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Histone Modifications: Crucial Elements for Damage Response and Chromatin Restoration

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Abstract

Eukaryotic genomes are packaged into chromatin from repeated nucleosome arrays in which DNA sequences wrap around histones. Chromatin organization has profound influence on DNA-templated processes such as transcription, DNA replication and repair. Recent studies have also revealed chromatin dynamics as an active contributor to diverse DNA damage responses. Here, we review recent progress in histone modification related to DNA damage response and post-repair chromatin restoration at the sites of DNA damage. We discuss how the timing and features of histone modifications would provide the initial as well as the final guidance for DNA damage response, and the prospect that modifications may challenge the epigenetic stability of repaired cells and serve as damage memory in chromatin.

In eukaryotic cells, genomic DNA is packaged into chromatin, a highly architectural DNA-histone complex that encodes both genetic and epigenetic information governing specific patterns of gene expression and determining cell identity (Luger, 2006). The basic building block for chromatin is the nucleosome, which consists of DNA in 147 bp length wrapped around a histone octamer, containing a tetramer of histone H3 and H4, flanked by two H2A–H2B dimers (Kornberg, 1977). Nucleosomes with linker DNAs, each about 20–60 bp, form an approximately 10 nm diameter "beads-on-a-string" structure where a linker histone H1 contacts the exit and entry of DNA strand on the nucleosome. The "beads-on-a-string" structure coils into a 30 nm diameter helical structure known as the 30 nm filament. This level of chromatin structure is thought to be the form of euchromatin, which is highly dynamic and unfolds into a 10 nm fiber. Variations within nucleosomal module can be achieved by DNA methylation (Klose and Bird, 2006), histone post-translational modifications (PTMs) (Kouzarides, 2007), incorporation of distinct histone variants (Sarma and Reinberg, 2005), and association with chromatin-binding proteins (Bernstein et al, 2007). Superimposed upon DNA sequences, the histone modification and DNA methylation constitute a layer of heritable epigenetic information regulating chromatin structure and DNA accessibility.

Many different PTMs on histones include acetylation, methylation, phosphorylation, ubiquitination, *et cetera* (Kouzarides, 2007). These modifications occur primarily at specific positions within the amino-terminal of the histone tails. In general, lysine acetylation is related to chromatin accessibility and transcription. The effect of lysine acetylation is in part

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due to the neutralization of the charges of histone tails, which interact with the DNA backbone. Additionally, lysine acetylation functions through recruiting other protein factors, e.g., bromodomain proteins to a specific region of the genome, for mediating transcription and anti-silencing functions (LeRoy et al, 2008; Ladurner et al, 2003; Matangkasombut and Buratowski, 2003). Lysine methylation, on the other hand, has a different impact on transcription, depending on the positions and degree of methylation. Methylation of H3 lysine (H3K) 4 and 36 is associated with transcribed domains, whereas methylation of H3K9, H3K27 and H4K20 appears to correlate with transcriptional repression. In yeast, methylated H3K4-interacting chromodomain protein, Chd1, was found to be a component of Spt-Ada-Gcn5 acetyltransferase (SAGA) complex, and the interaction between methylated H3K4 and the Chd1 chromodomain recruits SAGA complex to chromatin (Pray-Grant et al, 2005). Human Chd1 binds to methylated H3K4 through its tandem chromodomains, linking the recognition of histone modifications to non-covalent chromatin remodeling (Sims, III et al, 2005). In contrast, methylated H3K9 and H3K27 are recognized by heterochromatin protein 1 (HP1) and Polycomb repressive complexes (PRC). The binding of HP1 and PRC to methylated H3 is influenced by the “methyl states” (Fischle et al, 2003). For example, HP1 binds to both di- and tri-methylated H3K9, while PRC2 mediates tri-methylation at H3K27 to become tri-methyl H3k27. This tri-methyl H3 mark is recognized by PRC1 that in turn ubiquitinates histone H2A at K119, whereas Ring2/Ring1b E3 ubiquitin ligase serves as the core component of PRC1 (Wang et al, 2004). In mice, PRC1 links ubiquitination of histone H2A to heritable gene silencing and X chromosome inactivation (de Napoles et al, 2004). Recent studies indicated that ubiquitinated H2A (uH2A) is enriched at high-density CpG promoters at repressed genes (Kallin et al, 2009). How H2A ubiquitination regulates gene transcription or “repressed state” of chromatin remains largely unknown.

