, the priority is to detect it and to signal it for repair. Even though these processes have been intensively studied, it is still not clear which factor arrives first at the break to recognize it and induce the DDR. Among all chromatin modifications linked to DSB damage response (Table 1), it is clear that phosphorylation of the H2A variant H2AX occurs within few minutes following the break, and is probably the first histone modification appearing in its vicinity (reviewed in (8,11)). This phosphorylation occurs in a unique conserved SQE motif in the C-terminal tail (at serine 129 (S129) of yeast H2A or S139 of the H2AX human variant, so-called γ -H2AX (12,13)). The kinases responsible for this modification have been identified as phosphatidylinositol 3-kinase-related kinases (PIKKs): the ataxia-telangiectasia mutated (ATM), ATM- and Rad3-related (ATR), and the DNA-dependant protein kinase (DNA-PK). ATM and DNA-PK principally function after ionizing radiation (IR) whereas ATR responds to replication stress and ultra-violet (UV) irradiation (14-16). In human cells, γ -H2AX spreads over more than 1Mb on each side of the break (50kb in yeast) (13,17-20), thus amplifying the repair signal, which make it easily detectable by immunofluorescence, and commonly used as a bio-marker of DNA-damage nuclear foci (for review, see (21)). Moreover, mice deficient for γ -H2AX are radiosensitive and show chromosomal aberrations, strengthening the critical role of γ -H2AX in DDR (22).

It is still poorly understood which DSB sensor induces H2AX kinase recruitment. Different models have been proposed to explain ATM relocalization and activation at the break. First, it has been suggested that conformational changes of the DNA activate ATM at the DSB. Another theory suggests that ATM activation is dependent on initial DNA damage detection by the MRN repair complex (Mre11, Rad50, Nbs1) (Fig. 1) (23). In addition, it has been shown that inactivation of human histone acetyltransferases (HAT) of H3 and H4, such as hMOF or TIP60, suppresses ATM activation (24-26). Surprisingly, it has been found that γ -H2AX foci do not form with the same dynamic on different chromatin regions after DNA-damage, but more efficiently in euchromatin (27,28). This phenomenon can be due to either less DSBs generated in heterochromatin, or because heterochromatin features inhibit the large spreading of repair marks near DSBs. In addition, Iacovoni et al. demonstrated that γ -H2AX spreads in a bidirectional but not necessarily symmetrical manner, being influenced by the transcription state of the gene present on the DNA surrounding the DSB (29). Another group demonstrated different dynamics and factors regulating γ -H2AX domains proximal or distal to the break (30). Finally, Durocher and Robert groups have also used γ -H2AX to map in high-resolution the genome-wide fragile sites (31).

Following H2AX phosphorylation, DDR and repair factors accumulate at the break. Indeed, repair factors and checkpoint proteins (MRN, MDC1, BRCA1, 53BP1, UBC13/RNF8, RNF168) and chromatin-remodeling complexes (INO80, SWR1, TIP60-p400) will form foci that colocalize with γ -H2AX (Fig. 1) (22,32-34). The phosphorylation of H2AX itself has been shown to not affect chromatin organization, but rather has a role in the localization of repair factors at the break (35). Although the presence of γ -H2AX is not required for the initial recruitment of signaling and repair factors (33), it is essential for their accumulation and retention at the break, and amplification of the signal (22,32,36). For example, MDC1 directly binds to γ -H2AX via its BRCT domain, and plays a critical role in the accumulation of Nbs1 (subunit of MRN), 53BP1 and ATM (Fig. 1) (37-39). As a consequence, Savic et al. have recently proposed a MDC1- and ATM-dependant γ -H2AX self-reinforcing mechanism that promotes a continued local H2AX phosphorylation (30). The very large domains of γ -H2AX surrounding DSBs are also thought to be binding platforms for cohesins that allow chromosome stability and keep DNA ends in close proximity for the repair process (19).

