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# Epigenetic Modifications in Double-Strand Break DNA Damage Signaling and Repair

Dorine Rossetto[1], Andrew W. Truman[2], Stephen J. Kron[2], and Jacques Côté[1]

## 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. These targets 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 posttranslational modifications of histones that are critical for DNA damage recognition and/or 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. *Clin Cancer Res; 16(18); 4543–52. ©2010 AACR.*

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 (DDR) 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 because it can lead to loss of genetic material (2). DSBs are mainly repaired by homologous recombination (HR) and nonhomologous 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 of the direct ligation of the two broken ends, which can produce short deletions.

In eukaryotic cells, 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 146 bp of DNA. The chromatin is a dynamic structure that regulates DNA accessibility during essential nuclear events, such as 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 biophysical features; posttranslational histone modifications mainly occurring on histone tails protruding from the nucleosome; ATP-dependent 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 and/or disassembly (3).

After DNA damage induction, the chromatin needs to be in an "open" state in order to allow the repair factors to access 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 DSB repair. Drugs that target the chromatin modifiers' or readers' implicated DNA-damage response have very high therapeutic potential (see refs. 4–6 and accompanying *Focus* reviews; refs. 7–10).

## 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

**Authors' Affiliations:** [1]Laval University Cancer Research Center, Hôtel-Dieu de Québec (CHUQ), Quebec City, Canada and [2]Department of Molecular Genetics and Cell Biology, Ludwig Center for Metastasis Research, The University of Chicago, Chicago, Illinois

**Corresponding Author:** Jacques Côté, Laval University, Hotel-Dieu de Quebec (CHUQ), 9 McMahon Street, Quebec City, QC G1R 2J6, Canada. Phone: 418-525-4444; Fax: 418-691-5439; E-mail: jacques.cote@crhdq.ulaval.ca.

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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 a few minutes following the break, and is probably the first histone modification appearing in its vicinity (reviewed in refs. 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; refs. 12, 13]. The kinases responsible for this modification have been identified as phosphatidylinositol 3-kinase–related kinases (PIKK): the ataxia-telangiectasia mutated (ATM), ATM- and Rad3-related (ATR), and the DNA-dependent protein kinase (DNA-PK). ATM and DNA-PK principally function after ionizing radiation, whereas ATR responds to replication stress and UV irradiation (14–16). In human cells, γ-H2AX spreads over more than 1 Mb on each side of the break (50 kb in yeast; refs. 13, 17–20), thus amplifying the repair signal, which makes it easily detectable by immunofluorescence and commonly used as a biomarker of DNA-damage nuclear foci (for review, see ref. 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 Mre11, Rad50, Nbs1 (MRN) repair complex (Fig. 1; ref. 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 form more efficiently in euchromatin (27, 28). This phenomenon can be either due to fewer DSBs being generated in heterochromatin, or to the fact that heterochromatin features inhibit the large spreading of repair marks near DSBs. In addition, Iacovoni and colleagues showed 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 showed different dynamics and factors regulating γ-H2AX domains proximal or distal to the break (30). Finally, the Durocher and Robert groups have also used γ-H2AX to map the genome-wide fragile sites in high resolution (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; refs. 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

**Table 1.** Histone modifications influencing DNA damage response

| DDR step | 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 | not identified |
| 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 | Deactetylation | Sin3/Rpd3, Sir2, Hst1/3/4 |
| | H4 S1 | Phosphorylation | 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 |

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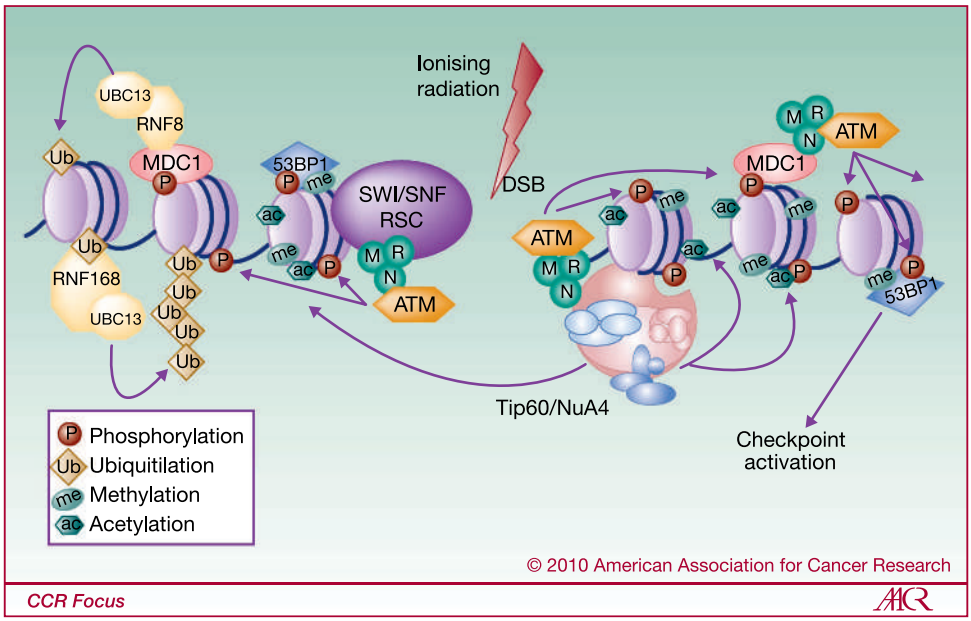