Obviously, chromatin is intimately associated with DNA-templated cellular processes and inevitably influences transcription, DNA replication and DNA repair. While chromatin structure presents a barrier to these processes, chromatin dynamics constitutes an active component of DNA damage response (DDR). Indeed, a number of histone modifications are now known to be involved in DDR. However, the functional significance of these modifications is not fully understood. Here, we review progress in our knowledge of histone modifications in response to ionizing radiation (IR) and ultraviolet (UV) light at specifically marked damage sites in genome. We discuss the implications of these histone modifications in cell cycle regulation, epigenetic stability and damage memory in chromatin.

Histone Modifications before DNA Repair

H2A/H2AX phosphorylation

Among the known PTMs, H2A phosphorylation is very well characterized and intensively studied. The H2A phosphorylation occurs at serine 128 in yeast or at 139 of the H2AX variant in humans (Downs et al, 2000; Rogakou et al, 1998). ATM and DNA-PK function redundantly to phosphorylate H2AX after IR (Stiff et al, 2004), while ATR phosphorylates H2AX in response to replication stress and UV irradiation (Ward and Chen, 2001). In yeast, phosphorylated H2A (γ H2A) can be detected as far as 50 Kb on either side of double-strand break (DSB) (Shroff et al, 2004). In human cells, γ H2AX involves ~2 Mb DNA region and forms foci that are easily detectable by immunofluorescence microscopy (Rogakou et al, 1998). Phosphorylation of H2AX generates a carboxyl-terminal phospho-motif that is then recognized by the tandem BRCT (BRCA1-carboxyl-terminal) domain-containing protein, MDC1 (mediator of DNA damage checkpoint 1, MDC1), a critical scaffold for mediating downstream events (Stucki et al, 2005). The recruitment of MDC1 to γ H2AX is an early event, leading to the recruitment of two downstream protein complexes: MRE11-RAD50-NBS50 (Chapman and Jackson, 2008; Melander et al, 2008; Spycher et al, 2008) and

UBC13/RNF8 ubiquitin ligase complex (Wang and Elledge, 2007; Kolas et al, 2007; Huen et al, 2007; Mailand et al, 2007).

Histone ubiquitination

As a consequence of recruitment of the UBC13/RNF8 complex, γ H2AX becomes ubiquitinated on chromatin surrounding the DSBs. The ubiquitination is initiated by UBC13/RNF8 ubiquitin ligase complex, and the ubiquitin conjugates are amplified by another ubiquitin ligase RNF168 (Doil et al, 2009; Stewart et al, 2009). RNF8 is recruited through interaction of its FHA domain with phosphorylated MDC1, using phosphorylated TQXF motifs in MDC1 as docking sites. Knocking down of RNF8 impairs G2/M checkpoint, and results in cellular sensitivity to low doses of IR. Thus, the MDC1- and RNF8-dependent histone ubiquitination is a critical event downstream of MDC1 binding. Ubiquitination creates K63-linked polyubiquitin chains on both γ H2AX and H2A, leading to subsequent recruitment of Rap80/Abraxas/Brca1/Brcc36 complex (Wang and Elledge, 2007). Not surprisingly, Ubc13-deficient cells are compromised for DSB repair by homologous recombination (HR) (Zhao et al, 2007). Although H2AX is a target of RNF8, mutations of all lysines in H2AX neither abrogate the accumulation of ubiquitin conjugates at DSBs nor affect the recruitment of downstream factors (Doil et al, 2009). Thus, the ubiquitination of histone H2A rather than γ H2AX may be the crucial event for subsequent signaling. Besides H2A and γ H2AX, H2B has also been found as a substrate of RNF8 (Wu et al, 2009). However, it is not known whether H2B ubiquitination contributes to DDR.

H2A ubiquitination by UBC13/RNF8 ubiquitin ligase complex also occurs at the sites of UV-induced DNA damage (Marteijn et al, 2009). Depletion of these enzymes causes UV hypersensitivity, without affecting nucleotide excision repair (NER), suggesting that UBC13 and RNF8 are only involved in UV-induced DDR. Similar to recruitment events at DSBs, RNF8 is recruited to the sites of UV damage in a MDC1 dependent manner, but requires ATR as well as NER-generated single-stranded repair intermediates. Thus, there may be a conserved common pathway of H2A ubiquitination for both DSBs and UV lesions.

