EXPERIMENTAL CELL RESEARCH 312 (2006) 2677–2686

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Review Article

Chromatin modulation and the DNA damage response

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ARTICLE INFORMATION

Article Chronology:
Received 19 June 2006
Accepted 19 June 2006
Available online 30 June 2006

Keywords:
DNA
Chromatin
DNA damage response
Histone

ABSTRACT

The ability to sense and respond appropriately to genetic lesions is vitally important to maintain the integrity of the genome. Emerging evidence indicates that various modulations to chromatin structure are centrally important to many aspects of the DNA damage response (DDR). Here, we discuss recently described roles for specific post-translational covalent modifications to histone proteins, as well as ATP-dependent chromatin remodelling, in DNA damage signalling and repair of DNA double strand breaks.

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Introduction

To avoid propagation of mutations that could lead to genomic instability and cancer, it is imperative that genomic DNA damage is detected and repaired before each cell division [1]. DNA damage detection and signalling are carried out by a signal transduction pathway termed the DNA damage response (DDR) and this pathway is therefore crucial for the maintenance of genomic integrity. Activation of the DDR induces cell cycle arrest, DNA repair, induction of a

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doi:10.1016/j.yexcr.2006.06.031

transcriptional programme and in cases of severe damage, apoptosis [2].

Of the many types of DNA damage, DNA double strand breaks (DSB) are one of the most severe because the genome potentially loses its continuity if the damage is not repaired. The cell can use one of two mechanisms for DSB repair: homologous recombination (HR), which allows for error-free repair, and non-homologous end joining (NHEJ), which is an error prone repair pathway [3,4].

In the cell, chromatin is the physiological template for all DNA transactions including transcription, replication and DNA repair. The fundamental subunit of chromatin is the nucleosome [5,6] consisting of 147 bp of DNA wrapped around a histone octamer comprised of two molecules each of the

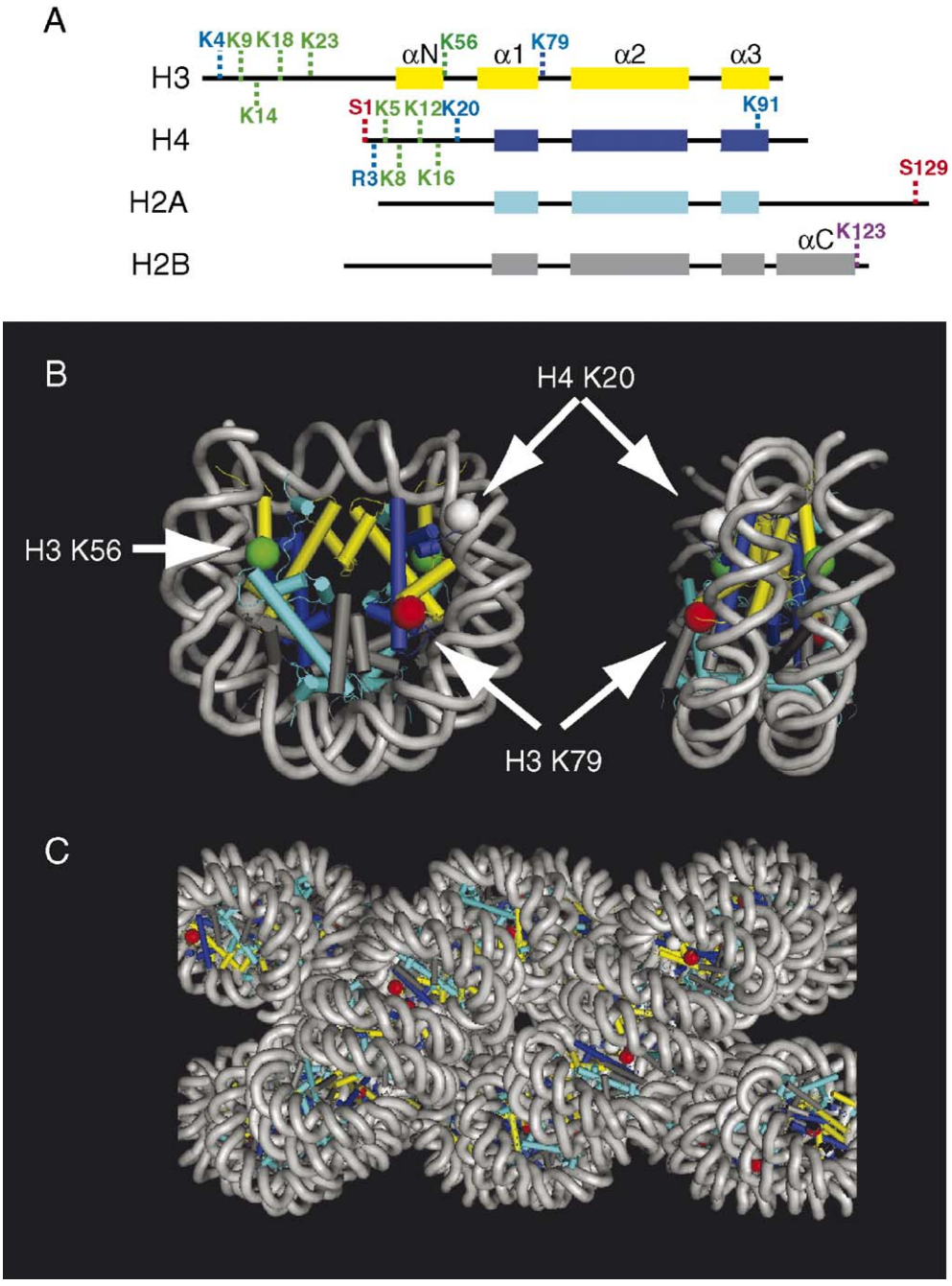


Fig. 1 – Histone covalent modifications. (A) Location of histone covalent modifications involved in the DNA DSB response in the linear histone sequence. Rectangles represent structured alpha-helical regions. Covalent modifications are color coded as follows: red for phosphorylation; blue for methylation; green for acetylation; and purple for ubiquitination. (B) Location of residues H3K79 (red sphere), H4K20 (white sphere) and H3K56 (green sphere) in the nucleosome, also indicated by the white arrows. (C) Location of H3K79 (red sphere) and H4K20 (white sphere) in an idealised higher order chromatin structure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

histones H2A, H2B, H3 and H4 (see Figs. 1A and B). Nucleosomes facilitate further compaction of the genome into higher order chromatin structures to allow packaging of the genomic DNA into the cell nucleus [7] (see Fig. 1C). In response to DNA damage, detection of lesions and repair of DNA must occur in this chromatin environment. Folding into chromatin alters the accessibility of the DNA to proteins involved in DNA transactions, and specialised mechanisms have evolved to deal with the chromatin-packaged state of DNA. These mechanisms include three ways of directly manipulating chromatin structure: covalent histone modifications, ATP-dependent chromatin remodelling and histone variant incorporation [8–10].

