

Complicated Tails: Histone Modifications and the DNA Damage Response

Minireview

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In recent years, several ATP-dependent chromatin-remodeling complexes and covalent histone modifications have been implicated in the response to double-stranded DNA breaks (DSBs). When a DSB occurs, cells must identify the DSB, activate the DNA damage checkpoint, and repair the break. Chromatin modification appears to be important but not essential for each of these processes, yet its precise mechanistic roles are only beginning to come into focus. Here, we discuss the role of chromatin in signaling by the DNA damage checkpoint pathway.

On induction of a DSB, the 5' strand of DNA is selectively degraded. This resection is a required processing step for some forms of DNA repair, such as homologous recombination and single-strand annealing. Both of these repair pathways are homology based, and production of a single-stranded DNA (ssDNA) end aids in the identification of complementary sequences. Resection also serves to effectively amplify the signal emanating from a single DSB, as many checkpoint proteins are thought to recognize the ssDNA. The data discussed here suggest that the chromatin adjacent to this ssDNA may also have a role in repair and signaling.

Three classes of proteins are thought to initiate damage checkpoint signaling: two related PI3K-like kinases called ATR and ATM; a PCNA-like heterotrimeric ring called the 9-1-1 complex; and a loosely defined set of adaptor proteins, many of which contain BRCT domains. This last class is represented by the archetypal *S. cerevisiae* checkpoint protein Rad9 and two quite divergent proteins, *S. pombe* Crb2 and human 53BP1. While we will treat these three proteins as homologs here, it should be noted that they do not have extensive sequence similarity, nor do they function identically in yeast and man. These adaptor proteins are thought to promote the phosphorylation/activation of downstream serine/threonine kinases, such as scRad53, hChk2, and spChk1.

Studies have indicated that ATR/ATM and the 9-1-1 complex localize to sites of damage independently. ATM, called Tel1 in *S. cerevisiae* and *S. pombe*, associates with DNA damage through the MRN complex (composed of Mre11, Rad50, and Nbs1). ATR and the *S. cerevisiae* homolog Mec1 are thought to subsequently associate, indirectly, with the ssDNA uncovered by resection. Loading of the 9-1-1 complex has been suggested to occur at ssDNA/dsDNA junctions. Here, we will review data suggesting that both phosphorylation

and methylation of histones help target the adaptor protein Rad9/Crb2/53BP1 to DSBs.

H2AX Phosphorylation

Eukaryotic DNA is wrapped in a complex of eight histone molecules, two copies each of H2A, H2B, H3, and H4, generating a structure called the nucleosome. In addition, several variant forms of histones exist. For H2A, these include H2AX, H2AZ, MacroH2A, and H2A-Bbd (Kamakaka and Biggins, 2005). H2AX makes up a considerable portion of the H2A pool, ~2%–25% in mammals. Phosphorylation of the C terminus of H2AX by ATM/ATR (Tel1/Mec1 in budding yeast) is an evolutionarily conserved response to DSBs. In humans, the H2AX C-terminal tail is a short extension beyond a conserved core region that distinguishes this variant from the canonical H2A1 histone. Although the length of the tail can vary in different species, the SQ residues at the –4 and –3 positions from the C terminus (which represent the ATR/ATM consensus site) are invariant. Both *S. cerevisiae* and *S. pombe* lack a separate H2AX variant, yet ~90% of the H2A pool carries an analogous C-terminal serine that is also phosphorylated upon DNA damage. Phosphorylated mammalian H2AX and yeast H2A will both be referred to as γ -H2AX (reviewed by Pilch et al. [2003]).

Phosphorylation of H2AX is one of the earliest responses to DNA damage. Within minutes of ionizing radiation (IR), γ -H2AX foci have been observed by immunofluorescence in mammalian cells (Celeste et al., 2003; Celeste et al., 2002; Pilch et al., 2003). These damage-induced foci have been demonstrated to form at DSBs and increase in size over time. Laser scissors, which induce DSBs along the path of a laser across cells, induce a coincident pattern of γ -H2AX staining (Celeste et al., 2003). Moreover, when a site-specific DSB is induced by expressing the HO endonuclease in yeast, γ -H2AX has been shown by chromatin immunoprecipitation (ChIP) to associate with DNA adjacent to the break (Downs et al., 2004; Unal et al., 2004; van Attikum et al., 2004). Over time, γ -H2AX can be immunoprecipitated with genomic loci increasingly distal from the HO break, up to 50 kilobases away (Unal et al., 2004).