In an effort to purify and characterize histone ubiquitin ligases, Wang, H et al found an ubiquitin ligase activity capable of ubiquitinating all histones *in vitro* (Wang et al, 2006). The ligase was later characterized as CUL4-DDB-ROC1 complex, an enzyme that is known for DDB2 and XPC ubiquitination at UV damage sites (Sugasawa et al, 2005; El-Mahdy et al, 2006; Wang et al, 2005). A small fraction of Histone H3 and H4 (0.3% and 0.1%, respectively) is found ubiquitinated *in vivo*, and siRNA-mediated knockdown of CUL4A, B and DDB1 decreases the H3 and H4 ubiquitination levels. In addition, the dynamics of CUL4-DDB-ROC1-mediated H3 and H4 ubiquitination is similar to that of XPC. Indeed, further biochemical studies indicate that the H3 and H4 ubiquitination weakens the interaction between histones and DNA, and facilitates the recruitment of XPC repair factor to damaged DNA. These studies point to a role of H3 and H4 ubiquitination in chromatin disassembly at the sites of UV lesions (Wang et al, 2006).

Histone acetylation

A transient acetylation of H3 and H4 in their amino-terminal tails has been found to occur at DSBs in both mammalian and yeast cells (van and Gasser, 2005). The acetylation is mediated by TIP60 complex in mammals or its homologue NuA4, SWR1 and INO80 in yeast. The TIP60 complex comprises TIP60 HAT, Snf2-related p400, TRRAP and other components. This large multiprotein complex acetylates several lysine residues (H3 K14, K23, H4 K5, K8, K12 and K14) of core histones *in vitro* (Kimura and Horikoshi, 1998). Mutations of lysine residues in H4 tail confer sensitivity to DSB-generating agents (Tamburini and Tyler, 2005; Bird et al, 2002; Murr et al, 2006). TIP60 is recruited to DSBs,

where it binds to and acetylates H4 with cofactor TRRAP (Ikura et al, 2007). Depletion of TRRAP impairs recruitment of DNA repair protein 53BP1 and RAD51 but not MDC1, and affects DSB repair. It is not yet known how histone acetylation by TIP60 complex regulates chromatin organization. Recently, TIP60 is found to promote ubiquitination of γ H2AX (Ikura et al, 2007). The acetylation appears to be a prerequisite for ubiquitination of γ H2AX. Furthermore, the acetylation is mediated by a TIP60-UBC13 complex, and the acetylation leads to release of H2AX from damaged chromatin. Thus, the sequential acetylation and ubiquitination of H2AX may promote histone dynamics at DSBs.

Histone Modification after DNA Repair

In order to distinguish the distinct roles of histone modifications in the course of DDR, it is essential to separate the events occurring before and after the repair of DNA damage. It is becoming increasingly apparent that some histone modifications occur as “post repair” events, as they are linked to the restoration of chromatin occurring after DNA sequences are successfully restored. The plethora of histone modifications occurring upon DNA damage induction suggest that the chromatin in the vicinity of damaged sites undergoes substantial processing and rearrangements. Thus, it is intuitive to reason that histone modifications are critical elements in the execution of DNA damage response. The key question is whether the original chromatin organization is eventually restored faithfully or some lingering epigenetic alterations in the newly restored chromatin are set as the memory of the genomic repair?

Histone modification and DNA replication-coupled nucleosome assembly

During cell proliferation, histone modifications are tightly associated with DNA replication-coupled nucleosome assembly. As DNA replicates, the parental histones are randomly transferred onto either the leading or the lagging strand (Jackson, 1988). When the parental nucleosomes are disrupted, a nucleosome is separated into two H2A–H2B dimers and one H3–H4 tetramer. The latter is randomly transferred onto one of the nascent DNA strands. Newly synthesized H3 and H4 are deposited to nascent DNA strands to form a chromatin precursor, which then serves as the template for deposition of either new or old histone H2A and H2B (Smith and Stillman, 1991). Conceivably, the deposition of new histones is important for full restoration of nucleosome density onto two daughters DNA strands. The H3 and H4 deposition involves histone chaperones, and among them, chromatin-assembly factor 1 (CAF-1) plays a pivotal role in chromatin assembly during DNA replication and DNA repair (Hoek and Stillman, 2003; Gaillard et al, 1996; Green and Almouzni, 2003; Polo et al, 2006; Zhu et al, 2009). CAF-1 is an evolutionary conserved protein complex, consisting of p150, p60 and p48 subunits, with a unique ability to preferentially deposit newly synthesized H3 and H4 onto replicating DNA (Shibahara and Stillman, 1999). Human CAF-1 and HIRA form predeposition machineries with histone H3.1/H4 and H3.3/H4, respectively (Tagami et al, 2004). The CAF-1 predeposition machinery is necessary to mediate DNA synthesis-dependent nucleosome assembly. During DNA replication or DNA repair, CAF-1 is targeted to the sites of DNA synthesis through its direct interaction with DNA polymerase processivity factor PCNA. As the replication fork progresses, H3.1–H4 dimers are deposited onto nascent DNA strands (Benson et al, 2006). CAF-1 is aided by another H3–H4 chaperone anti-silencing function 1 (Asf1), which synergistically cooperates with CAF-1 in DNA repair- and replication-coupled chromatin assembly (Tyler et al, 1999; Mello et al, 2002).