**Fig. 1.** Model of histone modifications and chromatin remodeling during DNA DSB repair, step 1: Recognition and signaling of a DSB. γ-H2AX plays a key role in DNA damage signaling, acting as a platform of assembly for the repair factors as well as for checkpoint proteins. Immediately following the apparition of a DSB, the MRN complex binds DNA ends and participates in ATM kinase recruitment. ATM then rapidly phosphorylates the 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 bidirectionally spreads out from the DSB (approximately 2 Mb), 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 polyubiquitination 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.

BRCT domain, and plays a critical role in the accumulation of Nbs1 (subunit of MRN), 53BP1, and ATM (Fig. 1; refs. 37–39). As a consequence, Savic and colleagues have recently proposed a MDC1- and ATM-dependent 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 shown 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 an MDC1-dependent manner (40–42), resulting in the polyubiquitination of γ-H2AX and H2A at the DSB, and this is coordinated with other ubiquitin and sumo ligases (Table 1; refs. 34, 43). It has also been shown that RNF8-dependent H2A-ubiquitination is implicated in the recruitment of 53BP1 and BRCA1-Abraxas-RAP80 complex via direct binding of RAP80 with poly-UbH2AX (Fig. 2; refs. 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, evidence shows 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 homolog 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–dependent binding to γ-H2AX (Figs. 1 and 2). Furthermore, H4K91 monoubiquitination 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-dependent 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

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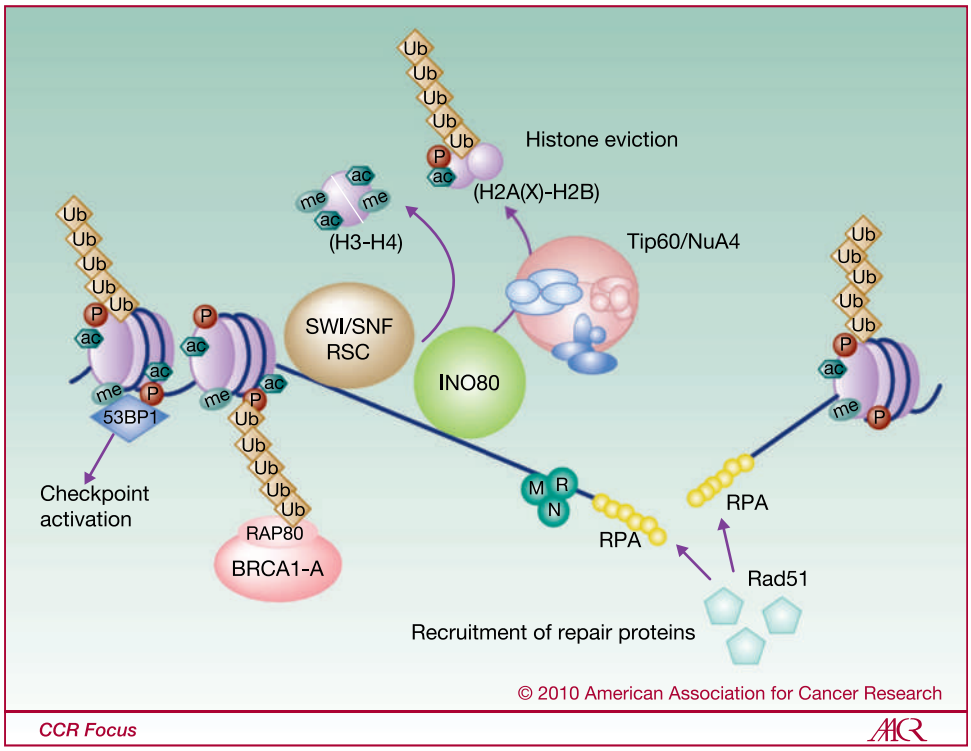


**Fig. 2.** Model of histone modifications and chromatin remodeling during DNA DSB repair, step 2: Opening of chromatin to repair the break. Once the DSB has been recognized and signaled, it is time to repair the break. Histones need to be removed from chromatin in the vicinity of the break to allow 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-dependent 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 to remove 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).