γ -H2AX is the best-characterized DNA-damage-induced modification, but it has been more recently demonstrated that ubiquitination also occurs rapidly at the break in response to DNA damage. In fact, γ -H2AX mediates the recruitment of the UBC13/RNF8 ubiquitin ligase complex, in a MDC1-dependant manner (40-42), resulting in the poly-ubiquitination of γ -H2AX and H2A at the DSB and this is coordinated with other ubiquitin and sumo ligases (Table1) (34,43). It has also been shown that RNF8-dependant H2A-ubiquitination is implicated in the recruitment of 53BP1 and BRCA1-Abraxas-RAP80 complex via direct binding of RAP80 with poly-UbH2AX (Fig. 2) (44-46). In addition, specific mono-ubiquitination of H2A has been reported at damage sites and can participate in local chromatin remodeling (47).

Methylation of histones H3K79 and H4K20 has been shown to be important in the DSB repair pathway. Even if these marks are not induced by DDR but are constitutively present on chromatin, there are evidences that H4K20me and H3K79me help in the recruitment of repair factors at the DSB. In fission yeast, H4K20me allows the recruitment of Crb2 (fission yeast homologue of 53BP1) through its Tudor domain (48), and Crb2 can also bind γ -H2AX through its BRCT domain (49). Mammalian 53BP1 can directly bind H4K20me2 (50) and this binding may work in conjunction with its BRCT domain-dependant binding to γ -H2AX (Fig1 and 2). Furthermore, H4K91 mono-ubiquitination by hBBAP plays a role in association with H4K20me in 53BP1 recruitment during DDR (51). In budding yeast, methylation of H3K79, catalyzed by yDot1 and promoted by H2BK123Ub, has been implicated in DNA repair. Both H3K79me and H2AS129ph are required for the recruitment of yRad9 (h53BP1) to chromatin, through direct recognition of histone marks by its Tudor and BRCT domains, allowing Rad53/Chk2-dependant checkpoint activation (52-55). It has been suggested that yRad9 is then phosphorylated by ATM, oligomerizes and forms a platform for DDR proteins resulting in checkpoint activation (53). The specific role of these constitutive methyl-marks during DNA repair could be explained by the fact that DNA damage may induce chromatin conformation changes, leading to the exposition of K20me and K79me, which then act like docking sites for the recruitment of signal inducers such as the checkpoint protein 53BP1; but some data cast doubts on such a model (52).

More recently, Xiao et al. identified that WSTF tyrosine (Y) kinase constitutively phosphorylates H2AX on Y142, and this phosphorylation is critical for the DDR (56). They show that H2AX Y142ph decreases when γ -H2AX is induced. They propose that dephosphorylation of H2AX Y142 could enhance MDC1 and ATM recruitment to extend and maintain γ -H2AX after DSB formation. At the same time, EYA1 was shown to be the phosphatase targeting H2AX Y142ph, influencing apoptotic or repair complexes recruitment to γ -H2AX in response to DNA damage (57).

3/ DNA-repair factors access to DNA: Chromatin needs to be remodeled at the break