The DNA replication-coupled nucleosome assembly is important for inheritance of epigenetic information during DNA replication and repair (Groth et al, 2007). In these processes, PCNA molecules are used as a hub to couple chromatin restoration to replication. The PCNA recruits a number of chromatin-modulating enzymes, e.g., DNA

methyltransferase DNMT1 and CAF-1, to re-establish epigenetic memory based on DNA methylation and histone modifications. Both DNMT1 and CAF-1 also have the ability to recruit histone modifier enzymes for PTMs. For example, DNMT1 interacts with H3K9 methyltransferase G9a (Esteve et al, 2006) and EZH2 component of PRC2 (Vire et al, 2006), which catalyses trimethylation of H3K27. PRC2 binds to H3K27me₃ and colocalizes with this modification in G1 phase, and with the sites of ongoing DNA replication (Hansen et al, 2008). The ability of PRC2 to recognize a previously established mark triggers its renewal (Margueron et al, 2009). DNMT1 also forms a complex with G9a, which is required for recruitment of G9a to replication sites and for maintenance of H3K9 methylation at epigenetically silenced rDNA repeat (Esteve et al, 2006). Unlike DNMT1, CAF-1 reproduces the nucleosome density on replicated DNA throughout the genome. Nevertheless, recent evidence indicated that CAF-1 plays a role in maintaining the repressed state of silenced chromatin (Houlard et al, 2006). In embryonic stem cells, depletion of CAF-1 p150 results in the alteration of epigenetic histone methylation marks at the level of pericentric heterochromatin. Additionally, CAF-1 is found to link replication-coupled chromatin assembly to DNA and histone H3K9 methylation (Reese et al, 2003; Sarraf and Stancheva, 2004). CAF-1 forms a replication-dependent complex with H3K9 methyltransferase SETDB1 and MBD1, a protein that binds to methylated CpG dinucleotide. This complex is required for maintenance of H3K9 methylation and stable silencing of certain genes in proliferating cells. During DNA replication, MBD1 recruits SETDB1 to CAF-1 p150 and mediates H3K9 methylation in replication-coupled chromatin assembly. A similar mechanism also operates for H3K9 methylation of newly synthesized H3 deposited by CAF-1 in pericentric heterochromatin (Loyola et al, 2009). In this case, SETDB1 associates with HP1 α -CAF-1 complex and mono-methylates H3K9 on non-nucleosomal histone H3, providing H3K9me₁ for further trimethylation in pericentric regions. While both HP1 and CAF-1 are reported to localize to the sites of DNA damage (Green and Almouzni, 2003; Luijsterburg et al, 2009), it is not known whether HP1 α -CAF-1-SETDB1 complex works in DNA repair-dependent nucleosome assembly.

Histone H3K56 acetylation

Recent studies have uncovered a connection between H3K56 acetylation and chromatin assembly following DNA replication and DSB repair (Chen et al, 2008; Li et al, 2008). It was found in yeast that H3K56 acetylation drives Asf1-dependent re-assembly of chromatin after DSB repair (Chen et al, 2008). Mutation of K56 to glutamine, mimicking permanent acetylation, partially bypasses the requirement of Asf1 in resistance to DSB-generating agents. Nevertheless, DSB repair itself was operational in Asf1 mutants. On the other hand, lack of H3K56 acetylation in HAT Rtt109 mutants leads to persistent activation (phosphorylation) of checkpoint protein Rad53. These observations indicated that restoration of chromatin, driven by acetylated H3K56, is a signal for the completion of DSB repair. Similarly, CAF-1 is involved in acetylated H3K56-driven chromatin restoration (Li et al, 2008). H3K56 acetylation promotes the association of histone H3 with CAF-1 and Rtt109, and facilitates CAF-1-dependent nucleosome assembly. Thus, acetylated H3K56 coordinates the function of H3-H4 chaperones in nucleosome assembly.

