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Review

H2AX: the histone guardian of the genome

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Abstract

At close hand to one's genomic material are the histones that make up the nucleosome. Standing guard, one variant stays hidden doubling as one of the core histones. But, thanks to its prime positioning, a variation in the tail of H2AX enables rapid modification of the histone code in response to DNA damage. A role for H2AX phosphorylation has been demonstrated in DNA repair, cell cycle checkpoints, regulated gene recombination events, and tumor suppression. In this review, we summarize what we have learned about this marker of DNA breaks, and highlight some of the questions that remain to be elucidated about the physiological role of H2AX. We also suggest a model in which chromatin restructuring mediated by H2AX phosphorylation serves to concentrate DNA repair/signaling factors and/or tether DNA ends together, which could explain the pleotropic phenotypes observed in its absence.
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1. Introduction: the tale of a tail

Just as the nucleic acid base is the fundamental repeating unit of DNA, the nucleosome forms the basic building block of chromatin. Within each nucleosome, 147 base-pairs of DNA are wrapped 1.7 times around a central core of eight histone protein molecules (an octamer consisting of two copies each of H2A, H2B, H3, and H4 histones) that form a 100 kDa protein complex [1]. Each core histone contains a "globular domain," which is necessary for histone–histone and histone–DNA contacts, as well as a "tail" motif in both the COOH and NH2-terminal regions, which are targets of post-translational modification. Further compaction is achieved by the linker histone H1, which compresses linear nucleosome arrays into a 30 nm chromatin fiber. Although histones are highly conserved proteins, cells contain alternative versions of the canonical core histones with distinct amino acid sequences. Indeed, functional isoforms are scattered throughout the mammalian genome (see http://genome.nhgri.nih.gov/histones/chrmap.shtml for specific details of the human genome). Recent studies have indicated that some of these histone variants have specialized biological functions beyond the packaging of DNA into nucleosomes. Here, we review the experiments that provided a growing understanding of the role of the histone H2A variant H2AX in cellular metabolism and the maintenance of genomic stability.

First identified in 1980 in human cells as an electrophoretic isoform of the core histone H2A [2], H2AX was subsequently sequenced in the late 80's [3]. These early studies noted the presence of a short COOH terminal tail not found in any other mammalian H2A isoforms, but being present—with various degrees of homology and linker lengths—in the unique H2A homologue of lower eukaryotes [3,4]. H2AX constitutes a major H2A species, and its levels vary from 2–25% of the mammalian histone H2A pool depending on the cell line or tissue examined [5,6]. Like other members of the H2A family, H2AX can be phosphorylated on Ser 1, acetylated on Lys 5 and ubiquitinated on Lys 119 [7]. But what makes H2AX unique is a highly conserved serine residue located 4 amino acids from the COOH terminus, which is rapidly phosphorylated upon the exposure of cells to DNA damage [5].

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2. γ-H2AX marks the spot

The presence of two evolutionary conserved pathways, homologous end joining (NHEJ) and homologous recombination (HR), for the repair of DNA double strand breaks (DSBs) highlights the threat to genomic integrity caused by free DNA ends [8]. A role for H2AX in the DNA damage response was first suggested by William Bonner and co-workers who used two-dimensional gel analysis to show that phosphorylated H2AX formed rapidly following exposure of cells to ionizing radiation [5]. The phosphorylation site was mapped to the serine 139 residue present within the conserved COOH terminal tail of H2AX. In addition, they found that the amount of H2AX that was γ-phosphorylated per DSB corresponded to a very large region, estimated to contain about 2 megabases of chromatin or thousands of nucleosomes [5]. Subsequently, the development of a phospho-specific anti-γ-H2AX antibody confirmed that, indeed, H2AX is massively phosphorylated in the chromatin surrounding DSBs, in what has been come to be known as nuclear foci [9]. H2AX was found to be essentially immobile in chromatin, suggesting that phosphorylation-dephosphorylation cycles do not follow a diffusional exchange mechanism [10]. Because of the rapid induction and amplification of phospho-H2AX, and the 1:1 correspondence between the number of γ-H2AX foci and the number of DSBs [11], γ-H2AX recognizing antibodies have become a gold standard to detect the presence of DSBs.