Histones have a high content of lysines and arginines, and a significant number of serines and threonines. All four residues are, by their polar nature, typically located on the exposed exterior surfaces of the histone octamer. The amine and hydroxyl functional groups of these side chains are reactive under biochemical conditions and therefore readily undergo post-translational modifications. In particular, differences in electrostatic properties between the modified and unmodified forms of these residues can significantly affect interactions between the histones and DNA or other proteins. Histone modifications implicated in the DNA damage response include phosphorylation, methylation, acetylation and ubiquitination (Table 1).

ATP-dependent chromatin remodelling complexes physically manipulate chromatin structure, for example by repositioning or disrupting nucleosomes. It is known that many of these complexes are involved in gene regulation and transcription [11]. More recently, roles for some chromatin remodelling complexes in the DDR have been defined (see below).

Manipulation of chromatin structure is required for many aspects of the DNA damage response and, accordingly, genetic studies have revealed that mutants of histone modifying proteins and chromatin remodellers often show sensitivity to genotoxins. However, genetic analysis of the individual roles played by these enzymes in the DDR is complicated by a functional redundancy between individual enzymes and their multiples roles in manipulating the chromatin environment.

This review will focus on interactions between chromatin and the DDR pathway and how these interactions facilitate DNA damage signalling and repair.

γH2A(X) in DNA DSB signalling and repair

Several histone post-translational modifications are involved in the DNA damage response (Table 1). The most studied of these modifications, also termed marks, is the phosphorylation of serine 139 on the C-terminal tail of histone H2AX, or the equivalent residue of yeast H2A, serine 129, by phosphatidyl inositol kinase-like kinases (PIKKs). In this review, the term γH2A(X) will be used when referring to this modification in both yeast and higher cells. Note that the numbering of residues for human H2AX does not include the initiating methionine residue, whereas in yeast H2A this residue is included. Also these histone modifications are termed H2AXS139ph in higher cells or H2AS129ph in budding yeast using a recently described nomenclature [12]. H2A(X) phosphorylation is induced after DNA damage and has become a standard marker of DSBs. γH2A(X) function in the DNA damage response has been reviewed extensively in [13–18] and here we will only highlight some of its more recently described roles in the DDR.

Extensive phosphorylation of H2A(X) is an early and ubiquitous event after a DSB, extending over 60 kb in yeast and up to 1 Mb in higher eukaryotes on each side of the break [13]. γH2A(X) is necessary for the damage-induced focal accumulation of proteins involved in checkpoint signalling, DNA repair and even chromatin remodelling. Importantly, this modification is not needed for the initial recruitment to DSBs of key DDR proteins believed to be involved in DNA damage sensing, such as Nbs1 or 53Bp1 [19]. Some proteins, for example Mdc1, bind directly to γH2A(X) via an interaction between the Ser139 phosphate and the BRCT domains of Mdc1 [20]. However, direct interaction with γH2A(X) has not been demonstrated for all proteins recruited to the site of DNA damage. The recruitment of many of these proteins may be facilitated by exposure of other histone modifications or other

Table 1 – Summary of functions of covalent histone modifications involved in the DNA double strand break response, and the enzymes responsible for these modifications

Modification	Modification site	Enzyme	Function	References
Phosphorylation	H2A S129	Mec1, Tel1	Stable retention of DDR checkpoint proteins at DSB, DSB repair	[19,33]
Phosphorylation	H4 S1	CK2	DNA damage regulated kinase that phosphorylates H4S1, linked to histone deacetylation	[46,75–77]
Methylation	H3 K79	Dot1	Required for 53Bp1, Rad9 recruitment to DSB, checkpoint activation in *S. cerevisiae*, marks active chromatin	[26–28,30]
Methylation	H3 K4	Set1	Involved in checkpoint activation in *S. cerevisiae*, H3K4me2, H3K4me3 mark 5′ region of active genes, H3K4me1 localised to silenced chromatin	[26,28,78,79]
Methylation	H4 K20	$Set9^{Sp}$	Involved in DDR in *S. pombe*, not in other chromatin processes, marks silenced chromatin in higher eukaryotes (varying reports on whether monomethylation is localised to active genes)	[32,80–82]
Acetylation	H3 K9, H3 K14, H3 K18, H3 K23, H3 K27, H3 K56 H4 K5, H4 K8, H4 K12, H4 K16, H4 K91	Esa1, Gcn5, Hat1	Mark active chromatin in *S. cerevisiae*, H3K56 acetylation functions in the DDR, H4K91 acetylation is involved in chromatin assembly	[50,83]
Ubiquitination	H2B K123	Rad6–Bre1	Involved in checkpoint activation in *S. cerevisiae*, required for di and trimethylation of H3K4 and H3K79	[29,84]

docking sites by γH2A(X)-dependent chromatin remodelling ([14] and see below).

Proteins recruited in a γH2A(X)-dependent manner are involved in a number of different functions. γH2A(X) is necessary for signal amplification at low doses of damage [14,16], for tethering DSB ends [14], as well as DSB-induced cohesin recruitment [13] and chromatin remodelling [21], roles that relate to checkpoint and/or DNA repair mechanisms. It is likely that additional roles, both related and unrelated to checkpoint and/or DNA repair mechanisms, will be revealed by further study.

The checkpoint and repair functions attributed to γH2A(X) have been difficult to identify until recently because of the relatively mild damage sensitivity phenotype of unphosphorylatable H2A(X) mutants. Recent genetic studies have revealed strong synthetic phenotypes in both checkpoint signalling and repair, illustrating the crucial role this modification plays in these two processes. For example, *Schizosaccharomyces pombe* H2A S129A mutants have a completely abolished checkpoint when coupled to a Crb2 T215A mutant. This phenotype, significantly stronger than the partial defect seen in either single mutant, indicates that γH2A signalling through Crb2 is a key part of the checkpoint response in fission yeast [22]. In *Saccharomyces cerevisiae*, efficient repair of DSBs requires both γH2A and H3K79me ([32] and see below). In the chicken DT40 cell line, cells incapable of phosphorylating H2AX are mildly sensitive to DNA damage and display only a mild defect in HR (Eiichiro Sonoda and Yasunari Takami, personal communication). However, when the inability to form γH2AX was combined with loss of *Xrcc3* function, one of the five Rad51 paralogues involved in HR, synthetic lethality was observed (note that chicken genes follow mouse nomenclature). This suggests that γH2AX can partially substitute for Rad51 paralogues in the repair of endogenously generated lesions.

Finally, the dephosphorylation and removal of this epitope has been recently shown to be a significant step in turning off the DNA damage response. Dephosphorylation of γH2A(X) is catalysed by PP2A in humans and the Pph3 subunit of the H2A phosphatase Complex (HTP-C) in *S. cerevisiae* [22–24]. Both enzymes have a high specific activity for their γH2A(X) substrates *in vitro*, localise to DSB sites and their suppression leads to persistent γH2A(X) foci. In yeast, lack of Pph3 does not affect normal DNA repair, although cells are defective in checkpoint recovery. Furthermore, γH2A(X) has been observed to persist on disorganised chromatin through mitosis, thought to represent an aberrant chromatin structure caused by illegitimate rejoining [25]. This suggests that active dephosphorylation of γH2A(X) near the break is an important step in signalling successful DNA repair. It is clear that the complexity of the roles played by γH2A(X) in the DNA damage response remain to be fully resolved.