To achieve accurate DNA damage repair, the chromatin needs to be opened in order to facilitate the access for the repair factors at the site of the DNA lesion. Histone-modifiers and ATP-dependant chromatin remodelers are recruited at the break to modulate the chromatin architecture. The destabilization of the nucleosome is thought to require acetylation of histones through the action of histone acetyltransferases (HATs) such as hTip60/yNuA4 (reviewed in (58)). Human Tip60 and yeast NuA4 HAT, as well as the INO80 and SWR1 Swi2-family ATP-dependant remodelers are recruited to the DSB (Fig. 2), and they can directly interact with γ -H2AX through their common yArp4 subunit (mammalian BAF53) (17,59-61). hTip60/yNuA4 is one the first modifiers appearing at the break where it acetylates H4 and H2A, and promotes the relaxation of the chromatin at the DSB (17,62,63). The mammalian TIP60 complex comprises both Tip60 HAT homologous of yEsa1 in NuA4, and p400/Domino homologous of ySWR1 (64). This overlap of HAT and remodeling activities show that they probably act together on chromatin. Indeed, a study in *Drosophila* showed that TIP60 can acetylate DNA-damage induced phosphoH2Av (a γ -H2AX-like histone variant), mediating the exchange with an unmodified H2Av at the DSB (65). However, whether this model applies to other species is currently unclear even though two studies showed persistence of γ -H2AX in mammalian cells depleted of Tip60 activity (66,67). SWR1 is known to remodel chromatin through its ability to incorporate the H2A variant H2AZ at promoters and subtelomeric regions (68-70). Evidence from yeast models suggest that SWR1 may play a role in H2AZ deposition into chromatin surrounding DSB in absence of INO80 (71). In addition, Altaf et al. have recently reported that H2A and H4 acetylation by yNuA4 directly stimulates SWR1-dependant incorporation of H2AZ-containing H2A-H2B dimers into the nucleosome (72). However, the model of SWR1-dependant H2AZ incorporation at DSBs has been debated by Van Hattikum et al. whose studies have shown that there is no accumulation of H2AZ at the DSB during repair (60). Nevertheless, Kalocsay et al. have recently shown that H2AZ is transiently deposited close to the break, and its sumoylation in combination to Rad51 DNA-binding participates in the relocalization of a persistent DSB to the nuclear periphery (73). Thus, further studies need to investigate the function of SWR1 during DSB repair and whether Tip60/NuA4-dependant acetylation of H2A and H4 may be required for γ -H2AX exchange at the break. One report suggests that Tip60-dependant acetylation and removal of H2AX functions through stimulation of the histone ubiquitination by UBC13 (67).

yINO80 complex is rapidly recruited close to the DSB by the direct interaction of its Arp4 and/or Nhp10 subunits with γ -H2AX and influenced by NuA4-dependant acetylation (17,59,61). INO80 has been reported to mediate removal of core histones, containing or not H2AZ and H2AX, from region surrounding the DSB (Fig. 2). This nucleosome remodeling then allows resection of DNA at the DSB (60,74). Furthermore, studies have also shown that yINO80 is required for maintaining high level of γ -H2AX during DNA-repair (71).

Another chromatin remodeler, RSC (member of the SWI/SNF family) is present at the break before INO80 and SWR1. In opposition to INO80 and SWR1, its presence at the break is not γ -H2AX-dependant, and it was shown to interact with MRN (Fig. 1) (75,76). RSC has been shown to be required for yeast Tel1/Mec1 kinases (homologues of mammalian ATM/ATR) and yRad9 recruitment at the break (77), suggesting that RSC is an early sensor of the DSB (66). However, it is not clear whether RSC or MRN appears first at the break, their recruitment being dependant on each other (76,78). Furthermore, it has been proposed that in mammalian cells, chromatin decondensation in the vicinity of DSB is dependant on ATP-dependant chromatin remodelers, but not on phosphorylation of H2AX, suggesting a very early function in DSB repair (79). In fact, mammalian SWI/SNF remodeler is critical for

efficient induction of γ -H2AX (80), since inhibition of its catalytic core subunits, BRG1 and Brm, compromises phosphorylation of H2AX and γ -H2AX foci formation. More recently, Lee et al. have shown that SWI/SNF binds to γ -H2AX-containing nucleosomes via an interaction between its bromodomain-containing BRG1 subunit with GCN5-dependant acetylated H3, and this binding is important for DSB repair (81).

Altogether, data from the literature indicate that a specific combinations of chromatin marks and ATP-dependent chromatin remodeling allows binding of DNA repair factors and healing of the DNA lesion.

4/ Signaling the end of repair: restoration of chromatin to its initial state

Modulation of chromatin architecture mediated by chromatin modifiers and remodelers is an essential process for DNA damage repair. First, it allows repair factors to access to the damaged DNA (as discussed above), but it is also an important mechanism for switching off the DNA-damage signal. Thus, after repair has been completed, the cell clears the marks associated to DNA-damage signal and restores chromatin organization to its initial state. These processes are essential to recover from the checkpoint arrest and re-enter the cell cycle.