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; however, some data cast doubts on such a model (52).

More recently, Xiao and colleagues 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 complex recruitment to γ-H2AX in response to DNA damage (57).

## 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 access for the repair factors at the site of the DNA lesion. Histone modifiers and ATP-dependent 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 HATs such as hTip60/yNuA4 (reviewed in ref. 58). Human Tip60 and yeast NuA4 HAT, as well as the INO80 and SWR1 Swi2-family ATP-dependent remodelers, are recruited to the DSB (Fig. 2), and they can directly interact with γ-H2AX through their common yArp4 subunit (mammalian BAF53; refs. 17, 59–61). hTip60/yNuA4 is one of 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

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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 suggests that SWR1 may play a role in H2AZ deposition into chromatin surrounding DSB in absence of INO80 (71). In addition, Altaf and colleagues have recently reported that H2A and H4 acetylation by yNuA4 directly stimulates SWR1-dependent incorporation of H2AZ-containing H2A-H2B dimers into the nucleosome (72). However, the model of SWR1-dependent H2AZ incorporation at DSBs has been debated by Van Hattikum and colleagues, whose studies have shown that there is no accumulation of H2AZ at the DSB during repair (60). Nevertheless, Kalocsay and colleagues have recently shown that H2AZ is transiently deposited close to the break, and its sumoylation, in combination with 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-dependent acetylation of H2A and H4 may be required for γ-H2AX exchange at the break. One report suggests that Tip60-dependent 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-dependent acetylation (17, 59, 61). INO80 has been reported to mediate removal of core histones, containing or not containing H2AZ and H2AX, from the 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 a 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–dependent, and it was shown to interact with MRN (Fig. 1; refs. 75, 76). RSC has been shown to be required for yeast Tel1/Mec1 kinases (homologs 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 dependent on each other (76, 78). Furthermore, it has been proposed that in mammalian cells, chromatin decondensation in the vicinity of DSB is dependent on ATP-dependent 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), because inhibition of its catalytic core subunits, BRG1 and Brm, compromises phosphorylation of H2AX and γ-H2AX foci formation. More recently, Lee and colleagues have shown that SWI/SNF binds to γ-H2AX–containing nucleosomes via an interaction between its bromodomain-containing BRG1 subunit with GCN5-dependent acetylated H3, and this binding is important for DSB repair (81).

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

## Signaling the End of Repair: Restoration of Chromatin to Its Initial State

Modulation of chromatin architecture that is mediated by chromatin modifiers and remodelers is an essential process for DNA damage repair. First, it allows repair factors access to the damaged DNA (as discussed above), and 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 with the DNA damage signal and restores chromatin organization to its initial state. These processes are essential to recover from the checkpoint arrest and reenter 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 the 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 the 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 as the γ-H2AX phosphatase, whereas 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-dependent dephosphorylation seems 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, colocalizes with γ-H2AX foci, and regulates γ-H2AX dephosphorylation during recovery (87). It is interesting to notice that yeast γ-H2AX spreads out over 50 kb surrounding the DSB, but is less abundant as close as 1 or 2 kb 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 and/or removal, depending on the position relative to the break (30).

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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; refs. 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, the Sin3/Rpd3 HDAC complex interacts with the casein kinase [casein kinase 2 (CK2)] responsible for H4S1 phosphorylation, and this modification has been shown to increase at the break at the end of repair (Fig. 3; refs. 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–dependent manner. Phosphorylation of H2BS14 by hMst1 kinase has a role in apoptotic-dependent chromatin compaction, so we can propose that an 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 Chromatin Assembly Factor 1 (CAF1), 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 ref. 95). To date, no evidence shows 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 DSBs, and with the help of Asf1, deposits H3-H4 onto the DNA (Fig. 3; refs. 96–99). CAF1 is also required for hRing1b-dependent 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;