H3K7me in DNA DSB signalling and repair

Another covalent histone modification implicated in the DNA damage response is the methylation of histone H3 at lysine 79 (H3K79me) [26–28]. Unlike γH2A(X), this modification is not induced by DNA damage and is constitutively present on chromatin. The enzyme responsible for this modification is the evolutionarily conserved histone methyltransferase, Dot1. The modification is localised to regions of euchromatin in human cells [29] and in *S. cerevisiae* up to 90% of chromatin is methylated at this residue [30]. Methylation of H3K79 peaks in G1 and S phase, and in budding yeast this cell cycle regulation is, at least in part, due to regulation of *DOT1* transcription.

Huyen et al. [27] have proposed that H3K79me is responsible for recruiting human 53BP1 and its budding yeast functional homologue, Rad9, to sites of DNA DSBs via conserved hydrophobic residues in the Tudor domain of these proteins. The Tudor domain is a member of the 'Royal Family' of chromodomains that bind methyllysines [31] and mutation of this domain abrogates the binding of 53BP1 to methylated H3 [27]. Similarly, it has been reported in fission yeast that methylation of H4K20 is required for recruitment of Crb2 [32]. Crb2, along with 53BP1, is a member of the 'Rad9-like' family of proteins, and Sanders et al. [32] postulate that the Crb2 Tudor domain could be responsible for its interaction with H4K20. As illustrated in Fig. 1B, H3K79 and H4K20 are located on the outside of the nucleosome and so these residues are buried in higher order chromatin structure (Fig. 1C). The model proposed, also valid for Crb2 and H4K20me [32], is that induction of a DSB leads to the passive relaxation of higher order chromatin structure surrounding the break site, allowing 53BP1 to access H3K79me and to act at the early sensing step of the DNA damage response [27]. Once recruited to the site of damage, 53BP1 could in turn recruit other proteins to activate the checkpoint response [27]. It has also been proposed that recruitment of Rad9 to H3K79me in budding yeast initiates a similar cascade [26,28]. This model is attractive because the constitutively methylated H3K79 residue can act as a 'ready-made mark' of DNA damage, allowing the rapid sensing of DSBs and subsequent checkpoint activation. Indeed, results in budding yeast support this model, at least in G1 arrested cells, as mutants of the Rad9 Tudor domain, H3K79, or Dot1 completely abrogate checkpoint activation and result in a defective cell cycle delay following ionising radiation (IR) ([26,28] and our unpublished results). Surprisingly, however, when these mutant cells are arrested in G2 and subjected to IR, there is only a slight, but reproducible, defect in checkpoint activation, as measured by phosphorylation of Rad53 and cell cycle progression through mitosis ([28] and our unpublished results). Therefore, contrary to the model proposed above, methylation of H3K79 plays only a minor role in checkpoint activation in G2. However, it is clear that Rad9 recruitment to DSBs in the G2 phase is dependent on both γH2AX and methylation of H3K79 by Dot1 [28,33], and that this recruitment is only detectable at late stages of the DNA damage response, where it promotes efficient repair of DSBs by homologous recombination [33].

In higher cells, H3K79me is also likely to be involved in DSB repair, as 53Bp1 has been implicated in NHEJ. Using the genetically tractable chicken cell line DT40, Nakamura et al. [34] showed an epistatic relationship between chicken *53Bp1* and components of this repair pathway. Furthermore, in this study, $53Bp1^{-/-}$ cells were shown to be fully proficient for their intra-S and G2/M checkpoints, suggesting that the primary role of 53Bp1 in these vertebrate cells is in DSB repair, and that

if it is involved in checkpoint regulation, it must act redundantly with other checkpoint proteins. In human and mouse cells, 53BP1 has been reported to have a minor role in checkpoint regulation which is only detectable at low doses of ionising radiation [35–37] after DNA damage. Thus, 53BP1, like its yeast orthologues Rad9 and Crb2, is likely to play multiple roles in the DNA damage response, ranging from checkpoint signalling early in the response, to DNA repair *per se* at later steps in the response. By recruiting 53BP1, H3K79me is likely to also be involved in both signalling and repair.

Histone acetylation in the manipulation of chromatin structure and recruitment at a DSB

Histone acetylation, as well as functioning in protein recruitment, also functions in the relaxation of chromatin structure. Acetylation of lysines in the N-terminal tails of histones H3 and H4 removes the positive charge on the side chain and destabilises higher order chromatin structure [11]. In gene expression, this acetylation-dependent relaxation of chromatin occurs in transcriptionally active regions [38–40] and this process has also been shown to occur in response to a DSB. The acetylation status of histones is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) and some of these HATs and HDACs are recruited to the region flanking a DSB induced by the HO endonuclease in budding yeast [41,42]. The HATs Esa1 [43], Gcn5 [44] and Hat1 [45] are recruited to a 5-kb region flanking a DSB. Furthermore, changes in acetylation levels that occur during repair can be correlated with the recruitment of HATs or HDACs. The arrival of HATs at a DSB mirrors the increase in acetylation levels of histone H3 at residues K9, K14, K18, K23 and K27 and of histone H4 at residues K5, K8, K12 and K16 [41]. The subsequent recruitment of the HDACs Sir2 [41], Rpd3 [46] and Hst1 [41] 2 h after the induction of the break coincides with the observed decrease in acetylation levels at the site of damage.

The earliest known acetylation event following a DSB is the acetylation of the histone H4 N-terminal tail by the NuA4 complex, which occurs within 1 h of HO induction [42,43,46]. Esa1, the HAT subunit of the NuA4 complex, is conserved in mammalian cells. Its human orthologue, Tip60, is responsible for the acetylation of the N-terminal tail of histone H4, which appears to facilitate the subsequent loading of a subset of repair proteins, including 53Bp1, Brca1 and Rad51 [47]. Furthermore, hypotonic salt treatments that induce relaxation of chromatin structure reverse DDR protein recruitment defects caused by lack of H4 acetylation [41,47]. However, localisation of other DDR proteins, such as Mdc1 and Nbs1, is unaffected by lack of acetylation.

Regarding DNA repair, the modulation of chromatin structure caused by acetylation is thought to facilitate access of DSB repair proteins to the lesion [48]. Indeed, it is possible that the degree of acetylation could direct the lesion into alternative repair pathways. This hypothesis is supported by the observation in yeast cells that acetylation of one lysine residue in the histone H4 N-terminal tail is sufficient to direct the cell into a replication coupled repair pathway, whereas acetylation of more than one lysine is necessary for repair via NHEJ [43].

