Review

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Environmental responses mediated by histone variants

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Fluctuations in the ambient environment can trigger chromatin disruptions, involving replacement of nucleosomes or exchange of their histone subunits. Unlike canonical histones, which are available only during S-phase, replication-independent histone variants are present throughout the cell cycle and are adapted for chromatin repair. The H2A.Z variant mediates responses to environmental perturbations including fluctuations in temperature and seasonal variation. Phosphorylation of histone H2A.X rapidly marks double-strand DNA breaks for chromatin repair, which is mediated by both H2A and H3 histone variants. Other histones are used as weapons in conflicts between parasites and their hosts, which suggests broad involvement of histone variants in environmental responses beyond chromatin repair.

Histone variants are available to respond

Eukaryotic organisms must respond to environmental changes with changes in gene expression to survive. Although we often think of environmental responses in terms of whole-organism responses, including growth, movement, learning, homeostasis, and immunity, ultimately all of these involve changes in gene expression in the relevant nuclei of the organism, and hence involve changes to the epigenomic landscape that provide access to genes that are packaged in nucleosomes. One mode of altering chromatin is through the deployment of histone 'variants', non-allelic paralogs of the four 'canonical' core histones (H2A, H2B, H3, and H4) that package the genome into nucleosomes at replication. Histone variants substitute for their canonical counterparts, thereby changing the properties of nucleosomes. In recent years, histone variants have been shown to be involved in several modes of environmental responses.

Histone variants are distinguished from canonical histones not only by their amino acid sequences and physical properties but also by their incorporation into chromatin outside of replication. This ability to use different deposition modes makes them adaptable to respond to environmental stimuli, which typically are not synchronous with replication. Indeed the term 'variants' is something of a misnomer because in many single-celled eukaryotes the variants may be the sole or primary histones, and are deployed throughout the cell cycle as these organisms respond to their environments [1].

H2A.Z and responsiveness

Mediation of responsiveness to the environment is thought to be a major role of the histone variant H2A.Z, a universal variant with a single origin pre-dating the divergence of modern eukaryotes [2]. H2A.Z has roles in a variety of seemingly contradictory processes including gene activation, heterochromatic silencing, transcriptional memory, and others. It is found surrounding the nucleosome-deficient regions at gene transcription start-sites (TSSs), especially at the first (+1) nucleosome of genes, but also in gene bodies ([3,4] for reviews).

Although H2A.Z is essential in many organisms, in the budding yeast *Saccharomyces cerevisiae*, H2A.Z (also known as Htz1 in *Saccharomyces*) is non-essential, but an early study showed that *htz1Δ* mutants are sensitive to heat and defective in the ability to grow on galactose, indicating a failure to induce the *GAL* genes [5]. The mutants failed to recruit efficiently RNA polymerase II (PolII) and TATA-binding protein to the *GAL 1-10* promoter, and had a global increase in DNA accessibility to micrococcal nuclease when grown on galactose. In another study *htz1Δ* mutants were found to be defective in growth in oleate medium [6], which induces widespread activation of genes involved in mitochondrial and peroxisomal lipid metabolism. Acetylated H2A.Z is necessary for the full induction of otherwise repressed oleate-responsive genes, and for the efficient recruitment of TATA-binding protein to oleate-responsive gene promoters. H2A.Z nucleosomes are disassembled upon induction, and this is thought to provide access for the transcriptional machinery because in yeast the +1 nucleosome overlaps the TSS. In a meta-analysis, a significant excess of genes with high levels of H2A.Z in the coding region were also upregulated by environmental stress in *Saccharomyces*, and in the fission yeast *Schizosaccharomyces pombe* a significant excess of genes enriched for H2A.Z in the coding region were involved in meiosis and genotoxic stress [7].