Phosphorylation of H2AX is induced in response to DSBs originating from diverse origins including external damage [5,9,12], replication fork collision [13,14], apoptosis [15], and dysfunctional telomeres [16,17]. Many immunofluorescence-based studies have exploited the sensitivity of γ-H2AX foci to address questions about the role, timing and distribution of DSBs. For example, quantification of γ-H2AX foci has been used to demonstrate that NHEJ is important in all cell cycle phases, whereas HR occurs predominantly in the S/G_2M cell cycle phases [18]. Studies in mouse germ cells revealed that γ-H2AX staining (and therefore DSB formation) preceded the synapsis of homologous chromosomes, indicating that the breaks during meiotic recombination formed even before the homologous chromosomes are aligned [19]. V(D)J and class-switch recombination in lymphocytes also rely on DSB intermediates that are repaired by NHEJ. γ-H2AX formation was detected at loci actively undergoing such alterations in the antigen receptor genes [16,20,21]. The role of H2AX and its associated foci in programmed gene rearrangements is discussed in detail below.

3. Who rings the H2AX serine 139 bell?

Following the generation of DSBs a rapid kinase-based signaling pathway is activated that coordinates DNA repair with the induction cell-cycle checkpoints [22,23]. The principal mediators in this pathway are the phosphatidylinositol-3 kinase-like family of kinases (PIKK). At least four PIKK family members are involved in the transduction of the signal that originates at broken DNA: ataxia telangiectasia mutated (ATM) ATM-and Rad3-related (ATR) ATM related kinase (ATX), and DNA dependent protein kinase (DNA-PK) [24]. While ATM seems to be the principal kinase that responds to ionizing radiation, the ATR-Chk1 pathway is predominant in the signaling pathway triggered by stalled replication forks or ultraviolet light [25]. Studies so far indicate that this distinction also holds true for H2AX phosphorylation [14,26,27]; that is, H2AX is phosphorylated by ATR in response to DNA replication stress [14], and primarily by ATM in response to low levels of ionizing radiation [27,28]. Moreover, formation of γ-H2AX triggered by uncapped telomeres and meiotic recombination associated DSBs are also largely dependent on ATM [16,17,29]. However, there is functional redundancy among the pathways that activate H2AX, since H2AX phosphorylation is detectable in individual kinase dead mutants ($ATM^{-/-}$, $DNA\text{-}PK^{-/-}$, and $ATR^{-/-}$) [14,27–30].

Finally, H2AX phosphorylation has been identified over the condensed XY chromosomes in male meiotic prophase I [19]. Interestingly, this pattern of phosphorylation is independent of meiotic recombination-associated DSBs [19,31] and independent of ATM and DNA-PK [31]. Thus, the kinase(s) as well as the signal responsible for this massive H2AX phosphorylation over the sex chromosomes remains to be elucidated.

4. Race to the break

The recruitment of DNA damage signaling and repair proteins to sites of genomic damage constitutes a primary event triggered by DNA damage. Many components of the DNA damage response, including ATM, BRCA1, 53BP1, MDC1, RAD51, and the MRE11/RAD50/NBS1 (MRN) complex [12,21,32–36] form ionizing radiation induced foci (IRIF) that co-localize with γ-H2AX foci. These nuclear micro-domains are thought to contain hundreds to thousands of molecules that accumulate in the vicinity of a DSB. Several lines of evidence led to the hypothesis that γ-H2AX is essential for the the recruitment of repair/signaling proteins to DNA damage: (a) γ-H2AX IRIF formation exhibits rapid kinetics that precedes repair factor assembly into IRIF [5,12]; (b) H2AX, and more precisely γ-H2AX [37,38] is required for foci formation for numerous factors including 53BP1, NBS1, BRCA1 and MDC1 [12,35,39,40]; (c) γ-H2AX physically interacts with NBS1, 53BP1 and MDC1 [35,38,41,42]. However, γ-H2AX-mediated foci formation is not equivalent to factor recruitment [37]. First, although several factors do not form foci in $H2AX^{-/-}$ cells, they are largely phosphorylated upon exposure to IR even in the absence of H2AX [37]. In the case of NBS1 and CHK2, this PIKK-mediated phosphorylation has been shown to occur