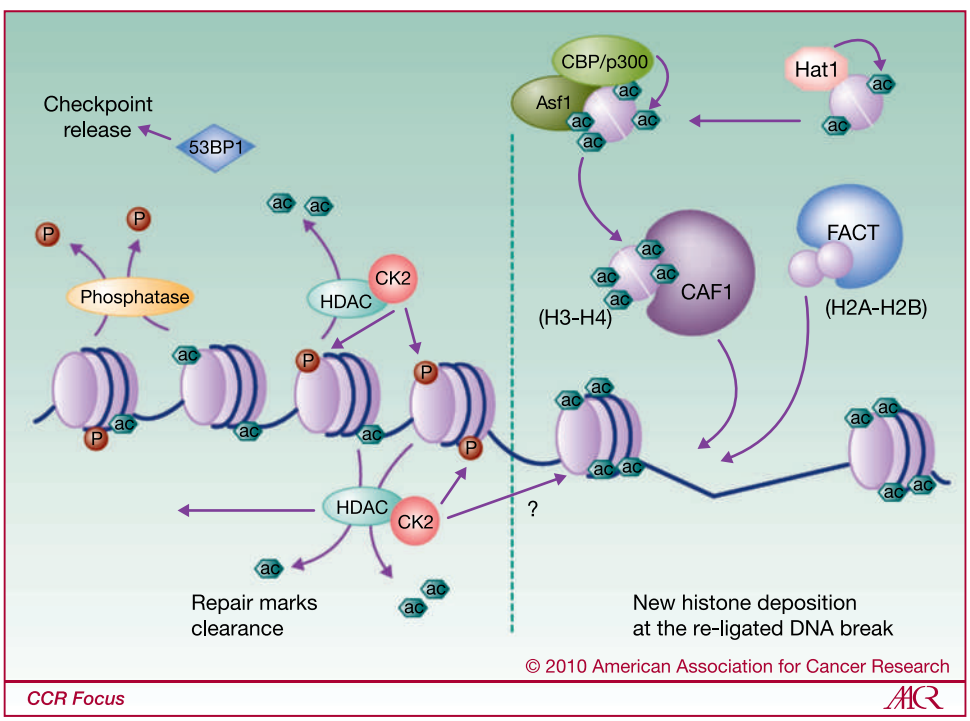


**Fig. 3.** Model of histone modifications and chromatin remodeling during DNA DSB repair, step 3: Chromatin restoration after DNA break repair. When repair of the DSB is completed, the chromatin needs to be restored, and the repair-specific histone marks need to be removed in order to release repair 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 though 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. ySin3/Rpd3 HDAC associates with CK2, which is responsible for subsequent phosphorylation of H4S1, reinforcing nucleosome stability by blocking reacetylation.

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refs. 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; ref. 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, whereas 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.

## Conclusion and Future Directions

Maintenance of genomic stability in eukaryotic cells requires a tight regulation of histone modifications that accompany DDR. Although DNA-damage repair kinetics have been extensively studied, the exact order of histone modifiers, repair factors, and remodeler recruitment remains imprecise. It seems 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 the full process of DNA DSB repair. It is clear that the timing and cross-talk between histone marks are critical in the process of the chromatin dynamic.

Moreover, in addition to 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). Furthermore, loss of H3T11 phosphorylation by hChk1 has been shown to repress transcription of cyclinB and cdk1 after DNA damage induction, through loss of Gcn5-dependent 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, as well as to investigate the direct or indirect effect of DNA damage on nonrepair processes such as specific transcription regulation.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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## References

1. De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis 2004;19:169–85.
2. Peterson CL, Cote J. Cellular machineries for chromosomal DNA repair. Genes Dev 2004;18:602–16.
3. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007;128:707–19.
4. Balch C, Montgomery JS, Paik HI, et al. New anti-cancer strategies: epigenetic therapies and biomarkers. Front Biosci 2005;10:1897–931.
5. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 2007;8:286–98.
6. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128:683–92.
7. Plummer R. Perspective on the pipeline of drugs being developed with modulation of DNA damage as a target. Clin Cancer Res 2010;16:4527–31.
8. Redon CE, Nakamura AJ, Zhang Y, et al. Histone γH2AX and Poly (ADP ribose) as clinical pharmacodynamic biomarkers. Clin Cancer Res 2010;16:4532–42.
9. Annunziata CM, O'Shaughnessy J. Poly(ADP-Ribose) Polymerase as a novel therapeutic target in cancer. Clin Cancer Res 2010;16:4517–26.
10. Chan N, Bristow RG. "Contextual" synthetic lethality/loss of heterozygosity: tumor hypoxia and modification of DNA repair. Clin Cancer Res 2010;16:4553–60.
11. Thiriet C, Hayes JJ. Chromatin in need of a fix: phosphorylation of H2AX connects chromatin to DNA repair. Mol Cell 2005;18:617–22.
12. Downs JA, Lowndes NF, Jackson SP. A role for Saccharomyces cerevisiae histone H2A in DNA repair. Nature 2000;408:1001–4.
13. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 1998;273:5858–68.
14. Ward IM, Chen J. Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. J Biol Chem 2001;276:47759–62.
15. Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lobrich M, Jeggo PA. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. Cancer Res 2004;64:2390–6.