γ -H2AX appears to be important for promoting efficient repair in both mice and yeast. H2AX^{-/-} knockout mice are viable yet sensitive to IR (Celeste et al., 2002). H2AX^{-/-} mouse embryonic fibroblasts (MEFs) have more spontaneous chromosomal aberrations than their wild-type counterparts and generate more breaks when exposed to IR. H2AX^{-/-} cells are also slower to repair IR-induced damage (Celeste et al., 2002). Similar phenotypes were observed in yeast carrying an H2A serine to alanine (AQ) mutation, which prevents H2A from being phosphorylated in response to damage. Studies examining *S. cerevisiae* and *S. pombe* H2A-AQ mutants report increased sensitivity to several DNA damaging agents, such as MMS, camptothecin, and IR, all of which generate DSBs (Downs et al., 2000; Nakamura et al., 2004). This sensitivity is far less than that conferred by checkpoint and repair mutants, suggesting γ -H2AX

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may contribute to but is not essential for both processes.

One mechanism by which γ -H2AX could promote repair is to recruit repair machinery to damage sites; however, localization studies argue against this notion. DSBs are repaired by nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ promotes the religation of two broken ends of a DSB, whereas HR uses a homologous template, preferably a sister chromatid, for repair-induced replication leading to rejoining of the broken ends. The HR protein Rad51 forms IR-induced foci in H2AX^{-/-} mouse MEFs (Celeste et al., 2002). Similarly, the *S. pombe* HR protein Rad22 forms foci equally well in wild-type or H2A-AQ mutants after exposure to IR (Nakamura et al., 2004). On the other hand, the MRN complex, which functions in both NHEJ and HR in yeast, is initially recruited to sites of damage, but this is followed by a partial or complete loss of focus formation in the absence of H2AX (Celeste et al., 2003; Celeste et al., 2002; Kobayashi et al., 2002). As with recruitment of the checkpoint adaptors (below), this may indicate that the MRN complex has both an initial means of localizing to breaks (independent of γ -H2AX) and a secondary binding interface (dependent on γ -H2AX). This latter interaction could be through a direct association of Nbs1 with γ -H2AX (Kobayashi et al., 2002). Since the MRN complex also targets ATM to damage sites, this might allow γ -H2AX to spread via a sequential cycle of phosphorylation and binding.

One compelling link to repair is through the γ -H2AX-dependent association of cohesins to DSBs (Strom et al., 2004; Unal et al., 2004). Cohesin complexes are the physical links between sister chromatids that are established during S phase and maintained until mitosis occurs. Recent studies in *S. cerevisiae* demonstrate that cohesin complexes can, in fact, be loaded de novo outside of S phase in response to an HO-induced DSB (Strom et al., 2004; Unal et al., 2004). This new loading is largely dependent upon H2A phosphorylation and spans an area that overlaps with γ -H2AX spreading (Unal et al., 2004). Furthermore, repair of IR damage is slower in the absence of de novo cohesin loading, suggesting that cohesion promotes HR when a sister chromatid is used as a template (Strom et al., 2004).

An emerging picture of γ -H2AX function is that it promotes effective repair in multiple ways. Although it is clear that γ -H2AX is not essential for checkpoint signaling, several lines of evidence suggest that γ -H2AX may promote the accumulation of checkpoint adaptors. Mouse H2AX^{-/-} cells were capable of eliciting a checkpoint response to high-dose IR but were defective in triggering the G2/M checkpoint when treated with low-dose IR (Celeste et al., 2002). Despite having a partially functional checkpoint, damage-induced foci of the checkpoint effectors NBS1, 53BP1, and BRCA1, all of which carry BRCT domains, were greatly diminished (Celeste et al., 2003). More detailed analysis showed that, similar to the above-mentioned MRN complex, these proteins could transiently form damage-induced foci without H2AX, but this localization was not sustained, suggesting a role in maintenance of the checkpoint (Celeste et al., 2003). Curiously, the role of the BRCT domain in γ -H2AX binding differs between proteins. While the BRCT domain is important for NBS1 binding to a phospho-H2A peptide, 53BP1's BRCT do-

main is not required for its γ -H2AX binding or focus formation (Kobayashi et al., 2002; Ward et al., 2003).