precisely at DSBs [43]. Second, some of the proteins that do not form IRIF in H2AX$^{-/-}$ cells are essential for life (i.e., NBS1 or BRCA1), presumably because they are critical components of the repair/signaling pathways that deal with spontaneous DSBs. Finally, although NBS1 or 53BP1 IRIF formation is impaired in the absence of H2AX, recent experiments using irradiation- and laser-induced DSBs demonstrate that the actual recruitment of repair/signaling factors is independent of H2AX [37]. Given that H2AX is also phosphorylated in response to replication stress [14], it would be interesting to know whether the assembly of damage-induced foci during replication is dependent on H2AX.

What is then the relationship between initial recruitment and subsequent accumulation of factors that that produce foci? To integrate these disparate phenomenon, we proposed a two-stage recruitment model in which γ-H2AX modulates the accumulation of repair/signaling proteins in the chromatin regions distal to a DSB, following their initial (H2AX-independent) migration to DSBs (Fig. 1) [37]. The retention and subsequent increase in the local concentration of factors may be mediated via weak interactions between the SQ motif in the H2AX tail (thousands of which are modified by phosphorylation), and specific domains of repair/signaling proteins. Such a model might be rigorously tested by analyzing the retention of GFP-tagged proteins in living cells subjected to local DNA damage. Additionally, H2AX phosphorylation may have a direct effect on chromatin structure in the region surrounding a DSB (Fig. 1) [31,44]. In this way, IRIF formation could also arise by the H2AX-dependent concentration of chromatin, rather than via direct protein–protein interactions at the lesion [45].

Unlike some of the other factors that are phosphorylated at sites of DSBs (e.g. Chk2, Nbs1), the activating intermolecular auto-phosphorylation of ATM can be induced at a distance by changes in chromatin stucture [46]. Whether ATM directly senses these DSB-induced chromatin alterations or requires DNA “sensors” to transmit the damage signal is still not clear. The MRN complex is required for optimal ATM activation in response to DSBs [47]; however, it has been noted that cells expressing a form of NBS1 lacking the phospho-Ser/Thr-binding Forkhead associated domain show impaired MRN recruitement to DSBs [43]. This may indicate the existence of a positive feedback loop between MRN and PIKK that is required for both favorable PIKK activation and optimal assembly of MRN on DSBs. In summary, we still have a great deal to learn about how the ATM-dependent checkpoint is activated in response to DSBs. Further analysis of chromatin structure and epigenetic modifications at DNA damage sites will be necessary in order to understand how chromatin perturbations trigger the DNA damage response.