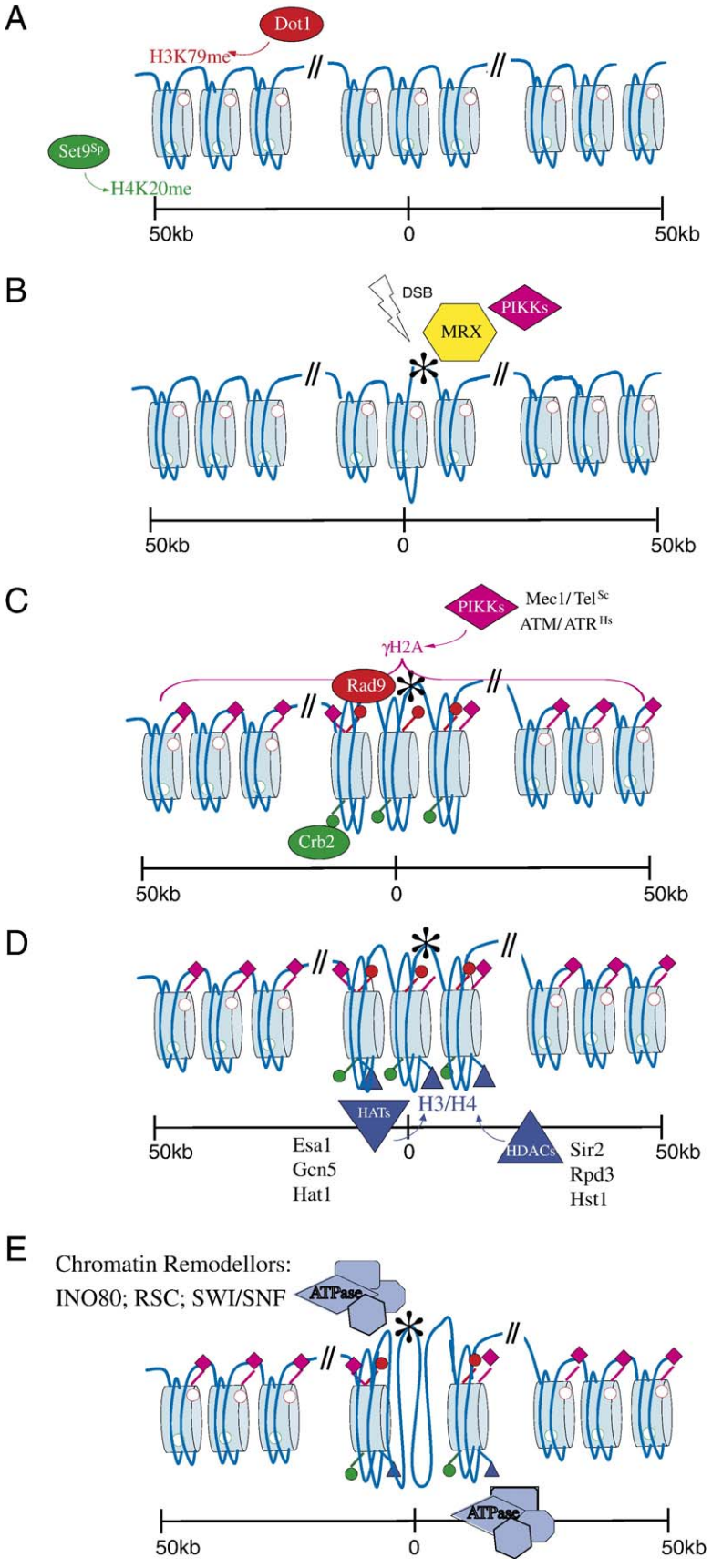
The choice of repair pathway might also depend on the direct regulation of the enzymatic activity of HATs and HDACs. There is evidence that DNA-PK, a PIKK with functions in NHEJ, can inactivate the Gcn5 HAT by phosphorylation [49]. As maximal acetylation levels have been shown to occur during HR [41], this suggests that the inhibition of this HAT may direct cells towards NHEJ rather than HR.

It is interesting to note that the population of histone H3 that is deposited behind the replication fork is acetylated at K56 [50]. The key structural position of this lysine, in contact with the phosphodiester backbone at the entry and exit point of the nucleosome core (Figs. 1A and B), results in a relaxed nucleosomal structure upon acetylation [51]. Mutation of this residue causes sensitivity to DSB-inducing agents during replication, suggesting a specific role in DSB repair pathway during S phase [50]. Deposition of acetylated H3K56 might prime DNA for repair in S phase, alleviating the need to recruit specific HAT activities during DNA replication [50]. Deacetylation of this residue usually occurs upon entering the G2 phase but, in response to damage, levels of acetylation are maintained in G2, in a Rad9, and therefore checkpoint-dependent manner [50]. This indicates that one function of the DNA damage checkpoint is to promote appropriate DNA repair by negatively regulating the level of deacetylation of H3K56ac, likely by regulating HDAC activities.

The function of histone acetylation during DSB repair is further complicated by the role of this modification in direct protein recruitment, as acetylated lysines in specific protein contexts can be recognised by proteins containing bromo-domains [52]. Many repair proteins, as well as chromatin remodelling complexes, which are also recruited at DSB, contain bromo-domains. In addition, the recruitment of these complexes is also dependent on H4 acetylation. For example, Bdf1, a subunit of the SWR-C complex (see below) contains a double bromo-domain and specifically binds the acetylated lysines of the N-terminal tail of histone H4 [53]. Esa1 and its mammalian homologue Tip60 also contain bromo-domains and both proteins have been implicated in DSB repair. Finally, Tip60 has recently been shown to acetylate both ATM and DNA-PK, an event that appears to be required early in the activation of both proteins [54,55]. Intriguingly, this PIKK activation function could be dependent on the bromo-domains of Tip60, suggesting that Tip60 would first bind acetylated chromatin before activating these two PIKKs.

ATP-dependent chromatin remodelling in DNA repair

In addition to the covalent histone modifications mentioned above, the structure of chromatin can also be directly manipulated by ATP-dependent chromatin remodelling complexes. These complexes use the energy of ATP hydrolysis to facilitate chromatin remodelling, through nucleosome sliding, nucleosome disruption and exchange of histone components [8,56–59]. Although roles for many ATP-dependent remodelling complexes were first identified in transcriptional regulation, it has recently been shown that some of these complexes, for example the INO80, RSC, SWI/SNF and SWR-C complexes, are also recruited to the HO break in *S. cerevisiae* [22,42,60–63].


A
Dot1
H3K79me
Set9Sp
H4K20me
50kb
0
50kb
B
DSB
MRX
PIKKs
50kb
0
50kb
C
PIKKs
Mec1/ Tel Sc
ATM/ ATR Hs
γH2A
Rad9
Crb2
50kb
0
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D
HATs
H3/H4
50kb
Esa1
Gcn5
Hat1
0
HDACs
Sir2
Rpd3
Hst1
50kb
E
Chromatin Remodellors:
INO80; RSC; SWI/SNF
ATPase
ATPase
50kb
0
50kb

The INO80, SWR-C and SWI/SNF complexes are recruited between 30 and 60 min after HO induction. The RSC complex is recruited earlier, at 10 min after induction. The distinct kinetics with which these complexes are recruited may be relevant to the different roles they perform in the DNA damage response [63].