Whether H3K56 acetylation occurs as results of DSB repair in human cells is a matter of debate (Das et al, 2009; Tjeertes et al, 2009). Das, C et al showed that DNA damaging agents, such as IR, UV, hydroxyurea and MMS, induce histone H3K56 acetylation in human cells, and histone acetyl transferase CBP/p300 is responsible for such an acetylation. Acetylated H3K56 colonizes with γ H2AX immediately following IR, indicating that the acetylated H3K56 is enriched at the sites of DNA damage. Moreover, acetylated H3K56 is assembled into chromatin at the sites of DNA repair by CAF-1 and Asf1. By contrast, Tjeertes, JV et al observed that the H3K56 acetylation was rapidly and reversibly reduced in

response to DNA damage (Tjeertes et al, 2009). Histone acetyl-transferase GCN5/KAT2A, rather than CBP/p300 acetylates H3K56 *in vitro* and *in vivo*. Further work is needed to clarify this inconsistency and reveal the importance of H3K56 acetylation in DDR in mammalian cells.

Histone H2A ubiquitination

An UV-induced mono-ubiquitination of histone 2A in the vicinity of DNA lesions has been described (Bergink et al, 2006). In living cells, harboring green fluorescent protein (GFP)-ubiquitin fusion, bleaching of GFP fluorophore in UV-exposed regions was followed by rapid local accumulation of GFP-ubiquitin fusion. The UV-induced ubiquitination requires functional NER, and coincides with the sequestration of PCNA, suggesting that the ubiquitination is linked to DNA repair. Since ubiquitin mutations that eliminate all lysine residues do not affect the ubiquitination, mono-ubiquitination is the most probable character of this NER-dependent process. Histone 2A is the primary target and the ubiquitination occurs at K119. Further studies suggest that the observed H2A ubiquitination may be a post-repair process related to chromatin restoration (Zhu et al, 2009). As determined by the formation of uH2A foci at local UV damage sites, dynamics of uH2A and XPC foci formation exhibits a clear temporal difference. For example, XPC foci are seen in almost all the damaged cells within 15 min and completely disappear at 4 h after local UV irradiation. On the other hand, the uH2A foci persist for 24 h post irradiation, suggesting that the formation of uH2A foci is not directly associated with damage recognition. More importantly, the H3–H4 chaperone CAF-1 is required for uH2A foci formation. Depletion of CAF-1 p60 by siRNA impairs recruitment of CAF-1 to DNA damage and eliminates uH2A foci. Moreover, the H2A ubiquitin ligase Ring2/Ring1b, a component of PRC2, is not detected at damage sites (Zhu et al, 2009). Therefore, it is very likely that H2A ubiquitination occurs at newly assembled nucleosomes, or the uH2A is deposited on DNA repair region from an existing pool.

It must be noted that the UV-induced H2A ubiquitination differs from γ H2AX or H2A ubiquitination, observed following IR, in a number of ways. First, RNF8 and UBC13 are known to form K63-ubiquitin chain (Plans et al, 2006). Second, the ubiquitination by RNF8 is dependent on γ H2AX and MDC1, whose BRCT domain binds γ H2AX. Deficiency in γ H2AX abolished RNF8 recruitment and eliminated IR-induced ubiquitination after IR (Huen et al, 2007). In contrast, γ H2AX deficiency did not affect UV-induced H2A ubiquitination (Bergink et al, 2006). Therefore, H2AX ubiquitination is integral part of checkpoint signaling in response to DSBs and UV induced DNA damage, while NER-dependent H2A mono-ubiquitination is related to the chromatin restoration after repair of UV-induced lesions.