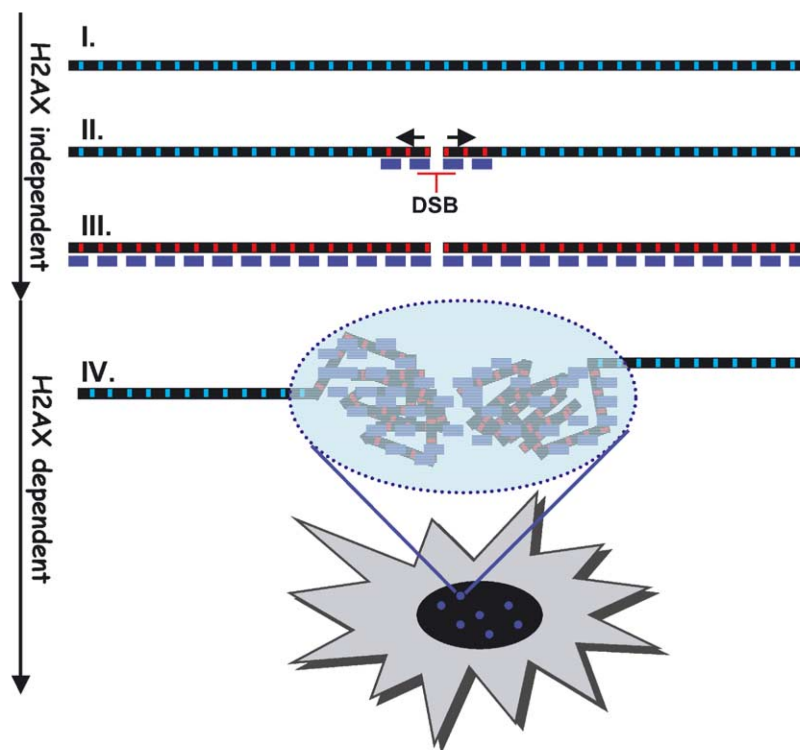

Fig. 1. Model for the role of H2AX phosphorylation in chromatin-and factor- condensation near DSBs. Generation of a DSB results in the early recruitment of repair/signaling factors (II, blue). A wave of H2AX phosphorylation spreads megabases from each side of the break (red, III). The restructuring of chromatin mediated by γ-H2AX serves to concentrate and retain factors in the vicinity of the lesion, and helps keep ends tethered together (IV).

5. The H2AX syndrome

Hereditary diseases affecting the cellular response to DSBs include ataxia telangiectasia (defective ATM), Nijmegen breakage syndrome (defective Nbs1) and Bloom's syndrome (defective BLM). The hallmarks of these disorders are growth defects, immunodeficiency, hypogonadism, hypersensitivity to specific DNA damaging agents, chromosomal fragility, and cancer predisposition. Mouse models in which specific components of the DSB repair/signaling pathway are disrupted recapitulate most of the pleotropic features found in chromosomal instability syndromes [48]. H2AX-deficient mice resemble chromosomal instability disorders in that they show radiosensitivity, male specific infertility, small size, and reduced levels of secondary immunoglobulin isotypes [39]. More recently, it has been shown that H2AX deficiency also modifies tumor susceptibility in mice [49,50]. The availability of H2AX-deficient cells and mice has allowed us to gain greater insight into the cellular function of what was previously known only as a cytological marker of DSBs.

6. Role of H2AX in DNA repair

The first experiment that demonstrated a role for H2AX in DNA repair was a genetic study performed in *Saccharomyces cerevisiae* [51]. Elimination of the unique C-terminal H2A serine residue in *yeast* led to an impairment in NHEJ [51]. More recently, it was shown that in *S. cerevisiae* H2A Ser 129 is critical for the efficient repair of DSBs during DNA replication [52]. The analysis of H2AX-deficient ES cells and mice showed that H2AX is not essential for NHEJ or HR in mammalian cells, but does appear to modulate both pathways [20,39,40,44]. Though γ-H2AX facilitates faithful repair, the biochemical mechanism remains unclear. One possibility is that phosphorylation of H2AX increases the likelihood of assembling a functional repair complex by increasing the local concentration of repair factors near the lesion. Another, non- exclusive possibility is that chromatin reorganization facilitated by γ-H2AX could prevent the premature separation of broken ends, a function that would safeguard against potentially tumorigenic chromosome rearrangements (Figs. 1 and 2).