γ -H2AX also appears to have a role in adaptor function in *S. pombe*. After low levels of IR, the H2A-AQ mutant can appropriately phosphorylate downstream checkpoint components and mediate a checkpoint arrest (Nakamura et al., 2004). This checkpoint competency is challenged with increasing amounts of irradiation. H2A-AQ mutants cannot form Crb2 foci at sites of damage. In vitro, Crb2 is able to bind phosphorylated but not unphosphorylated H2A peptides, suggesting a direct interaction (Nakamura et al., 2004). Thus, γ -H2AX is important but not essential for Crb2 response at DSBs.

Chromatin remodeling complexes are an additional class of proteins that are recruited to damage via γ -H2AX. Recently, three separate *S. cerevisiae* remodeling complexes, namely NuA4, Ino80, and Swr1, have been shown to specifically interact with γ -H2AX or a phospho-H2A peptide and localize to DNA proximal to an induced HO break (Bird et al., 2002; Downs et al., 2004; Morrison et al., 2004; van Attikum et al., 2004). NuA4 is a histone acetyltransferase complex whose catalytic subunit, Esa1, acetylates the N-terminal tail of H4, which is important for resistance to DNA-damaging agents (Bird et al., 2002). The Ino80 and Swr1 complexes both contain a catalytic subunit in the SWI/SNF family of ATP-dependent chromatin remodeling enzymes. The Ino80 complex can remodel and slide nucleosomes in vitro, whereas the Swr1 complex exchanges H2A for H2AZ in vitro and in vivo (Ehrenhofer-Murray, 2004; Kamakaka and Biggins, 2005). These complexes are localized to DNA proximal to an induced HO break. Preventing the function of Ino80, NuA4, or Swr1 sensitizes cells to DSB-inducing agents (Bird et al., 2002; Downs et al., 2004; Morrison et al., 2004; van Attikum et al., 2004).

Ino80, NuA4, and Swr1 could promote the access to or processing of DNA by repair proteins. Chromatin remodeling may also lead to the removal of phosphorylated histones. This intriguing activity has been recently suggested by Kusch et al., who examined the *Drosophila melanogaster* Tip60 chromatin remodeling complex, which contains subunits homologous to both the yeast NuA4 and Swr1 complexes (Kusch et al., 2004). Tip60 specifically acetylates the phosphorylated form of the fly histone variant H2Av (a.k.a. H2AvD), and this acetylation promotes the removal of phospho-H2Av from nucleosomes. H2Av is phosphorylated upon damage in a manner analogous to H2AX but also has characteristics of H2AZ. Loss of Tip60 in vivo results in the persistence of damage-induced phospho-H2Av foci (Kusch et al., 2004), presumably because of a lack of H2Av eviction. However, it is not yet known whether this exchange promotes repair or simply allows cells to recover after repair. Furthermore, it is unclear whether damage-induced exchange is a property of mammalian H2AZ or H2AX, since H2AX is reported to be immobile in chromatin (Pilch et al., 2003).

Histone Methylation

Histone methylations are best known for their role in gene silencing and heterochromatin formation. These posttranslational modifications are carried out by histone methyl-transferases (HMT), which covalently modify lysines and arginines on histones. These modifications, in combination with acetylations, are thought to

inscribe a histone pattern that recruits factors that affect transcription (Ehrenhofer-Murray, 2004). Recently, histone ubiquitinations, acetylations, and methylations have been implicated in the DNA damage checkpoint and repair pathways (see Table S1 in the Supplemental Data available with this article online). We will focus specifically on histone methylations and their role in recruiting checkpoint adaptors to damage sites.

Bulk levels of histone methylations do not appear to be induced after DNA damage (Huyen et al., 2004). Nonetheless, histone methylations contribute to the checkpoint by directly interacting with checkpoint components. In mammals, methylation of lysine 79 on H3 (H3-K79-Me) is important for localization of 53BP1 (Huyen et al., 2004). Cells deficient in Dot1, the HMT responsible for lysine 79 methylation, are unable to form 53BP1 foci after damage. The requirement for H3-K79 methylation in 53BP1 focus formation is most likely due to a direct interaction between H3 and 53BP1, since 53BP1 can bind H3-K79-Me in vitro (Huyen et al., 2004).