In order to signal to the cell that the repair process is achieved, γ -H2AX is eliminated from chromatin surrounding the repaired DSB, by either eviction or dephosphorylation (Fig. 3). It has been speculated that the presence of SWR1 at the break may allow γ -H2AX removal from chromatin surrounding DSB (82). Some data suggest that yINO80 and ySWR1 function antagonistically at the DSB, yINO80 maintaining the high level of γ -H2AX and ySWR1 replacing it with H2AZ variant (71). Another simple mechanism to get rid of γ -H2AX would be its dephosphorylation (Fig. 3). The HTP-C complex, containing the Pph3 phosphatase catalytic subunit, has been identified in yeast to be the γ -H2AX phosphatase, while the human phosphatase function is attributed to both PP2A and PP4C (83-86). These reports suggest that yPph3 and hPP4C (human Pph3 ortholog) have a function in the checkpoint termination. Interestingly, yPph3 is thought to dephosphorylate γ -H2AX after its removal from chromatin, whereas hPP4C-dependant dephosphorylation appears to take place in the chromatin. It still remains to be determined if hPP2A dephosphorylates γ -H2AX directly on chromatin or displaced γ -H2AX. Very recently, other phosphatases have been characterized, hPP6 and hWip1 (87,88). hPP6 has been shown to contribute to γ -H2AX dephosphorylation and subsequent checkpoint release (88). hWip1, whose expression is induced after DNA-damage, can bind to chromatin, co-localizes with γ -H2AX foci and regulates γ -H2AX dephosphorylation during recovery (87). It is interesting to notice that yeast γ -H2AX spread out over 50kb surrounding the DSB, but is less abundant as close as 1 or 2kb from the DSB, where INO80 and MRN are most present (17,20). It is then logical to speculate that γ -H2AX is evicted from chromatin proximal to DSB, whereas it is dephosphorylated in the chromatin farther away. This model is supported by two different dynamics of γ -H2AX accumulation/removal depending on the position relative to the break (30).

As we described above, the relaxation of chromatin for efficient repair involves HAT activities at the DSB, where they induce an increase of acetylation. Several histone deacetylases (HDAC) have thus been implicated in the DDR, but mainly once repair has been completed (Fig. 3) (89,90). In opposition to HAT inducing chromatin “opening”, HDAC may have a role in chromatin restoration. In yeast, Sin3/Rpd3, Sir2 and Hst1 HDAC have been shown to facilitate DNA repair (89-91). In addition, mammalian Hdac3 has been linked to DNA damage repair, but its exact role is still unknown (92).

In yeast, Sin3/Rpd3 HDAC complex interacts with the casein kinase CK2 responsible for H4S1 phosphorylation, and this modification has been shown to increase at the break at the end of repair (Fig. 3) (91,93). Interestingly, the phosphorylation of H4S1 inhibits acetylation of the adjacent lysine residues by NuA4, suggesting that H4S1ph appears at the break after repair completion to prevent new acetylation and to stabilize the nucleosome.

In mammalian cells, histone H2B has also been reported to be phosphorylated on S14 following DNA damage (94). H2BS14ph appears at late time points and accumulates in repair foci in a γ -H2AX-dependant manner. Phosphorylation of H2BS14 by hMst1 kinase has a role in apoptotic-dependant chromatin compaction, so we can propose that increased level of H2BS14ph at the site of repaired DNA may contribute to chromatin stabilization after restoration.

It is logical to predict that histone chaperones such as CAF1 (Chromatin Assembly Factor 1), Asf1 or FACT, are involved and required for chromatin remodeling. Indeed, they are recruited to the site of DNA damage, where they mediate nucleosome disassembly and reassembly (reviewed in (95)). To date, there is no evidence that chaperones play an active role in chromatin disassembly during DNA repair, whereas they are clearly implicated in nucleosome reassembly. In yeast and human, the CAF1 chaperone is recruited to UV-damaged sites and DSB, and with the help of Asf1, deposits H3-H4 onto the DNA (Fig. 3) (96-99). CAF1 is also required for hRing1b-dependant H2AK119 mono-ubiquitination, a mark involved in chromatin restoration after UV-induced damages (100). Asf1 association with yeast Rtt109 and human CBP/p300 or Gcn5 HAT is essential for H3K56 acetylation and, in yeast, acetylation of H3K56 is required for effective DDR (Fig. 3) (101-108). It has been suggested that Asf1 and CAF1 function would be mainly required for checkpoint recovery and chromatin restoration after repair, and that H3K56ac would signal the completion on chromatin reassembly (109,110), followed by its deacetylation by Hst2/Hst3 sirtuins (111,112).