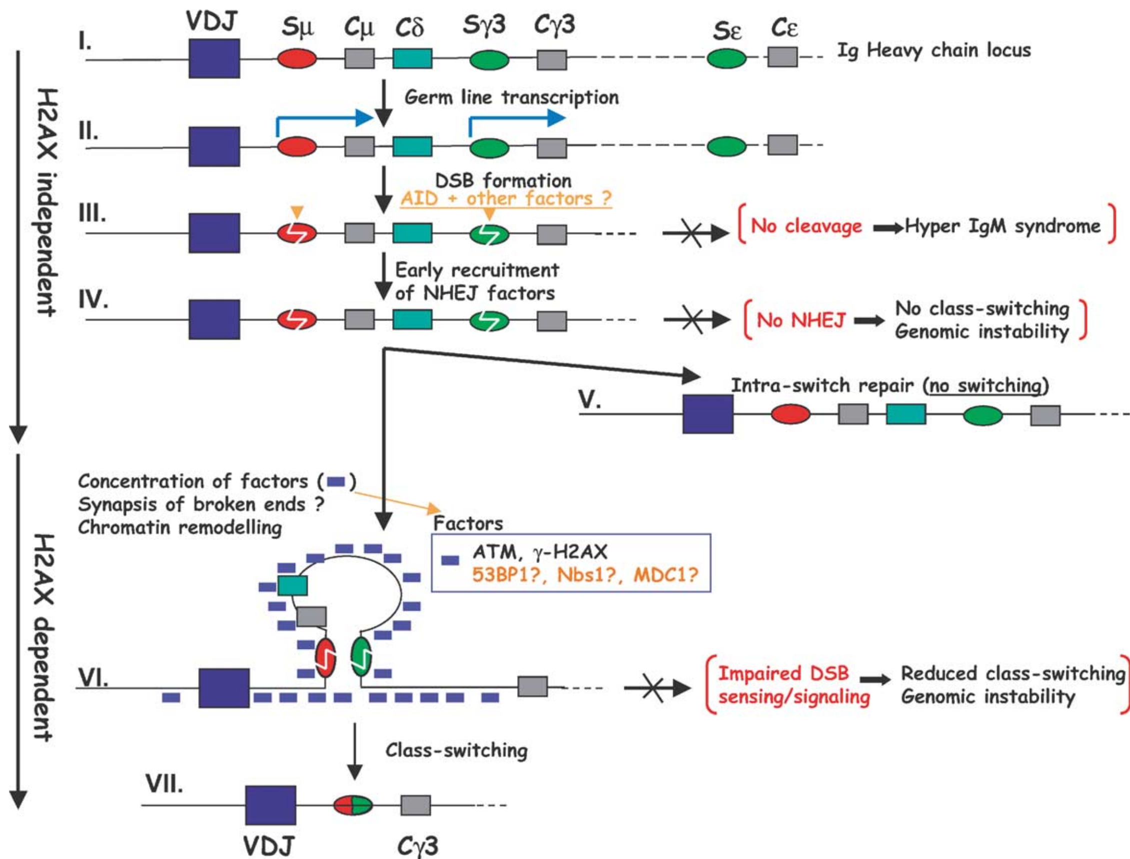

Fig. 2. Schematic representation of class switch recombination (CSR), and potential role of H2AX. CSR requires germ-line transcription of participating switch (S) regions, which is induced by a combination of activators and cytokines (II). These stimuli also induce expression of AID (III). AID and potential co-factors catalyze the CSR reaction by introduction of DSBs, either via RNA editing or by direct deamination of DNA (III). Induction of DSBs results in the recruitment of NHEJ factors (IV), stimulates the phoshorylation of H2AX (VI) and the assembly of Nbs1 into γ-H2AX foci. The breaks can be resolved by the direct ligation of the ends (intra-switch recombination, V), which is dependent on NHEJ, or by intrachromosomal deletion that results in an isotype switch (VI, VII). This example shows a switch from IgM to IgG3. γ-H2AX, ATM, 53BP1, Nbs1 and MDC1 are hypothesized to facilitate switch regions synapsis via restructuring of chromatin (VI), as described in Fig. 1.

7. Role of H2AX in genomic stability and cell cycle checkpoints

Genomic instability is a general term used to describe a genetic propensity for an increase in chromosomal pathology secondary to inaccurate repair or deficiency in cell cycle checkpoints. Typically, the instability can be visualized as chromosomal breaks, translocations, or aneuploidy. H2AX-deficient mouse embryo fibroblasts and T cells contain chromosomal breaks and translocations. However, $H2AX^{-/-}$ B-cells do not show such aberrations, presumably because the apoptotic machinery eliminates B cells that carry chromosomal pathology [26,39]. Consistent with this idea, loss of checkpoint regulation by p53 deletion produces chromosomal aberrations that can be seen in all $H2AX^{-/-}$ $p53^{-/-}$ cell types [49].

Although rapidly phosphorylated in response to IR, H2AX is largely dispensable for checkpoint responses to high dose γ-irradiation [39,51,52]. In contrast, $H2AX^{-/-}$ cells exposed to low doses of irradiation that generate few DSBs fail to properly arrest at the G_2 phase of the cell cycle and progress to mitosis [28]. This defective G_2/M checkpoint response seems to be linked to the impaired accumulation of factors such as 53BP1 at DSBs, which may be an essential amplification step at threshold levels of DNA damage [28,53,54]. However, the precise contribution of the G_2/M checkpoint defect to the overall genomic instability manifested in H2AX deficient cells is not yet known.