S. cerevisiae seems to share this mechanism. Deletion of *DOT1* or mutation of H3-K79 impairs Rad53 phosphorylation after DNA damage (Giannattasio et al., 2005). As with 53BP1, Rad9 binds K79-methylated H3 in vitro (Huyen et al., 2004). Similar to the loss of γ -H2AX, loss of H3-K79 methylation does not entirely eliminate the checkpoint, suggesting that an independent mechanism for the recruitment of Rad9 must exist (Giannattasio et al., 2005).

S. pombe also uses histone methylation to recruit the adaptor protein Crb2 to damage, although it apparently uses a different methylation site, lysine 20 on histone H4 (Sanders et al., 2004). The methylation on H4-K20 requires the HMT Set9, and studies have shown that *set9*-deleted cells were more sensitive than wild-type to several types of DNA damage. *crb2* mutants are much more damage sensitive than *set9* (or H2A-AQ) mutants, consistent with the model of alternative recruitment mechanisms for adaptor proteins. The number of Crb2 foci is reduced in cells lacking *set9* (Sanders et al., 2004), and the G2 checkpoint is partially defective, as indicated by premature entry into mitosis. Moreover, the downstream checkpoint kinase Chk1 accumulates in its unphosphorylated form, also suggesting defects in checkpoint signaling. However, a complete loss of checkpoint function was only seen when a *set9* deletion was combined with mutations in other checkpoint genes.

From the examples above, it is evident that histone methylations play a part in the DNA damage checkpoint pathway, even though the methylation sites used are not entirely conserved. Mammals and budding yeast employ H3-K79-Me, whereas *S. pombe* uses H4-K20-Me. Despite this, the tandem Tudor domain, which is found in each of these checkpoint adaptor proteins and is thought to bind the methylated histone, is conserved (Huyen et al., 2004). Tudor domains have been characterized in several proteins that recognize methylated proteins and have structural and sequence similarities to other methyl binding domains, such as Chromo domains. Huyen et al. showed that mutations in the Tudor domain of 53BP1 eliminate its ability to form damaged-induced foci and bind H3-K79-Me containing chromatin in vitro (Huyen et al., 2004).