Recent studies have demonstrated the requirement of specific DNA damage response proteins for this recruitment of chromatin remodelling complexes to the HO break. γH2A is required for the recruitment of the INO80 complex [61,62], via its Arp4 and Nhp10 subunits [42,61]. Arp4 is also present in the SWR-C complex, and because SWR-C and γH2A interact, it is thought that the SWR-C complex is also recruited to DSBs through γH2A interaction [42]. However, it remains to be determined whether a component specific to SWR-C is indeed recruited to the HO break in a γH2A-dependent manner.

Recruitment of the RSC chromatin remodelling complex absolutely requires Mre11 and partially depends on yKu70 [63]. Protein interaction studies suggest that this recruitment is mediated through interactions between the Rsc1 and Rsc2 subunits and Mre11. Furthermore, mutations in the Rsc1 bromo-domains, which abrogate its interaction with Mre11, suggest this interaction could be mediated by an acetylated residue of Mre11 [63].

Once recruited to the DSB, these complexes function in DSB repair. A recent report demonstrates that nucleosomes are displaced from the region flanking an HO break, and that this displacement requires the INO80 complex [64]. Nucleosome displacement may facilitate the generation of ssDNA [62] or the consequent formation of the Rad51 filament [64]. Paradoxically, nucleosome displacement does not require γH2A, although γH2A is required for recruitment of INO80. This can be explained because INO80 is present at the Matα locus before HO induction, presumably in its transcriptional role, and this γH2A-independent pool of INO80 is sufficient for nucleosome displacement [64]. Further INO80 is recruited to the HO break through γH2A after DSB induction, and this second, γH2A-dependent population is thought to be involved in the strand invasion step of HR. Strand invasion also requires the SWI/SNF complex [60]. This complex is required for the recruitment of Rad51 and Rad52, and it has been proposed that SWI/SNF may facilitate clearing of nucleosomes surrounding the break to facilitate homology searching [60].

The RSC complex also plays a direct role in HR. Chai et al. [60] have suggested that RSC is needed for dissociation of the invading strand from the donor strand before ligation because RSC mutants are defective in the ligation step of HR. However, RSC mutants were also shown to be defective in NHEJ repair [63]. Additionally, it is possible that RSC is involved in cohesin loading at the break due to its analogous role loading cohesin in the cell cycle [65]. It is known that cohesin is also recruited to an HO break in a γH2A and Mre11-dependent manner [66], suggesting that Mre11 recruits RSC in order to facilitate the loading of cohesin at the break [60,63]. Clearly, chromatin remodelling complexes play diverse and complex roles in the DNA damage response.

Discussion

Interactions between chromatin and DNA damage response proteins are central to the cellular response to double strand breaks. These interactions may be required at all stages of the DDR pathway, from DNA damage detection and signalling to various stages of DNA repair. Emerging evidence indicates that the chromatin surrounding a DSB is modified for two principal purposes: direct recruitment of DDR proteins, with recruitment often being mediated by specific covalent histone modifications, and modification of compacted chromatin structure to facilitate DNA repair.

The most studied modification to chromatin at DSBs is the phosphorylation of H2A(X). This modification clearly plays important, if redundant, roles in the DDR, but it is still unclear how H2A(X) phosphorylation by the PIKKs is stimulated. In the model proposed by Lisby et al. [67], the Mre11/Rad50/Xrs2 (or MRX) complex is the first to be recruited to a DSB (see Fig. 2B).

However, the mechanism of MRX recruitment is unclear. Recognition of the DSB could involve direct binding of MRX to DNA ends formed by the DSB itself, or, alternatively, a chromatin remodelling event. Any such remodelling event would obviously be γH2A independent and could also be involved in exposing H3K79me. Along with MRX, the yeast homologues of human ATM and ATR, namely Tel1 and Mec1, are also recruited early in the DDR [67–71]. Once loaded at the site of damage in an Mre11-dependent manner, these PIKKs are responsible for phosphorylation of H2A (Fig. 2C).

γH2A has been shown to be required for recruitment of both HATs and chromatin remodellers (Figs. 2D and E). It is possible that the relaxation of chromatin structure by the action of HATs and remodelling complexes could expose the

Fig. 2 – Possible roles for histone modifications in the DNA damage response. (A) In undamaged chromatin, constitutively modified H3K79 (and H4K20 in *S. pombe* and higher cells) remains buried in higher order chromatin structure (see also Fig. 1C). (B) Following a DSB, the MRX (or MRN in higher cells) complex and PIKKs are initially recruited to activate the DDR. The asterisk symbolises a DSB. (C) H2A(X) becomes phosphorylated and at the same time H3K79 (and H4K20) may also become exposed in an Mre11-dependent manner (see text) allowing 'Rad9-like' proteins with Tudor domains to dock onto these modified residues. (D) Following H2A(X) phosphorylation, HATs and chromatin remodelling complexes are recruited in a stepwise manner. Acetylation reduces the affinity of DNA for histone proteins, allowing DNA repair proteins access to the lesion. (E) ATP-dependent chromatin remodelling complexes facilitate the disruption, exchange or sliding of nucleosomes surrounding a DSB and direct the cell into appropriate repair pathways. Open circles indicate methylated residues (either H3K79me or H4K20me) buried within the compacted nucleosomes. Filled circles indicate exposed residues that specifically mark damaged chromatin. The filled diamonds indicates γH2A(X). The filled blue triangle indicates acetylated histone residues. Larger symbols represent protein complexes as indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

constitutively methylated H3K79me. Following exposure of this residue, Rad9 is recruited to the site of DNA damage where it initially functions in checkpoint activation, but also has an additional role in DSB repair [33]. The role for Rad9 in DSB repair is dependent on H3K79me, occurs late in the DDR and concomitantly to the chromatin remodelling complexes mentioned above [33,42,62]. However, separately from this role in repair, H3K79me is also necessary for rapid checkpoint activation, as it is needed for Rad9 hyper-phosphorylation and Rad53 phosphorylation in the G1 phase within minutes of damage. At this early stage, HATs and chromatin remodelling complexes such as the NuA4, INO80, SWR-C and SWI/SNF complexes have not yet been recruited [42,62], suggesting that another pathway must be responsible for exposure of H3K79me. This pathway would likely be independent of γH2A because Rad9 activation does not require γH2A. One possibility, as put forward by Huyen et al. [27], is that DSBs lead to passive relaxation of chromatin in the region of the break, thus exposing the normally inaccessible H3K79 residue and leading to checkpoint activation. However, the exposure of that residue in response to a DSB is not necessary for the initial damage detection step involving MRX and PIKK kinases, as Mre11 and Ddc2 foci still form when H3K79 is not methylated (our unpublished results).