8. Role of H2AX in growth

Like other mouse models of genomic instability, H2AX deficient mice are small in size [39]. This growth defect is distributed proportionally throughout the entire organism. Similarly, $H2AX^{-/-}$ mouse embryonic fibroblasts (MEFs) exhibit impaired growth and senesce after only 3–4 passages in culture [39], a phenotype partially alleviated by p53 deficiency [49]. In human cells, senescence is associated with telomere erosion that follows every cell division [55]. Moreover, a number of proteins implicated in the DNA damage response have been shown to play an active role in telomere maintenance. Interestingly, H2AX phosphorylation has been detected in fusogenic telomeres in cells in which the capping protein TRF2 has been depleted, as well as in human fibroblasts undergoing senescence [16,17]. However, in contrast to the NHEJ components Ku80 or DNA ligase IV [56,57], H2AX is not essential for chromosomal fusions arising from either critically shortened or de-protected telomeres [29]; neither does H2AX deficiency significantly affect the telomere length [29]. Since H2AX is largely dispensable for somatic telomere maintenance, the $H2AX^{-/-}$ growth defect is likely to be due to the response to chromosomal aberrations that arise spontaneously in primary cells, rather than a consequence of modifications in telomere structure.

9. Role of H2AX in meiosis

Meiosis is a cellular differentiation program during which physiological DSBs are created and repaired, giving rise to recombination events between parental chromosomes. Meiotic recombination takes place in the prophase stage within the first of two meiotic divisions, and is triggered by DSBs generated by the Spo11 transesterase [58]. When the distribution of γ-H2AX is analyzed in mouse spermatocytes, it shows two distinct patterns of staining [19]. On the one hand, there is a Spo11-dependent γ-H2AX formation associated with meiotic DSBs on all chromosomes (occurring at the leptotene–zygotene transition). In the subsequent pachytene stage, only the XY chromosomes (but not the autosomes) are covered by γ-H2AX. This sex chromosome-specific staining is still apparent in $Spo11^{-/-}$ mice [19]. During meiotic prophase in male mammals the X- and Y-chromosomes condense to form a macrochromatin body termed the sex- or XY-body within which the X- and Y-linked genes are transcriptionally repressed [59]. Confirming that the γ-H2AX staining detectable over the sex chromosomes has a physiological role, the X- and Y-chromosomes of histone H2AX-deficient spermatocytes fail to condense to form a sex-body, do not initiate meiotic sex chromosome inactivation and exhibit severe defects in the pairing of X-Y chromosomes [31]. Since γ-H2AX staining over the sex body is independent of programmed Spo11-mediated DSBs, it is still a mystery as to what what triggers this massive phosphorylation. However, these observations suggest the possibility that γ-H2AX-dependent chromatin condensation may not be a meiosis exclusive phenomenon, but that phosphorylation of H2AX might also affect chromatin condensation and transcriptional inactivation during the repair of DNA double strand breaks in mitotic cells (Fig. 1).

Prior to the generation of the meiotic DSBs, the ends of all chromosomes (telomeres) cluster within a limited region of the nuclear envelop, forming a so-called “bouquet”. This clustering of telomeres is thought to insure proper homolog pairing and synapsis of the homolog axes [60–62]. Interestingly both ATM [63] and more recently H2AX [29] have been shown to regulate the persistence of the telomere bouquet. The exit from the bouquet stage may therefore be coupled to the completion of the repair of meiotic DSBs. In this scenario, the extended duration of the bouquet stage in H2AX deficient spermatocytes may reflect an altered recombination and/or signaling defect in these cells. Whether the meiotic functions of H2AX are mediated solely via the phosphorylation of its tail is presently unknown.