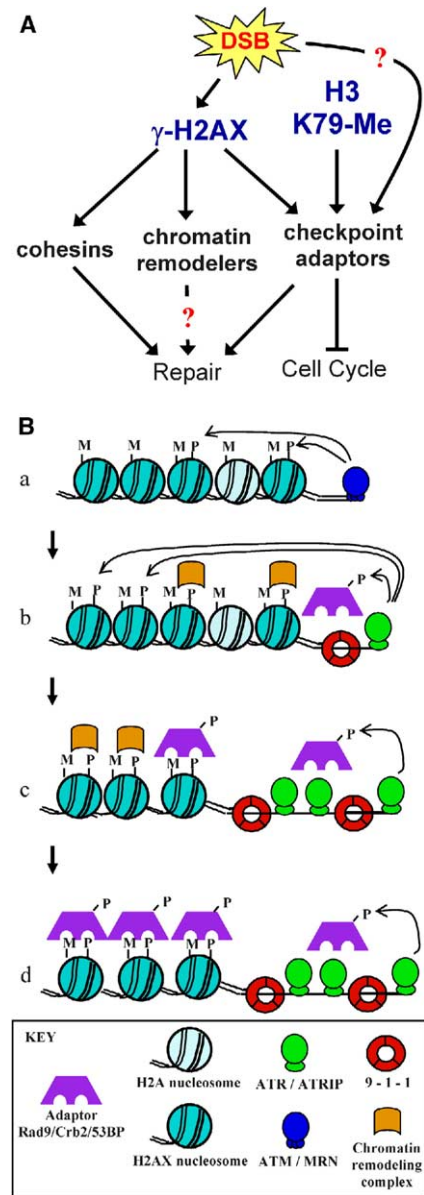


Figure 1. Models of Recruitment to a DSB via Histone Modifications (A) γ -H2AX is induced by DSBs to recruit cohesins, chromatin remodeling complexes, and checkpoint adaptors to the damage site. The H3-K79-Me (H4-K20-Me in *S. pombe*) is a constitutive modification that contributes to recruiting checkpoint adaptors to DSBs. (B) (Ba) The ATM kinase is recruited to the broken end through the MRN complex, where it mediates H2AX phosphorylation. (Bb) After the initiation of resection, both ATR (and its associated ATRIP subunit) and 9-1-1 are recruited to the resected ssDNA. ATR phosphorylates the adaptor protein and expands upon the initial H2AX phosphorylation. Chromatin remodeling complexes are recruited to γ -H2AX. (Bc) Chromatin remodelers may facilitate presentation of H3/H4 methylations. (Bd) Adaptors could interact cooperatively with H3/H4 methylation and γ -H2AX.

Conclusions

Collectively, these data represent a complicated picture of Rad9/Crb2/53BP1 recruitment (Figure 1A). The complication stems largely from three facts. First, the recruitment of the adaptor proteins appears to depend upon many different modifications, only a subset of

which are known to be DNA damage inducible. Second, some of these modifications seem to recruit several proteins to chromatin, leaving open the possibility that the observed dependencies could be indirect. Finally, neither chromatin remodeling nor histone modifications are absolutely required for these adaptor proteins to function in the DNA damage response.

In an effort to combine these data into a coherent picture, we present a speculative model (Figure 1B). Upon induction of a DSB, ATM initially associates with DSB ends and phosphorylates H2AX. After the initiation of resection, ATM is replaced in part by ATR, which maintains and expands the γ -H2AX phosphorylation. γ -H2AX then recruits several chromatin remodelers, including the Ino80 and Nu4A complexes. The activity of these complexes may promote the exposure of preexisting H4-K20-Me or H3-K79-Me modifications on nearby nucleosomes or could facilitate repair. Recruitment of Rad9/Crb2/53BP1 could then be driven by a cooperative association of these adaptors with both γ -H2AX and K-Me histone in a manner that may be aided by remodeling enzymes. Regardless of the exact mechanism, the data reviewed here place the majority of the recruited Rad9/Crb2/53BP1 at the unresected, intact chromatin, significantly distal to the ssDNA-associated Mec1/Rad3/ATR kinase.

The use of a constitutive histone modification to mediate the binding of checkpoint proteins to DNA damage sites is both intriguing and confusing. In yeast, K79-methylated H3 is abundant and accounts for ~90% of the H3 in the cell (van Leeuwen et al., 2002). Thus, H3-K79-Me may simply be part of a constitutive protein/protein interaction domain. Alternatively, methylation of H3-K79 could furnish an added level of regulation. It is not yet known whether the level of or access to methylated H3-K79 proximal to a DSB is altered. However, the fact that most of the genome is bound to H3-K79-Me strongly suggests that this modification is not sufficient to target the adaptors to DSBs in the absence of γ -H2AX.

Despite the fact that loss of either H2AX phosphorylation or H3-K79-Me/H4-K20-Me compromises Rad9/Crb2/53BP1 function, these checkpoint adaptors still respond to DNA damage and, at least in the case of Rad9 and Crb2, retain an active function in the checkpoint pathway. Further experiments may show that Rad9/Crb2/53BP1 activity is entirely eliminated by the simultaneous disruption of both γ -H2AX and H4-K20/H3-K79 methylation. Alternatively, Rad9/Crb2/53BP1 might remain damage responsive in these mutants because it has another mechanism for associating with damage sites, independent of nucleosomes. For example, a smaller fraction of Rad9/Crb2/53BP1 could associate with the resected ssDNA, either directly or through an association with other checkpoint proteins. Thus, despite a reduction in focus intensity in cells lacking these histone modifications, a functional pool of checkpoint adaptors might remain at damaged sites. Why, then, is it critical to create a local pool of checkpoint adaptors through multiple means of recruitment? Is the chromatin-associated adaptor functionally distinct and acting at a different step in the checkpoint pathway?

The answers to these questions will have to await further studies to unravel these complicated tails.

Supplemental Data

Supplemental Data include one table and can be found with this minireview online at <http://www.cell.com/cgi/content/full/121/7/973/DC1>.

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