The model plant *Arabidopsis* has three genes encoding H2A.Z. Plants with mutations in two of these genes have gene expression profiles in which 65% of the genes that are differentially regulated from wild type overlap with those from plants mutant in the *PIE1* (photoperiod-independent

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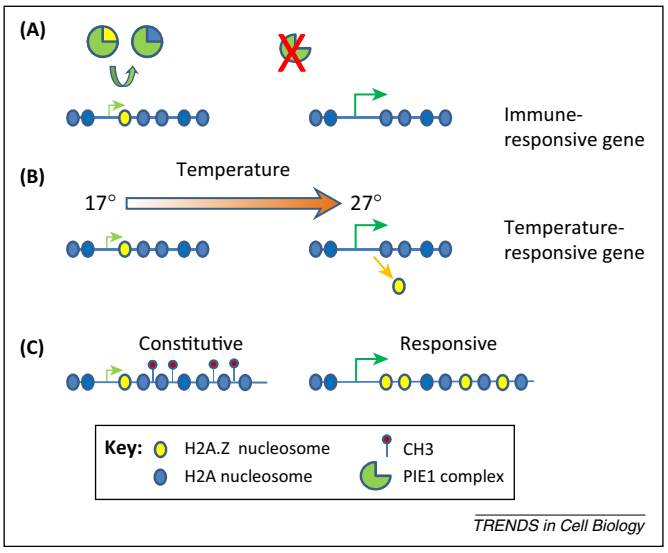

Figure 1. H2A.Z in *Arabidopsis*. **(A)** H2A.Z/H2B dimers are exchanged for H2A/H2B dimers by the PIE1 complex. Mutations (red X) in subunits (PIE1 or ARP6) of this complex result in misregulation of immunity response genes. **(B)** Increasing temperature results in reduced occupancy of H2A.Z at the +1 nucleosome and increased expression of temperature-regulated genes. **(C)** H2A.Z is present in the gene bodies of responsive genes, but is excluded by DNA methylation from constitutive genes.

early flowering 1) gene (Figure 1), which encodes the homolog of the Swr1 ATPase subunit of the SWR1 (Swi/Snf2-related) complex of yeast that replaces canonical H2A/H2B dimers with H2A.Z/H2B dimers [8]. The majority of these differentially regulated genes are related to salicylic acid-dependent immunity, in which increased salicylic acid levels trigger 'systematic acquired resistance' involving changes in expression of more than 1000 genes. In another study, a genetic screen found that mutations in the *ARP6* (actin-related protein 6) gene, which encodes a different subunit of the PIE1 complex, phenocopy double H2A.Z mutants and control the ambient temperature response in *Arabidopsis* [9]. With increasing temperature, H2A.Z nucleosomes are depleted at the +1 nucleosome of genes that are upregulated by temperature, suggesting that they limit expression at lower temperatures. This depletion is also seen in *arp6* mutants, leading to constitutive expression of temperature-inducible genes, suggesting that H2A.Z may serve as a thermo-sensor in plants. A third study found that H2A.Z enrichment in gene bodies is correlated with lower expression and with higher gene responsiveness [10,11]. Misregulated genes in triple mutants lacking nearly all H2A.Z were enriched in gene ontology terms related to immune response, temperature response, and in other categories related to the perception of external cues. In the H2A.Z triple mutant DNA methylation was little altered even in misregulated genes, suggesting that the previously observed anti-correlation between H2A.Z and DNA methylation [10] was primarily due to the exclusion of H2A.Z by DNA methylation. The authors proposed that H2A.Z facilitates regulation of responsive genes, whereas gene body methylation evolved to stabilize constitutive expression of housekeeping genes by excluding H2A.Z [11].

How might H2A.Z facilitate responsiveness? H2A.Z helps to recruit PolII in yeast [5] and facilitates assembly of both active and repressive chromatin complexes at promoters and enhancers in mouse embryonic stem cells [12]. Acetylation of H2A.Z is necessary for gene induction [13], and induces a conformational change in H2A.Z nucleosomes *in vitro* [14], suggesting that acetylation might act as an activation switch. H2A.Z also increases the activity of ISWI (imitation switch) family chromatin remodelers [15], further suggesting that H2A.Z promotes changes in chromatin accessibility. In addition, H2A.Z increases the rate of elongation through a yeast fusion gene [16]. Mapping of elongating and arrested PolII transcripts *in vivo* at nucleotide resolution in *Drosophila* S2 cells revealed that entry into the +1 nucleosome presents a significant barrier to transcription, whereas gene body nucleosomes present lower barriers. H2A.Z nucleosomes reduce the barrier to transcription, and anti-correlate with nucleosome occupancy, PolII stalling, and H3/H4 turnover, suggesting that H2A.Z/H2B dimers are more easily lost than H2A/H2B dimers, facilitating PolII transit while preserving H3/H4 tetramers [17]. Together these observations suggest that H2A.Z facilitates binding of both activating and repressive complexes by keeping key genome regions accessible [18], making it ideally suited to regulate responsive genes.