Concluding Remarks

In recent years, a remarkable progress has been made in revealing PTMs in DDR. A careful comparison of damage, modification types, and the time when PTMs occur, suggests that the PTMs help organize DDR for the prompt execution of checkpoint signaling, DNA repair, and ultimate restoration of chromatin. Ubiquitination and acetylation seem to be two pervasive modes of these modifications. Timing and features of modifications appear to be critical factors in determining the physiological effect of a specific modification. For example, H3 acetylation occurs both before and after DSB repair. The former, acetylation at H3K14 and H3K23, leads to γ H2AX ubiquitination, checkpoint activation, and DNA repair (Ikura et al, 2007). While the latter, H3 acetylation at K56, leads to restoration of chromatin and termination of checkpoint activation (Chen et al, 2008). Also, the same concept seems true for histone ubiquitination. The γ H2AX and H2A ubiquitination lead to checkpoint signaling, whereas the H2A ubiquitination after NER is associated with chromatin

restoration. These scenarios and information to-date raise an important question whether these modifications are an extension of histone code albeit in the realm of DDR? Answer to this question lies in our future understanding of detailed molecular mechanisms by which each modification functions in specific DDR pathway.

It is hypothesized that immediately upon induction of DNA damage, chromatin is locally destabilized to facilitate the access of repair machinery to DNA lesions. After DNA repair is completed, chromatin is restored to its original state in the vicinity of damage sites. This “access-repair-restore” model conceptually describes how DNA repair might occur in chromatin environment (Smerdon, 1991; Green and Almouzni, 2002). Now, histone modifications can also be integrated into this model (Fig. 1) and ask whether faithful repair of DNA sequences is always achieved with the preservation of epigenetic information? A recent study indicated that DNA damage leaves its mark on chromatin through incorporation of new H3.1 histones into the sites of UV repair (Polo et al, 2006). In the same vein, incorporations of acetylated H3K56 and uH2A into chromatin can be envisioned as analogous cellular events. Thus, these modifications too would be considered as DNA repair marks on chromatin.

Would these unique marks threaten the cell’s epigenetic stability with pathological consequences? To answer this question, it is important to know whether the specific marks constitute transient or permanent features within the cell. For instance, it has been found that USP3, a deubiquitinating enzyme, is required for deubiquitination of ubiquitin-conjugates of H2A, dephosphorylation of γ H2AX, and for S phase progression after DNA damage (Nicassio et al, 2007), suggesting that ubiquitin may be removed from H2A for checkpoint recovery or adaptation. On the other hand, RNF8 ubiquitin ligase is also required for mitotic exit in cell cycle (Plans et al, 2007). Given that the H2A ubiquitination regulates gene transcription or “repressed state” of chromatin, any uH2A remaining in the repaired chromatin would be expected to have a regional effect on genomic function. Therefore, the challenge for future studies is to understand how these modifications are processed further to maintain epigenetic integrity of the cell, or if they do become epigenetic marks, how they would impact DNA-templated processes in the “once-damaged” chromatin.

Abbreviations

Asf1	anti-silencing function 1
CAF-1	chromatin-assembly factor 1
DDR	DNA damage response
DSB	double-strand break
GFP	green fluorescent protein
H	histone
HP1	heterochromatin protein 1
HR	homologous recombination
IR	ionizing radiation
K	lysine
MDC1	mediator of DNA damage checkpoint 1
NER	nucleotide excision repair
PRC	polycomb repressive complex

PTM	post-translational modification
γH2AX	phosphorylated H2AX
SAGA	Spt-Ada-Gcn5 acetyltransferase
siRNA	small interference RNA
UV	ultraviolet light
uH2A	ubiquitinated H2A

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Fig. 1.

Temporal interplay of histone modifications before and after DNA repair. Upon initial DNA damage, nucleosomal histones are subjected to various PTMs: H3 and H4 acetylation by TIP60 HAT, H2AX phosphorylation by ATM/ATR, γ H2AX and H2A ubiquitination by RNF8, or H3 and H4 ubiquitination by DDB-CUL4-ROC1. These modifications may cause nucleosome disassembly, or histone eviction, and facilitate access of the repair nanomachines to DNA lesions. When DNA repair is complete, repair-dependent chromatin assembly restores the original nucleosome density. The histones with new epigenetic marks i.e., the acetylated histone H3, the ubiquitinated H2A, and possibly the histones with other modifications are incorporated into newly assembled chromatin at the damage sites. After chromatin is restored, the checkpoint is terminated and cells resume cycling. Some PTMs would be eliminated and others retained to serve as a memory of damage, potentially challenging the epigenetic stability of the cells having undergone repair. The symbols with “P”, “Ub”, “Ac” and “Me” represent phospho, ubiquitin, acetyl and methyl groups, respectively.