The RSC complex is the earliest ATP-dependent chromatin remodelling complex to be detected at the HO break, 10 min after HO induction. It is therefore possible that RSC activity could be responsible for exposure of H3K79me very early in the DNA damage response. If this is the case, RSC mutations should cause checkpoint defects. Interestingly, it has been shown that an RSC mutant is defective in γH2A phosphorylation, as well as chromatin remodelling, at the site of DNA damage (Jessica Downs and Nick Kent, personal communication). RSC has also been shown to interact with Mre11 and requires MRX for its recruitment to the break [63]. This suggests that RSC is recruited very early in the DDR by MRX to alter chromatin structure and that this is required to activate PIKKs. This complex might therefore be an excellent candidate to act at the initiation step of the DDR. No doubt, as modulations to chromatin immediately following damage are not well characterised, much remains to be elucidated.

It is possible that the covalent modifications discussed in this review, phosphorylation, methylation and acetylation, act together to form a DDR-specific histone code, similar to that proposed for transcriptional regulation [72]. An example of this is Rad9 foci formation, which requires the presence of both H2A phosphorylation and H3K79 methylation. Another example is that di- and trimethylation of H3K79 and H3K4 requires prior mono-ubiquitination of H2BK123. In agreement with this, mutations of H2BK123 result in checkpoint defects similar to those seen in the absence of Dot1 [26]. A complex regulatory code also exists within the H4 N-terminal tail. Phosphorylation of H4S1 by casein kinase II (CK2) inhibits H4 acetylation by Esa1. CK2 is found in a complex with the HDAC Rpd3 and thus links inhibition of acetylation to deacetylation. Methylation of H4R3 alleviates this inhibitory effect [46]. This is similar to the phospho/methyl switch between H3S10ph and H3K9me3 in the H3 N-terminal tail that modulates heterochromatin protein 1 (HP1) binding during mitosis [73]. The added complexity of an underlying histone code makes the precise elucidation of the role of individual modifications challenging.

Other covalent modifications thought to be involved in the DDR, for example H4K91 acetylation [74], are yet to be characterised. Also, a function for other histone modifications not yet implicated in the DDR, for example SUMOylation, should not be ruled out. However, even at this early stage in the study of the role chromatin plays in the DDR, it is clear that the modulation of chromatin structure is vital for effective DSB signalling and repair.

Acknowledgments

We thank members of the Genome Stability Laboratory, in particular Muriel Grenon for their critical comments and suggestions. We thank Jessica Downs, Nick Kent, Eiichiro Sonoda and Yasunari Takami for communicating results prior to publication. Work in the Genome Stability Laboratory is supported by the Health Research Board of Ireland, Cancer Research Ireland and from the EU-IP6 Networks "RISC-RAD" and "DNA Repair". T.C. and J.F. are supported by Embark Initiative Scholarships from the Irish Research Council for Science, Engineering and Technology.

REFERENCES

[1] M.B. Kastan, J. Bartek, Cell-cycle checkpoints and cancer, Nature 432 (2004) 316–323.
[2] N.F. Lowndes, J.R. Murguia, Sensing and responding to DNA damage, Curr. Opin. Genet. Dev. 10 (2000) 17–25.
[3] M.R. Lieber, Y. Ma, U. Pannicke, K. Schwarz, Mechanism and regulation of human non-homologous DNA end-joining, Nat. Rev., Mol. Cell Biol. 4 (2003) 712–720.
[4] S.C. West, Molecular views of recombination proteins and their control, Nat. Rev., Mol. Cell Biol. 4 (2003) 435–445.
[5] R.D. Kornberg, Y. Lorch, Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome, Cell 98 (1999) 285–294.
[6] K. Luger, A.W. Mader, R.K. Richmond, D.F. Sargent, T.J. Richmond, Crystal structure of the nucleosome core particle at 2.8 A resolution, Nature 389 (1997) 251–260.
[7] T. Schalch, S. Duda, D.F. Sargent, T.J. Richmond, X-ray structure of a tetranucleosome and its implications for the chromatin fibre, Nature 436 (2005) 138–141.
[8] A. Saha, J. Wittmeyer, B.R. Cairns, Chromatin remodelling: the industrial revolution of DNA around histones, Nat. Rev., Mol. Cell Biol. 7 (2006) 437–447.
[9] S.L. Berger, Histone modifications in transcriptional regulation, Curr. Opin. Genet. Dev. 12 (2002) 142–148.
[10] D. Lydall, S. Whitehall, Chromatin and the DNA damage response, DNA Repair (Amst) 4 (2005) 1195–1207.
[11] G.J. Narlikar, H.Y. Fan, R.E. Kingston, Cooperation between complexes that regulate chromatin structure and transcription, Cell 108 (2002) 475–487.
[12] B.M. Turner, Reading signals on the nucleosome with a new nomenclature for modified histones, Nat. Struct. Mol. Biol. 12 (2005) 110–112.
[13] N.F. Lowndes, G.W. Toh, DNA repair: the importance of phosphorylating histone H2AX, Curr. Biol. 15 (2005) R99–R102.
[14] O. Fernandez-Capetillo, A. Lee, M. Nussenzweig, A. Nussenzweig, H2AX: the histone guardian of the genome, DNA Repair (Amst) 3 (2004) 959–967.