10. Role of H2AX tumor suppression

There is mounting evidence that genomic instability is a cause and not just a consequence of cancer development [8]. Although chromosomes in cells from H2AX deficient

mice contain frequent breaks and translocations, there is little or no increase in tumor development in H2AX$^{-/-}$ mice [49,50]. Apparently, H2AX$^{-/-}$ cells are protected from malignant transformation by the activity of DNA damage sensors, like p53. Such 'gatekeepers' [64] proteins provide a safeguard against genomic instability by arresting cell division or triggering apoptosis in cells harboring chromosome abnormalities. Consistent with this view, there is a dramatic increase in the onset of both lymphoid and solid tumors when the loss of one or both H2AX alleles occurs on a p53 deficient background [49,50]. These studies showed that H2AX was still expressed in tumors from H2AX$^{+/-}$p53$^{-/-}$ deficient mice, indicating that H2AX functions as a dosage-dependent or haplo-insufficient tumor suppressor. In fact, when H2AX levels are reduced to 50% of wild-type levels, the resultant decrease in γ-H2AX formation is insufficient to maintain genomic stability, and leads to increased levels of chromosomal aberrations, reduced growth rates and radiation sensitivity [49,50]. Although γ-H2AX foci are still present in H2AX$^{+/-}$ cells, it remains unclear whether subtle differences in the size and/or quantity of foci exist when half as many nucleosomes contain H2AX.

11. Role of H2AX in immune receptor rearrangements

During lymphocyte development, T and B cells undergo the process of somatic gene rearrangement known as Variable Diversity Joining (V(D)J) recombination to produce the primary antigen receptor repertoire. Antigen receptor diversification in lymphocytes is initiated by the RAG-1/2 endonuclease, which introduces DSBs adjacent to the antigen receptor segments (V, D, and J segments) [65]. The subsequent juxtaposition and ligation of V(D)J ends requires ubiquitously expressed proteins (Ku80, Ku70, DNA-PKcs, XRCC4, Ligase 4, and Artemis) that function in NHEJ. A role for H2AX in V(D)J recombination was suggested by the findings that RAG-dependent γ-H2AX foci accumulate at sites of V(D)J recombination in developing thymocytes [21], and that a subset of T and B cell lymphomas in H2AX$^{-/-}$p53$^{-/-}$ deficient mice harbor oncogenic translocations that arise as byproducts of aberrant V(D)J recombination [49,50]. However, V(D)J recombination appears to be largely intact in H2AX$^{-/-}$ mice with only a two-fold reduction in the absolute number of lymphocytes and no abnormalities in the formation of V(D)J recombination signal or coding joints [39,40]. This apparent disparity may be explained by the strong immunologic selection for productive V(D)J joints during lymphocyte development. Only those cells that make a productive rearrangement survive and are clonally expanded, whereas cells bearing non-productive rearrangements are deleted by apoptosis. Therefore, subtle defects in the capacity to repair DSBs during V(D)J recombination would be difficult to detect. Indeed, given the phenotype of H2AX$^{-/-}$p53$^{-/-}$ mice, it is likely that there is a reduced efficiency in the repair of V(D)J recombination-induced lesions, and that those H2AX$^{-/-}$ cells harboring genome-destabilizing lesions are eliminated in vivo by p53-dependent apoptosis.

B lymphocytes undergo a second genomic recombination reaction in response to antigenic stimulation called class switch recombination (CSR) (Fig. 2). This reaction replaces one immunoglobulin (Ig) constant gene for another, thereby changing the effector function of the Ig. CSR is initiated by activation-induced deaminase (AID) [66,67], a single stranded DNA deaminase that creates lesions that are resolved by NHEJ (Fig. 2III). For example, Ku80 or Ku70 deficient B cells are unable to complete the CSR reaction [68,69] and in the absence of DNA-PKcs, CSR is very inefficient [70,71] (Fig. 2IV). CSR is also defective in ATM$^{-/-}$ and H2AX$^{-/-}$ mice [20,39,44,72] and B cells undergoing CSR accumulate AID-dependent γ-H2AX foci at the Ig locus [20].

How H2AX facilitates CSR is not precisely known but γ-H2AX appears to play a role in promoting switch region synapsis [44] (Fig. 2VI). During the CSR reaction, multiple DSBs are created in Ig switch region DNA (Fig. 2III) and these can be resolved by local re-ligation (Fig. 2V) or by recombination between switch regions (Fig. 2VII). Although recombination between switch regions is abnormal in the absence of H2AX, short-range intra-switch region recombination proceeds normally [44] (Fig. 2V). Consistent with the idea that accurate long distance chromosome joining is affected by H2AX deficiency, there is an increased frequency of breaks and translocations near the IgH locus in H2AX$^{-/-}$p53$^{-/-}$ B cells undergoing CSR [49], and an increased susceptibility to mature B cell lymphomas with breakpoints in the vicinity of switch regions [50].