Published OnlineFirst September 7, 2010; DOI:10.1158/1078-0432.CCR-10-0513




16. An J, Huang YC, Xu QZ, et al. DNA-PKcs plays a dominant role in the regulation of H2AX phosphorylation in response to DNA damage and cell cycle progression. BMC Mol Biol 2010;11:18.
17. Downs JA, Allard S, Jobin-Robitaille O, et al. Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. Mol Cell 2004;16:979–90.
18. Rogakou EP, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in DNA double-strand breaks *in vivo*. J Cell Biol 1999;146:905–16.
19. Unal E, Arbel-Eden A, Sattler U, et al. DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol Cell 2004;16:991–1002.
20. Shroff R, Arbel-Eden A, Pilch D, et al. Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr Biol 2004;14:1703–11.
21. Mah LJ, El-Osta A, Karagiannis TC. γ-H2AX: a sensitive molecular marker of DNA damage and repair. Leukemia 2010;24:679–86.
22. Celeste A, Petersen S, Romanienko PJ, et al. Genomic instability in mice lacking histone H2AX. Science 2002;296:922–7.
23. Lee JH, Paull TT. Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. Oncogene 2007;26:7741–8.
24. Gupta A, Sharma GG, Young CS, et al. Involvement of human MOF in ATM function. Mol Cell Biol 2005;25:5292–305.
25. Sun Y, Jiang X, Chen S, Fernandes N, Price BD. A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. Proc Natl Acad Sci U S A 2005;102:13182–7.
26. Sharma GG, So S, Gupta A, et al. MOF and histone H4 acetylation at lysine 16 are critical for DNA damage response and double-strand break repair. Mol Cell Biol 2010;30:3582–95.
27. Kim JA, Kruhlak M, Dotiwala F, Nussenzweig A, Haber JE. Heterochromatin is refractory to γ-H2AX modification in yeast and mammals. J Cell Biol 2007;178:209–18.
28. Cowell IG, Sunter NJ, Singh PB, Austin CA, Durkacz BW, Tilby MJ. γ-H2AX foci form preferentially in euchromatin after ionising-radiation. PLoS ONE 2007;2:e1057.
29. Iacovoni JS, Caron P, Lassadi I, et al. High-resolution profiling of γ-H2AX around DNA double strand breaks in the mammalian genome. EMBO J 2010;29:1446–57.
30. Savic V, Yin B, Maas NL, et al. Formation of dynamic γ-H2AX domains along broken DNA strands is distinctly regulated by ATM and MDC1 and dependent upon H2AX densities in chromatin. Mol Cell 2009;34:298–310.
31. Szilard RK, Jacques PE, Laramee L, et al. Systematic identification of fragile sites via genome-wide location analysis of γ-H2AX. Nat Struct Mol Biol 2010;17:299–305.
32. Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr Biol 2000;10:886–95.
33. Celeste A, Fernandez-Capetillo O, Kruhlak MJ, et al. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat Cell Biol 2003;5:675–9.
34. van Attikum H, Gasser SM. Crosstalk between histone modifications during the DNA damage response. Trends Cell Biol 2009;19:207–17.
35. Fink M, Imholz D, Thoma F. Contribution of the serine 129 of histone H2A to chromatin structure. Mol Cell Biol 2007;27:3589–600.
36. Bassing CH, Chua KF, Sekiguchi J, et al. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. Proc Natl Acad Sci U S A 2002;99:8173–8.
37. Lukas C, Melander F, Stucki M, et al. Mdc1 couples DNA double-strand break recognition by Nbs1 with its H2AX-dependent chromatin retention. EMBO J 2004;23:2674–83.
38. Lou Z, Minter-Dykhouse K, Franco S, et al. MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. Mol Cell 2006;21:187–200.
39. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell 2005;123:1213–26.
40. Mailand N, Bekker-Jensen S, Faustrup H, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell 2007;131:887–900.
41. Kolas NK, Chapman JR, Nakada S, et al. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science 2007;318:1637–40.
42. Huen MS, Grant R, Manke I, et al. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell 2007;131:901–14.
43. Galanty Y, Belotserkovskaya R, Coates J, Polo S, Miller KM, Jackson SP. Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature 2009;462:935–9.
44. Sobhian B, Shao G, Lilli DR, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science 2007;316:1198–202.
45. Marteijn JA, Bekker-Jensen S, Mailand N, et al. Nucleotide excision repair-induced H2A ubiquitination is dependent on MDC1 and RNF8 and reveals a universal DNA damage response. J Cell Biol 2009;186:835–47.
46. Wang B, Elledge SJ. Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brca1/Brcc36 complex in response to DNA damage. Proc Natl Acad Sci U S A 2007;104:20759–63.
47. Bergink S, Salomons FA, Hoogstraten D, et al. DNA damage triggers nucleotide excision repair-dependent monoubiquitylation of histone H2A. Genes Dev 2006;20:1343–52.
48. Sanders SL, Portoso M, Mata J, Bahler J, Allshire RC, Kouzarides T. Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage. Cell 2004;119:603–14.
49. Nakamura TM, Moser BA, Du LL, Russell P. Cooperative control of Crb2 by ATM family and Cdc2 kinases is essential for the DNA damage checkpoint in fission yeast. Mol Cell Biol 2005;25:10721–30.
50. Botuyan MV, Lee J, Ward IM, et al. Structural basis for the methylation state-specific recognition of histone H4–20 by 53BP1 and Crb2 in DNA repair. Cell 2006;127:1361–73.
51. Yan Q, Dutt S, Xu R, et al. BBAP monoubiquitylates histone H4 at lysine 91 and selectively modulates the DNA damage response. Mol Cell 2009;36:110–20.
52. Javaheri A, Wysocki R, Jobin-Robitaille O, Altaf M, Cote J, Kron SJ. Yeast G1 DNA damage checkpoint regulation by H2A phosphorylation is independent of chromatin remodeling. Proc Natl Acad Sci U S A 2006;103:13771–6.
53. Nnakwe CC, Altaf M, Cote J, Kron SJ. Dissection of Rad9 BRCT domain function in the mitotic checkpoint response to telomere uncapping. DNA Repair (Amst) 2009;8:1452–61.
54. Wysocki R, Javaheri A, Allard S, Sha F, Cote J, Kron SJ. Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9. Mol Cell Biol 2005;25:8430–43.
55. Toh GW, O'Shaughnessy AM, Jimeno S, et al. Histone H2A phosphorylation and H3 methylation are required for a novel Rad9 DSB repair function following checkpoint activation. DNA Repair (Amst) 2006;5:693–703.
56. Xiao A, Li H, Shechter D, et al. WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. Nature 2009;457:57–62.
57. Cook PJ, Ju BG, Telese F, Wang X, Glass CK, Rosenfeld MG. Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. Nature 2009;458:591–6.
58. Carrozza MJ, Utley RT, Workman JL, Cote J. The diverse functions of histone acetyltransferase complexes. Trends Genet 2003;19:321–9.
59. Morrison AJ, Highland J, Krogan NJ, et al. INO80 and γ-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell 2004;119:767–75.
60. van Attikum H, Fritsch O, Gasser SM. Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. EMBO J 2007;26:4113–25.
61. van Attikum H, Fritsch O, Hohn B, Gasser SM. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell 2004;119:777–88.