[15] E.R. Foster, J.A. Downs, Histone H2A phosphorylation in DNA double-strand break repair, FEBS J. 272 (2005) 3231–3240.
[16] M. Stucki, S.P. Jackson, gammaH2AX and MDC1: anchoring the DNA-damage-response machinery to broken chromosomes, DNA Repair (Amst.) 5 (2006) 534–543.
[17] C. Thiriet, J.J. Hayes, Chromatin in need of a fix: phosphorylation of H2AX connects chromatin to DNA repair, Mol. Cell 18 (2005) 617–622.
[18] H. Wurtele, A. Verreault, Histone post-translational modifications and the response to DNA double-strand breaks, Curr. Opin. Cell Biol. 18 (2006) 137–144.
[19] A. Celeste, O. Fernandez-Capetillo, M.J. Kruhlak, D.R. Pilch, D.W. Staudt, A. Lee, R.F. Bonner, W.M. Bonner, A. Nussenzweig, Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks, Nat. Cell Biol. 5 (2003) 675–679.
[20] M. Stucki, J.A. Clapperton, D. Mohammad, M.B. Yaffe, S.J. Smerdon, S.P. Jackson, MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks, Cell 123 (2005) 1213–1226.
[21] H. van Attikum, S.M. Gasser, The histone code at DNA breaks: a guide to repair? Nat. Rev., Mol. Cell Biol. 6 (2005) 757–765.
[22] T.M. Nakamura, B.A. Moser, L.L. Du, P. Russell, Cooperative control of Crb2 by ATM family and Cdc2 kinases is essential for the DNA damage checkpoint in fission yeast, Mol. Cell. Biol. 25 (2005) 10721–10730.
[23] M.C. Keogh, J.A. Kim, M. Downey, J. Fillingham, D. Chowdhury, J.C. Harrison, M. Onishi, N. Datta, S. Galicia, A. Emili, J. Lieberman, X. Shen, S. Buratowski, J.E. Haber, D. Durocher, J.F. Greenblatt, N.J. Krogan, A phosphatase complex that dephosphorylates gammaH2AX regulates DNA damage checkpoint recovery, Nature 439 (2006) 497–501.
[24] D. Chowdhury, M.C. Keogh, H. Ishii, C.L. Peterson, S. Buratowski, J. Lieberman, gamma-H2AX dephosphorylation by protein phosphatase 2A facilitates DNA double-strand break repair, Mol. Cell 20 (2005) 801–809.
[25] M. Suzuki, K. Suzuki, S. Kodama, M. Watanabe, Phosphorylated histone H2AX foci persist on rejoined mitotic chromosomes in normal human diploid cells exposed to ionizing radiation, Radiat. Res. 165 (2006) 269–276.
[26] M. Giannattasio, F. Lazzaro, P. Plevani, M. Muzi-Falconi, The DNA damage checkpoint response requires histone H2B ubiquitination by Rad6–Bre1 and H3 methylation by Dot1, J. Biol. Chem. 280 (2005) 9879–9886.
[27] Y. Huyen, O. Zgheib, R.A. Ditullio Jr., V.G. Gorgoulis, P. Zacharatos, T.J. Petty, E.A. Sheston, H.S. Mellert, E.S. Stavridi, T.D. Halazonetis, Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks, Nature 432 (2004) 406–411.
[28] R. Wysocki, A. Javaheri, S. Allard, F. Sha, J. Cote, S.J. Kron, Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9, Mol. Cell. Biol. 25 (2005) 8430–8443.
[29] H.H. Ng, D.N. Ciccone, K.B. Morshead, M.A. Oettinger, K. Struhl, Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: a potential mechanism for position-effect variegation, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 1820–1825.
[30] F. van Leeuwen, P.R. Gafken, D.E. Gottschling, Dot1p modulates silencing in yeast by methylation of the nucleosome core, Cell 109 (2002) 745–756.
[31] S. Maurer-Stroh, N.J. Dickens, L. Hughes-Davies, T. Kouzarides, F. Eisenhaber, C.P. Ponting, The tudor domain 'Royal Family': tudor, plant agenet, chromo, PWWP and MBT domains, Trends Biochem. Sci. 28 (2003) 69–74.
[32] S.L. Sanders, M. Portoso, J. Mata, J. Bahler, R.C. Allshire, T. Kouzarides, Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage, Cell 119 (2004) 603–614.
[33] G.W. Toh, A.M. O'Shaughnessy, S. Jimeno, I.M. Dobbie, M. Grenon, S. Maffini, A. O'Rorke, N.F. Lowndes, Histone H2A phosphorylation and H3 methylation are required for a novel Rad9 DSB repair function following checkpoint activation, DNA Repair (Amst) 5 (2006) 693–703.
[34] K. Nakamura, W. Sakai, T. Kawamoto, R.T. Bree, N.F. Lowndes, S. Takeda, Y. Taniguchi, Genetic dissection of vertebrate 53BP1: a major role in non-homologous end joining of DNA double strand breaks, DNA Repair (Amst) 5 (2006) 741–749.
[35] O. Fernandez-Capetillo, H.T. Chen, A. Celeste, I. Ward, P.J. Romanienko, J.C. Morales, K. Naka, Z. Xia, R.D. Camerini-Otero, N. Motoyama, P.B. Carpenter, W.M. Bonner, J. Chen, A. Nussenzweig, DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1, Nat. Cell Biol. 4 (2002) 993–997.
[36] R.A. DiTullio Jr., T.A. Mochan, M. Venere, J. Bartkova, M. Sehested, J. Bartek, T.D. Halazonetis, 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer, Nat. Cell Biol. 4 (2002) 998–1002.
[37] I.M. Ward, K. Minn, J. van Deursen, J. Chen, p53 Binding protein 53BP1 is required for DNA damage responses and tumor suppression in mice, Mol. Cell. Biol. 23 (2003) 2556–2563.
[38] B. Dorigo, T. Schalch, K. Bystricky, T.J. Richmond, Chromatin fiber folding: requirement for the histone H4 N-terminal tail, J. Mol. Biol. 327 (2003) 85–96.
[39] F. Gordon, K. Luger, J.C. Hansen, The core histone N-terminal tail domains function independently and additively during salt-dependent oligomerization of nucleosomal arrays, J. Biol. Chem. 280 (2005) 33701–33706.
[40] C. Zheng, X. Lu, J.C. Hansen, J.J. Hayes, Salt-dependent intra- and internucleosomal interactions of the H3 tail domain in a model oligonucleosomal array, J. Biol. Chem. 280 (2005) 33552–33557.
[41] B.A. Tamburini, J.K. Tyler, Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair, Mol. Cell. Biol. 25 (2005) 4903–4913.
[42] J.A. Downs, S. Allard, O. Jobin-Robitaille, A. Javaheri, A. Auger, N. Bouchard, S.J. Kron, S.P. Jackson, J. Cote, Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites, Mol. Cell 16 (2004) 979–990.
[43] A.W. Bird, D.Y. Yu, M.G. Pray-Grant, Q. Qiu, K.E. Harmon, P.C. Megee, P.A. Grant, M.M. Smith, M.F. Christman, Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair, Nature 419 (2002) 411–415.
[44] Y. Teng, Y. Yu, R. Waters, The *Saccharomyces cerevisiae* histone acetyltransferase Gcn5 has a role in the photoreactivation and nucleotide excision repair of UV-induced cyclobutane pyrimidine dimers in the MFA2 gene, J. Mol. Biol. 316 (2002) 489–499.
[45] S. Qin, M.R. Parthun, Recruitment of the type B histone acetyltransferase Hat1p to chromatin is linked to DNA double-strand breaks, Mol. Cell. Biol 26 (2006) 3649–3658.
[46] R.T. Utley, N. Lacoste, O. Jobin-Robitaille, S. Allard, J. Cote, Regulation of NuA4 histone acetyltransferase activity in transcription and DNA repair by phosphorylation of histone H4, Mol. Cell. Biol. 25 (2005) 8179–8190.
[47] R. Murr, J.I. Loizou, Y.G. Yang, C. Cuenin, H. Li, Z.Q. Wang, Z. Herceg, Histone acetylation by Trrap-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks, Nat. Cell Biol. 8 (2006) 91–99.
[48] M. Downey, D. Durocher, Chromatin and DNA repair: the benefits of relaxation, Nat. Cell Biol. 8 (2006) 9–10.
[49] N.A. Barlev, V. Poltoratsky, T. Owen-Hughes, C. Ying, L. Liu, J.L. Workman, S.L. Berger, Repression of GCN5 histone acetyltransferase activity via bromodomain-mediated binding and phosphorylation by the Ku-DNA-dependent protein kinase complex, Mol. Cell. Biol. 18 (1998) 1349–1358.