12. Model for H2AX function

We speculate that the pleotropic phenotypes observed in the absence of H2AX- chromosomal instability and radiation sensitivity in mitotic cells, abnormalities in XY pairing and transcriptional inactivation in spermatocytes, defects in G_2/M checkpoint control, and reduced levels of class-switching are all due to two complementary structural functions provided by the phosphorylation marks on H2AX: a role in (1) the focal assembly and retention of factors in chromatin regions near the damaged site (2) the reorganization of chromatin structure. In this model, the induction of a DSB activates ATM, and recruits ATM together with NHEJ repair proteins and damage sensors such as MRN, 53BP1, and MDC1 to the lesion. ATM and DNA-PKcs would phosphorylate numerous substrates at the DSB including H2AX, MRN, 53B1, and MDC1. Reinforcement of PI-3K kinase activity and subsequent phosphorylation events would require MRN, and to a lesser extent H2AX, 53BP1 and MDC1. The model posits that H2AX phosphorylation spreads over a distance of several megabases from the damaged site, and that

spreading would both condense chromatin and concentrate repair factors in the vicinity break (Figs. 1 and 2). The focal assembly of the H2AX/MRN/ATM/53BP1/MDC1 complex and associated changes in chromatin folding would then facilitate the synapsis of broken ends.

One prediction of the model is that the severity of the phenotype in cells lacking H2AX would depend both on the extent of DNA damage [28], and on the proximity of the broken ends. Thus, different repair reactions might have distinct requirements for components of the H2AX/MRN/ATM/53BP1/MDC1 complex in the tethering of DNA ends. For example, absence of H2AX leads to a mild defect in V(D)J recombination, but this could be a consequence of a relatively stable post-cleavage synaptic complex reinforced by RAG-1/2 proteins [73,74]. Similarly, the intra-switch recombination reaction might be facilitated by H2AX independent recruitment of NHEJ factors and the MRN/ATM/53BP1/MDC complex (Fig. 2V). On the other hand, the synaptic reinforcement mediated by H2AX might be more critical for the repair of irradiation-induced breaks, or for long-range chromosome interactions between switch regions or between X and Y chromosomes during meiosis. As mentioned above, even a partial loss of H2AX abrogates some of these functions, leading to chromosomal instability and increased susceptibility to cancer.

In conclusion, our model proposes that γ-H2AX has two functions in DNA repair: a role in promoting changes in the structural configuration of chromatin and in chromatin tethering of repair factors, these functions may or may not be related, but both likely to be required for efficient synapsis of broken chromosome ends.

13. Implications for human disease

The histone H2AX gene, located 11 Mb telomeric to ATM at 11q23.3, is in a region commonly deleted or translocated in several human hematological malignancies and solid tumors [75]. Heterozygous deletion of chromosome bands 11q22-q23 is detected at a particularly high frequency in B cell chronic lymphocytic leukemia (B-CLL), mantle cell lymphoma (MCL), and T cell prolymphocytic leukemia (T-PLL), and is associated with rapid disease progression and poor survival in B-CLL [75,76]. Since somatic disruption of ATM only accounts for a distinct subset of tumors involving alterations in 11q [75], other tumor suppressor genes in this chromosomal region are likely to play a pathogenic role. In one report, constitutional H2AX mutations were not found in a screen of hereditary breast cancer families [77], and indeed no evidence for increased risk of breast malignancies was detected in the knockout mice. Further studies are therefore needed to directly address the contribution of H2AX haploinsufficiency in human malignancies associated with 11q deletions.

Given that H2AX deficient mice present all of the features of genomic instability syndromes and that the H2AX gene is located in close proximity to the ATM locus, it remains possible that the molecular basis for some AT-like patients that have been classified on the basis of physical mapping may reside in mutations in H2AX. Based on the results from the mouse model, the histone guardian of the genome, H2AX, may prove to be an important player in cancer-causing genomic instability in humans. One important avenue for basic research will be to determine precisely how chromatin structure is modified by the phosphorylation marks on H2AX and how this modification is linked to DNA damage repair and signaling.

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