Published OnlineFirst September 7, 2010; DOI:10.1158/1078-0432.CCR-10-0513




62. Bird AW, Yu DY, Pray-Grant MG, et al. Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. Nature 2002; 419:411–5.
63. Murr R, Loizou JI, Yang YG, et al. Histone acetylation by Trrap-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks. Nat Cell Biol 2006;8:91–9.
64. Auger A, Galarneau L, Altaf M, et al. Eaf1 is the platform for NuA4 molecular assembly that evolutionarily links chromatin acetylation to ATP-dependent exchange of histone H2A variants. Mol Cell Biol 2008;28:2257–70.
65. Kusch T, Florens L, Macdonald WH, et al. Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. Science 2004;306:2084–7.
66. Jha S, Shibata E, Dutta A. Human Rvb1/Tip49 is required for the histone acetyltransferase activity of Tip60/NuA4 and for the down-regulation of phosphorylation on H2AX after DNA damage. Mol Cell Biol 2008;28:2690–700.
67. Ikura T, Tashiro S, Kakino A, et al. DNA damage-dependent acetylation and ubiquitination of H2AX enhances chromatin dynamics. Mol Cell Biol 2007;27:7028–40.
68. Krogan NJ, Baetz K, Keogh MC, et al. Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. Proc Natl Acad Sci U S A 2004;101:13513–8.
69. Kobor MS, Venkatasubrahmanyam S, Meneghini MD, et al. A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biol 2004;2:E131.
70. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 2004;303:343–8.
71. Papamichos-Chronakis M, Krebs JE, Peterson CL. Interplay between Ino80 and Swr1 chromatin remodeling enzymes regulates cell cycle checkpoint adaptation in response to DNA damage. Genes Dev 2006;20:2437–49.
72. Altaf M, Auger A, Monnet-Saksouk J, et al. NuA4-dependent acetylation of nucleosomal histone H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex. J Biol Chem 2010;285: 15966–77.
73. Kalocsay M, Hiller NJ, Jentsch S. Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. Mol Cell 2009;33:335–43.
74. Tsukuda T, Fleming AB, Nickoloff JA, Osley MA. Chromatin remodelling at a DNA double-strand break site in Saccharomyces cerevisiae. Nature 2005;438:379–83.
75. Chai B, Huang J, Cairns BR, Laurent BC. Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. Genes Dev 2005;19:1656–61.
76. Shim EY, Ma JL, Oum JH, Yanez Y, Lee SE. The yeast chromatin remodeler RSC complex facilitates end joining repair of DNA double-strand breaks. Mol Cell Biol 2005;25:3934–44.
77. Liang B, Qiu J, Ratnakumar K, Laurent BC. RSC functions as an early double-strand-break sensor in the cell's response to DNA damage. Curr Biol 2007;17:1432–7.
78. Shim EY, Hong SJ, Oum JH, Yanez Y, Zhang Y, Lee SE. RSC mobilizes nucleosomes to improve accessibility of repair machinery to the damaged chromatin. Mol Cell Biol 2007;27:1602–13.
79. Kruhlak MJ, Celeste A, Dellaire G, et al. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. J Cell Biol 2006;172:823–34.
80. Park JH, Park EJ, Lee HS, et al. Mammalian SWI/SNF complexes facilitate DNA double-strand break repair by promoting γ-H2AX induction. EMBO J 2006;25:3986–97.
81. Lee HS, Park JH, Kim SJ, Kwon SJ, Kwon J. A cooperative activation loop among SWI/SNF, γ-H2AX and H3 acetylation for DNA double-strand break repair. EMBO J 2010;29:1434–45.
82. Altaf M, Saksouk N, Cote J. Histone modifications in response to DNA damage. Mutat Res 2007;618:81–90.
83. Chowdhury D, Keogh MC, Ishii H, Peterson CL, Buratowski S, Lieberman J. γ-H2AX dephosphorylation by protein phosphatase 2A facilitates DNA double-strand break repair. Mol Cell 2005;20: 801–9.