Histone variants in DNA damage and repair

H2A variants

Cells must constantly detect and repair damage to DNA from both endogenous and environmental sources, a process involving alterations to chromatin to provide access for repair enzymes and subsequent restoration of the chromatin state. Chromatin changes during the DNA damage response and double-strand break (DSB) repair have been reviewed extensively [19,20], and we will therefore focus on more recent results pertaining to histone variants.

One of the most severe environmental challenges to cells is repair of DSBs, which can be caused by ionizing radiation (IR), environmental chemicals, or free radicals generated by cellular processes (Figure 2A–D). The phosphorylation of the histone variant H2A.X in response to DSBs is an early step in a process that includes checkpoint activation and cell cycle arrest, recruitment of repair proteins, and repair through non-homologous end-joining (NHEJ) or homologous recombination (HR). H2A.X differs from canonical H2A by the addition of the C-terminal motif Ser-Gln-(Glu/Asp)-Φ (SGD/EΦ), where Φ represents a hydrophobic residue. It typically comprises about 10% of the total H2A in chromatin in mammals, and is the primary form of H2A in *Saccharomyces*. Within minutes after a DSB, the serine in the SGD/EΦ motif is targeted by a kinase of the phosphoinositide 3-kinase-like kinase (PIKK) family, producing a phosphorylated form known as γH2A.X [19]. In mammals γH2A.X is bound by the MDC1 protein (mediator of DNA damage checkpoint protein 1), which recruits the MRN (Mre11-Rad50-Nbs1) complex that binds to the DSB and promotes resectioning, and recruits and activates the PIKK family kinase ATM (ataxia telangiectasia mutated). ATM phosphorylates a broad set of proteins including checkpoint proteins, repair enzymes [21], and additional H2A.X nucleosomes, producing a domain of