[50] H. Masumoto, D. Hawke, R. Kobayashi, A. Verreault, A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response, Nature 436 (2005) 294–298.
[51] C.A. Davey, D.F. Sargent, K. Luger, A.W. Maeder, T.J. Richmond, Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution, J. Mol. Biol. 319 (2002) 1097–1113.
[52] X.J. Yang, Lysine acetylation and the bromodomain: a new partnership for signaling, BioEssays 26 (2004) 1076–1087.
[53] M.S. Kobor, S. Venkatasubrahmanyam, M.D. Meneghini, J.W. Gin, J.L. Jennings, A.J. Link, H.D. Madhani, J. Rine, A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin, PLoS Biol. 2 (2004) E131.
[54] X. Jiang, Y. Sun, S. Chen, K. Roy, B.D. Price, The FATC domains of PIKK proteins are functionally equivalent and participate in the Tip60-dependent activation of DNA-PKcs and ATM, J. Biol. Chem. 281 (2006) 15741–15746.
[55] Y. Sun, X. Jiang, S. Chen, N. Fernandes, B.D. Price, A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13182–13187.
[56] G. Mizuguchi, X. Shen, J. Landry, W.H. Wu, S. Sen, C. Wu, ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex, Science 303 (2004) 343–348.
[57] X. Shen, G. Mizuguchi, A. Hamiche, C. Wu, A chromatin remodelling complex involved in transcription and DNA processing, Nature 406 (2000) 541–544.
[58] T. Owen-Hughes, R.T. Utley, J. Cote, C.L. Peterson, J.L. Workman, Persistent site-specific remodeling of a nucleosome array by transient action of the SWI/SNF complex, Science 273 (1996) 513–516.
[59] B.R. Cairns, Y. Lorch, Y. Li, M. Zhang, L. Lacomis, H. Erdjument-Bromage, P. Tempst, J. Du, B. Laurent, R.D. Kornberg, RSC, an essential, abundant chromatin-remodeling complex, Cell 87 (1996) 1249–1260.
[60] B. Chai, J. Huang, B.R. Cairns, B.C. Laurent, Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair, Genes Dev. 19 (2005) 1656–1661.
[61] A.J. Morrison, J. Highland, N.J. Krogan, A. Arbel-Eden, J.F. Greenblatt, J.E. Haber, X. Shen, INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair, Cell 119 (2004) 767–775.
[62] H. van Attikum, O. Fritsch, B. Hohn, S.M. Gasser, Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair, Cell 119 (2004) 777–788.
[63] E.Y. Shim, J.L. Ma, J.H. Oum, Y. Yanez, S.E. Lee, The yeast chromatin remodeler RSC complex facilitates end joining repair of DNA double-strand breaks, Mol. Cell. Biol. 25 (2005) 3934–3944.
[64] T. Tsukuda, A.B. Fleming, J.A. Nickoloff, M.A. Osley, Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*, Nature 438 (2005) 379–383.
[65] J. Huang, J.M. Hsu, B.C. Laurent, The RSC nucleosome-remodeling complex is required for cohesin's association with chromosome arms, Mol. Cell 13 (2004) 739–750.
[66] E. Unal, A. Arbel-Eden, U. Sattler, R. Shroff, M. Lichten, J.E. Haber, D. Koshland, DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain, Mol. Cell 16 (2004) 991–1002.
[67] M. Lisby, J.H. Barlow, R.C. Burgess, R. Rothstein, Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins, Cell 118 (2004) 699–713.
[68] J. Rouse, S.P. Jackson, Lcd1p recruits Mec1p to DNA lesions in vitro and in vivo, Mol. Cell 9 (2002) 857–869.
[69] R. Shroff, A. Arbel-Eden, D. Pilch, G. Ira, W.M. Bonner, J.H. Petrini, J.E. Haber, M. Lichten, Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break, Curr. Biol. 14 (2004) 1703–1711.
[70] J.A. Melo, J. Cohen, D.P. Toczyski, Two checkpoint complexes are independently recruited to sites of DNA damage in vivo, Genes Dev. 15 (2001) 2809–2821.
[71] D. Nakada, K. Matsumoto, K. Sugimoto, ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism, Genes Dev. 17 (2003) 1957–1962.
[72] T. Jenuwein, C.D. Allis, Translating the histone code, Science 293 (2001) 1074–1080.
[73] T. Hirota, J.J. Lipp, B.H. Toh, J.M. Peters, Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin, Nature 438 (2005) 1176–1180.
[74] A. Klochendler-Yeivin, E. Picarsky, M. Yaniv, Increased DNA damage sensitivity and apoptosis in cells lacking the Snf5/Ini1 subunit of the SWI/SNF chromatin remodeling complex, Mol. Cell. Biol. 26 (2006) 2661–2674.
[75] W.L. Cheung, F.B. Turner, T. Krishnamoorthy, B. Wolner, S.H. Ahn, M. Foley, J.A. Dorsey, C.L. Peterson, S.L. Berger, C.D. Allis, Phosphorylation of histone H4 serine 1 during DNA damage requires casein kinase II in *S. cerevisiae*, Curr. Biol. 15 (2005) 656–660.
[76] A. Ghavidel, M.C. Schultz, TATA binding protein-associated CK2 transduces DNA damage signals to the RNA polymerase III transcriptional machinery, Cell 106 (2001) 575–584.
[77] D.P. Toczyski, D.J. Galgoczy, L.H. Hartwell, CDC5 and CKII control adaptation to the yeast DNA damage checkpoint, Cell 90 (1997) 1097–1106.
[78] H.H. Ng, F. Robert, R.A. Young, K. Struhl, Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity, Mol. Cell 11 (2003) 709–719.
[79] K. van Dijk, K.E. Marley, B.R. Jeong, J. Xu, J. Hesson, R.L. Cerny, J.H. Waterborg, H. Cerutti, Monomethyl histone H3 lysine 4 as an epigenetic mark for silenced euchromatin in Chlamydomonas, Plant Cell 17 (2005) 2439–2453.
[80] H. Talasz, H.H. Lindner, B. Sarg, W. Helliger, Histone H4-lysine 20 monomethylation is increased in promoter and coding regions of active genes and correlates with hyperacetylation, J. Biol. Chem. 280 (2005) 38814–38822.
[81] G. Schotta, M. Lachner, K. Sarma, A. Ebert, R. Sengupta, G. Reuter, D. Reinberg, T. Jenuwein, A silencing pathway to induce H3–K9 and H4–K20 trimethylation at constitutive heterochromatin, Genes Dev. 18 (2004) 1251–1262.
[82] K. Nishioka, J.C. Rice, K. Sarma, H. Erdjument-Bromage, J. Werner, Y. Wang, S. Chuikov, P. Valenzuela, P. Tempst, R. Steward, J.T. Lis, C.D. Allis, D. Reinberg, PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin, Mol. Cell 9 (2002) 1201–1213.
[83] J. Ye, X. Ai, E.E. Eugeni, L. Zhang, L.R. Carpenter, M.A. Jelinek, M.A. Freitas, M.R. Parthun, Histone H4 lysine 91 acetylation a core domain modification associated with chromatin assembly, Mol Cell 18 (2005) 123–130.
[84] M.D. Shahbazian, K. Zhang, M. Grunstein, Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1, Mol. Cell 19 (2005) 271–277.