In addition to H3K56ac, H3K14/K23 acetylation and acetyltransferase yHat1 are also linked to chromatin restoration. In fact, epistasis analysis has determined that yHat1 influences DSB-repair chromatin reassembly through an interaction with Asf1 but not CAF1 (Fig. 3) (113). Furthermore, Hat1 has been reported to acetylate free H4 on K5 and K12, and H4K5/K12ac would also play a role during DNA damage recovery (114,115). Finally, while histone H4 ubiquitination has been linked to DDR, it is also known that acetylation of the same residue is important for chromatin assembly after repair (116). Further studies will be required to investigate the exact function of these marks during chromatin reassembly following DSB repair.

5/Conclusion-Future directions

Maintenance of genomic stability in eukaryotic cells required a tight regulation of histone modifications that accompany DDR. Although DNA-damage repair kinetics have been extensively studied, the precise order of histone modifiers, repair factors and remodelers recruitment remains imprecise. It appears more evident that a number of recruited factors regulate each other's accumulation and activation, rendering the study of the specific function of each factor more difficult. Here, we have mentioned the role of histone modifications during DNA DSB repair full process. It is clear that the timing and the cross-talk between histone marks are critical in the process of chromatin dynamic.

Moreover, beside histone marks appearing at the DSB (to signal, recruit repair factors and promote chromatin remodeling), other histone modifications induced by DNA-damage have been identified but not discussed in this review. Recent studies have shown an effect of

DNA-damage induced histone modifications on transcription regulation. For example, ATM and histone ubiquitination have been linked to transcription silencing near DSBs (117). In addition, loss of H3T11 phosphorylation by hChk1 has been shown to repress transcription of cyclinB and cdk1 after DNA-damage induction, through loss of Gcn5-dependant promoter acetylation (118). hEco1, which is an acetyltransferase important for sister cohesion during S phase and DDR, has also been shown to repress transcription by interaction with the hLSD1 histone demethylase (119,120).

It will be interesting then to continue to investigate the cooperation of the factors involved in the repair of DNA DSBs, but also the direct or indirect effect of DNA damage on non-repair processes such as specific transcription regulation.

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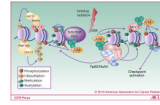


Figure 1.

Model of histone modifications and chromatin remodeling during DNA DSB repair, **Step 1:** Recognition and signaling of a DSB. γ -H2AX plays a key role in DNA-damage signaling, acting as a platform of assembly for the repair factors as well as for checkpoint proteins. Immediately following the apparition of a DSB, the MRN complex binds DNA-ends and participates in ATM kinase recruitment. ATM then rapidly phosphorylates H2AX histone variant at the site of the break. Phospho-H2AX, also called γ -H2AX allows the binding, retention and accumulation at the break of the complexes involved in the DDR. The simultaneous presence of the RSC remodeling complex at the break may facilitate the access of the recruited repair factors. Indeed, the mediator protein MDC1 is recruited to the DSB and binds γ -H2AX, where it promotes further ATM and MRN accumulation. As a consequence, γ -H2AX bi-directionally spreads out from the DSB (approximately 2Mb), thus increasing the accumulation of repair factors. MDC1 also recruits RNF8/UBC13 ubiquitin-ligase which ubiquitinates H2A and H2AX, which in turn, is recognized by RNF168-UBC13 H2AX-ubiquitin-ligase complex, resulting in the amplification of γ -H2AX poly-ubiquitination near the DSB. In parallel, γ -H2AX also permits TIP60 HAT recruitment at the break, followed by the acetylation of H2A and H4 histones and destabilization of the nucleosomes. In addition, phosphorylation of H2AX could induce conformational changes in the nucleosome, resulting in the exposition of H4K20me and H3K79me, recognized by the checkpoint protein 53BP1.