84. Keogh MC, Kim JA, Downey M, et al. A phosphatase complex that dephosphorylates γ-H2AX regulates DNA damage checkpoint recovery. Nature 2006;439:497–501.
85. Nakada S, Chen GI, Gingras AC, Durocher D. PP4 is a γ-H2AX phosphatase required for recovery from the DNA damage checkpoint. EMBO Rep 2008;9:1019–26.
86. Chowdhury D, Xu X, Zhong X, et al. A PP4-phosphatase complex dephosphorylates γ-H2AX generated during DNA replication. Mol Cell 2008;31:33–46.
87. Macurek L, Lindqvist A, Voets O, Kool J, Vos HR, Medema RH. Wip1 phosphatase is associated with chromatin and dephosphorylates γH2AX to promote checkpoint inhibition. Oncogene 2010;29: 2281–91.
88. Douglas P, Zhong J, Ye R, Moorhead GB, Xu X, Lees-Miller SP. Protein phosphatase 6 interacts with the DNA-dependent protein kinase catalytic subunit and dephosphorylates γ-H2AX. Mol Cell Biol 2010;30:1368–81.
89. Jazayeri A, McAinsh AD, Jackson SP. Saccharomyces cerevisiae Sin3p facilitates DNA double-strand break repair. Proc Natl Acad Sci U S A 2004;101:1644–9.
90. Tamburini BA, Tyler JK. Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair. Mol Cell Biol 2005;25:4903–13.
91. Utley RT, Lacoste N, Jobin-Robitaille O, Allard S, Cote J. Regulation of NuA4 histone acetyltransferase activity in transcription and DNA repair by phosphorylation of histone H4. Mol Cell Biol 2005;25:8179–90.
92. Bhaskara S, Chyla BJ, Amann JM, et al. Deletion of histone deacetylase 3 reveals critical roles in S phase progression and DNA damage control. Mol Cell 2008;30:61–72.
93. Cheung WL, Turner FB, Krishnamoorthy T, et al. Phosphorylation of histone H4 serine 1 during DNA damage requires casein kinase II in S. cerevisiae. Curr Biol 2005;15:656–60.
94. Fernandez-Capetillo O, Allis CD, Nussenzweig A. Phosphorylation of histone H2B at DNA double-strand breaks. J Exp Med 2004;199: 1671–7.
95. Ransom M, Dennehey BK, Tyler JK. Chaperoning histones during DNA replication and repair. Cell 2010;140:183–95.
96. Green CM, Almouzni G. Local action of the chromatin assembly factor CAF-1 at sites of nucleotide excision repair *in vivo*. EMBO J 2003;22:5163–74.
97. Nabatiyan A, Szuts D, Krude T. Induction of CAF-1 expression in response to DNA strand breaks in quiescent human cells. Mol Cell Biol 2006;26:1839–49.
98. Moggs JG, Grandi P, Quivy JP, et al. A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. Mol Cell Biol 2000;20:1206–18.
99. Polo SE, Roche D, Almouzni G. New histone incorporation marks sites of UV repair in human cells. Cell 2006;127:481–93.
100. Zhu Q, Wani G, Arab HH, El-Mahdy MA, Ray A, Wani AA. Chromatin restoration following nucleotide excision repair involves the incorporation of ubiquitinated H2A at damaged genomic sites. DNA Repair (Amst) 2009;8:262–73.
101. Hyland EM, Cosgrove MS, Molina H, et al. Insights into the role of histone H3 and histone H4 core modifiable residues in Saccharomyces cerevisiae. Mol Cell Biol 2005;25:10060–70.
102. Tjeertes JV, Miller KM, Jackson SP. Screen for DNA-damage-responsive histone modifications identifies H3K9Ac and H3K56Ac in human cells. EMBO J 2009;28:1878–89.
103. Das C, Lucia MS, Hansen KC, Tyler JK. CBP/p300-mediated acetylation of histone H3 on lysine 56. Nature 2009;459:113–7.
104. Masumoto H, Hawke D, Kobayashi R, Verreault A. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. Nature 2005;436:294–8.
105. Collins SR, Miller KM, Maas NL, et al. Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. Nature 2007;446:806–10.
106. Han J, Zhou H, Horazdovsky B, Zhang K, Xu RM, Zhang Z. Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. Science 2007;315:653–5.