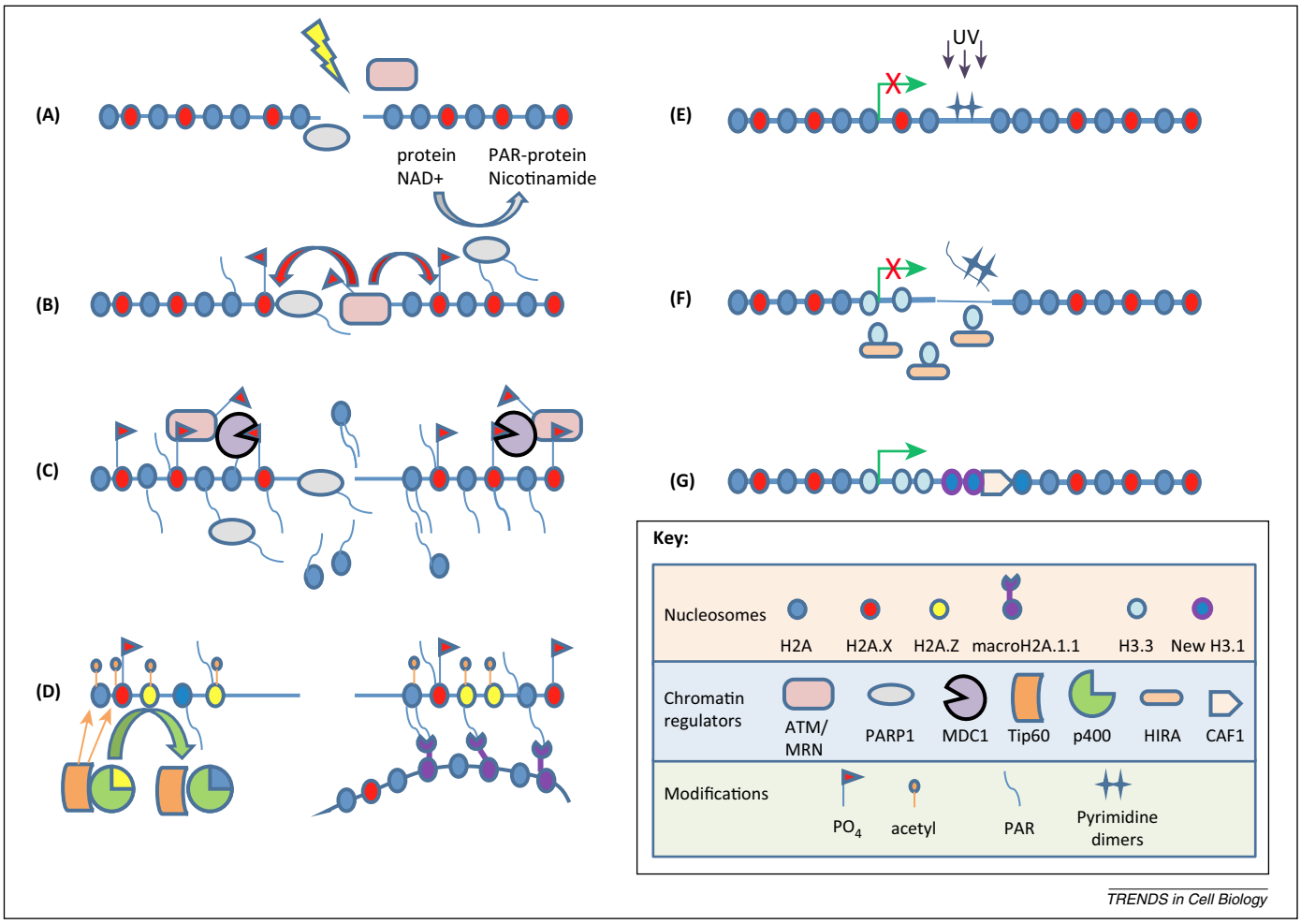

Figure 2. Histone variant transitions in DNA repair. (A–D) H2A variants in double-strand break repair. **(A)** Double-strand breaks are detected by PARP1 and ATM, which bind to DNA ends. **(B)** ATM autophosphorylates and targets H2A.X to form γH2A.X. Similarly, PARP1 undergoes auto-PARylation and targets histones and other proteins. **(C)** PARylated histones loosen the chromatin. MCD1 binds to both γH2A.X and ATM/MRN, establishing a large γH2A.X domain. **(D)** MacroH2A.1.1 binds to PAR through chromosome rearrangement. p400 exchanges H2A.Z/H2B dimers into chromatin by replacing H2A/H2B or H2A.X/H2B dimers. Through acetylation by Tip60, H2A.Z further loosens the chromatin for repair. (E-G) H3 variants in nucleotide excision repair. **(E)** Upon formation of a pyrimidine dimer, transcription is arrested. **(F)** HIRA assembles H3.3 into the damage site; the pyrimidine dimers are excised. **(G)** New DNA synthesis is followed by CAF1-mediated assembly of H3.1 Transcription competency is restored.

γH2A.X over 50 kb (*Saccharomyces*) or several hundred kb (mammals) around the break, although γH2A.X is depleted in the ~1 kb proximal to the break [22,23]. This γH2A.X domain is generally believed to concentrate cohesins as well as repair and checkpoint factors near the break to serve as a 'toolkit' for HR [19], although a proposed role in recruiting chromatin regulators has been questioned [24]. H2A.X mutant mice are viable and are able to activate checkpoints, but are growth-retarded, radiation-sensitive, and have defects in repair by HR. Mutant embryonic fibroblasts have a reduced mitotic index, poor proliferation, and increased chromosomal abnormalities [25]. In combination with a p53 deficiency, haploinsufficiency for H2A.X leads to increased tumor susceptibility [26]. H2A.X is missing entirely in some organisms such as *Caenorhabditis* [2], which favors NHEJ over HR.

DSBs are also sites of incorporation of H2A.Z, which is exchanged into chromatin by the p400 motor ATPase, a mammalian counterpart of the yeast SWR1 complex [27] and the PIE1 complex of *Arabidopsis*. p400 is part of the Tip60 (Tat-interacting protein of 60 kDa) chromatin-remodeling complex, which acetylates H2A.Z and H4, creating a more open chromatin structure. H2A.Z exchange is needed for ubiquitylation of the chromatin by the RNF8 (RING finger protein 8) ubiquitin ligase, and for recruiting downstream components of both the HR and NHEJ repair pathways [28]. Vertebrates have two paralogous H2A.Z genes, encoding H2A.Z.1 and H2A.Z.2, and in primates H2A.Z.2 additionally has two splice variants [29,30]. In chicken DT-40 cells, H2A.Z.2 but not H2A.Z.1 was mobilized and rapidly incorporated into damaged chromatin at DSBs, and H2A.Z.2-deficient cells were more sensitive to radiation than wild type or H2A.Z.1-deficient cells [31].