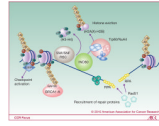


Figure 2.

Model of histone modifications and chromatin remodeling during DNA DSB repair, **Step 2:** Opening of chromatin to repair the break. Once the DSB has been recognized and signaled, it is time to repair the break. Histones need to be removed from chromatin in the vicinity of the break to allow access to the DNA to the repair factors. Chromatin-remodelers are then recruited to the DSB. TIP60 complex recruited at the DSB comprises HAT activity as well as histone exchange ability. Following acetylation-dependant nucleosome destabilization, TIP60 complex can remove H2A(X)-H2B histone dimers from chromatin at the break. INO80 is also recruited at the break where it helps removing histones close to the DSB. The SWI/SNF/RSC/BRG-1 remodeling complex is also present at the break where it can associate with γ -H2AX and promote histone eviction or exchange. Such histone eviction allows association of the ssDNA-binding protein RPA with resected DNA and subsequent recruitment of repair factors such as Rad51. Moreover, BRCA1-A repair complex accumulates at the break through direct interaction of its RAP80 subunit with poly-UbH2A(X).

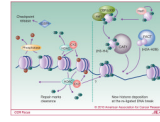


Figure 3.

Model of histone modifications and chromatin remodeling during DNA DSB repair, **Step 3:** Chromatin restoration after DNA-break repair. When repair of the DSB is completed, the chromatin needs to be restored and the repair specific histone marks need to be removed in order to release repair factors and cell cycle checkpoints. Thus, γ -H2AX has to disappear from the repaired site. Phosphatases such as PP2A and PP4C dephosphorylate γ -H2AX and allow release of checkpoint factors like 53BP1. In order to restore chromatin, new histones are deposited onto the DNA. Histone chaperones such as FACT and CAF1 have been implicated in this process. Moreover, H3-H4 histones deposited by CAF1 are first acetylated by Hat1, and then by CBP/p300/Rtt109-Asf1, as marks of new synthesized histones. This incorporation of new histones is thought to occur at the site of the repaired DNA. More distal to the site, repair marks are removed from nucleosomal histones in the chromatin context. Acetyl-marks associated with chromatin “opening” are eliminated by HDACs. γ Sin3/Rpd3 HDAC associates with Casein Kinase 2 (CK2) that is responsible for subsequent phosphorylation of H4S1, reinforcing nucleosome stability by blocking re-acetylation.

Table1

Histone modifications influencing DNA-damage response.

Step of DDR	Histone residue (human)	Type of modification	Enzyme
1/ Signaling	H2AX S139	Phosphorylation	ATM/ATR, DNA-PK
	H2A/H2AX	Ubiquitination	RNF8/RNF168
	H4 K20	Methylation	Set8/Suv4-20
	H3 K79	Methylation	Dot1
	H2AX Y142	Dephosphorylation	EYA1
	H4 K91	Mono-ubiquitination	BBAP
	H2AZ K126/133	Sumoylation	?
2/ Opening	H4/H2A(X)	Acetylation	Tip60/yNuA4
	H3 K9	Acetylation	Gcn5, CBP/p300
3/ Restoring	H2AX S139	Dephosphorylation	yPph3/hPP4, PP2A, PP6, Wip1
	H3/H4 K	Deacetylation	Sin3/Rpd3, Sir2, Hst1/3/4
	H4 S1	Phosphorylation	Casein Kinase 2 (CK2)
	H2B S14	Phosphorylation	Ste20
	H3 K56	Acetylation	yRtt109, CBP/p300, Gcn5
	H3 K14, K23	Acetylation	Gcn5
	H4 K5, K12	Acetylation	Hat1
	H4 K91	Acetylation	Hat1
	H2A K119	Mono-ubiquitination	Ring1b/Ring2