Published OnlineFirst September 7, 2010; DOI:10.1158/1078-0432.CCR-10-0513




**107.** Driscoll R, Hudson A, Jackson SP. Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. Science 2007;315:649–52.

**108.** Tsubota T, Berndsen CE, Erkmann JA, et al. Histone H3–56 acetylation is catalyzed by histone chaperone-dependent complexes. Mol Cell 2007;25:703–12.

**109.** Kim JA, Haber JE. Chromatin assembly factors Asf1 and CAF-1 have overlapping roles in deactivating the DNA damage checkpoint when DNA repair is complete. Proc Natl Acad Sci U S A 2009;106:1151–6.

**110.** Chen CC, Carson JJ, Feser J, et al. Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. Cell 2008;134:231–43.

**111.** Maas NL, Miller KM, DeFazio LG, Toczyski DP. Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4. Mol Cell 2006;23:109–19.

**112.** Celic I, Masumoto H, Griffith WP, et al. The sirtuins hst3 and Hst4p preserve genome integrity by controlling histone h3 lysine 56 deacetylation. Curr Biol 2006;16:1280–9.

**113.** Qin S, Parthun MR. Histone H3 and the histone acetyltransferase Hat1p contribute to DNA double-strand break repair. Mol Cell Biol 2002;22:8353–65.

**114.** Barman HK, Takami Y, Ono T, et al. Histone acetyltransferase 1 is dispensable for replication-coupled chromatin assembly but contributes to recover DNA damages created following replication blockage in vertebrate cells. Biochem Biophys Res Commun 2006;345:1547–57.

**115.** Verreault A, Kaufman PD, Kobayashi R, Stillman B. Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase. Curr Biol 1998;8:96–108.

**116.** Ye J, Ai X, Eugeni EE, et al. Histone H4 lysine 91 acetylation a core domain modification associated with chromatin assembly. Mol Cell 2005;18:123–30.

**117.** Shanbhag NM, Rafalska-Metcalf IU, Balane-Bolivar C, Janicki SM, Greenberg RA. ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. Cell 2010;141:970–81.

**118.** Shimada M, Niida H, Zineldeen DH, et al. Chk1 is a histone H3 threonine 11 kinase that regulates DNA damage-induced transcriptional repression. Cell 2008;132:221–32.

**119.** Choi HK, Kim BJ, Seo JH, Kang JS, Cho H, Kim ST. Cohesion establishment factor, Eco1 represses transcription via association with histone demethylase, LSD1. Biochem Biophys Res Commun 2010;394:1063–8.

**120.** Heidinger-Pauli JM, Unal E, Koshland D. Distinct targets of the Eco1 acetyltransferase modulate cohesion in S phase and in response to DNA damage. Mol Cell 2009;34:311–21.