In *Saccharomyces*, if a break cannot be repaired, it can be relocated to the nuclear envelope in a process that depends on H2A.Z sumoylated on its C-terminal tail [32]. Cells will eventually overcome the checkpoint and return to the cell cycle (checkpoint adaptation), but this depends on the removal of H2A.Z by the chromatin remodeler INO80 (inositol-requiring 80) [33], which replaces H2A.Z/H2B dimers with canonical dimers [34]. In an *ino80* mutant, levels of γH2A.X are reduced and levels of H2A.Z are increased around DSBs. Deletion of the *SWR1* or H2A.Z (*HTZ1*) genes alleviates the block to adaptation

[33]. Furthermore, the sensitivity of *ino80* mutants to DNA damage agents and replication blocks is also alleviated by H2A.Z in which the lysines in the N-terminal tail have been mutated to glutamines to mimic acetylation, suggesting that INO80 removes unacetylated H2A.Z [34]. Whether through this activity or some other process, INO80 increases the mobility of chromatin, both at the DSB and elsewhere, and this is thought to facilitate the search for a donor sequence for HR [35].

Other H2A variants also are recruited to DSBs. The variant macroH2A is characterized by a large 'macro domain' C-terminal to the histone-fold domain. As with H2A.Z variants, vertebrates have two paralogous genes encoding macroH2A.1 and macroH2A.2, and macroH2A.1 additionally has two splice variants, macroH2A.1.1 and macroH2A.1.2 [1]. A very early event in DSB repair is the activation of poly-ADP-ribose polymerase 1 (PARP1) by binding to DNA ends. PARP1 is an enzyme that adds ADP-ribose moieties (ADPR) from NAD^+ to form poly-ADP-ribose (PAR) on a variety of proteins including itself and histones [36]. Chromatin around DSBs undergoes rapid PARylation, which is associated with relaxation of chromatin [37]. The macrodomain of macroH2A.1.1, but not those of macroH2A.1.2 and macroH2A.2, can bind to ADPR, and binds to PAR on PARP1 and other proteins [38]. Chromatin around DSBs accumulates tagged macroH2A.1.1 co-extensive with γH2A.X phosphorylation. However, this macroH2A.1.1 is not exchanged into chromatin, and is recruited to DSBs apparently only by interaction of the macrodomain with PAR on chromatin proteins [39]. When tagged macroH2A.1.1 was expressed in HeLa cells with undetectable endogenous macroH2A.1.1, microirradiated sites were PARylated, H2A.X was phosphorylated (γH2A.X), and macroH2A.1.1 was recruited within 5 min. Because most macroH2A.1.1 is present in nucleosomes and is tightly associated with chromatin, recruitment to DSBs by binding to PAR likely represents rearrangement of macroH2A.1.1-containing chromatin to the DSB. This interpretation was supported by a transient increase in DNA density at DSBs. PARylation levels at the DSB decrease over time and macroH2A.1.1 binding is lost while γH2A.X still persists. The function of macroH2A.1.1-mediated chromatin rearrangement is unclear, but it is associated with increased γH2A.X and reduced recruitment of Ku70 (named from 'Ku antigen'), a protein required for repair by NHEJ [38]. Loss of macroH2A.1 results in increased sensitivity to irradiation and an increase in NHEJ [39], suggesting a possible role in promoting HR, perhaps by promoting chromatin rearrangement that facilitates homology searching.

In mouse embryonic fibroblasts, expression of GFP-tagged H2A.B (H2A.Bbd), which wraps less DNA than canonical H2A, transiently incorporates into sites of DNA synthesis during S-phase and during DNA repair [40]. The functional relevance to wild type cells of this ectopic expression is uncertain, and may merely reflect opportunistic but unstable assembly. However, the resulting shorter cell cycle and increased sensitivity to irradiation resemble Hodgkins' lymphoma cells, in which H2A.B is overexpressed. Increased sensitivity to irradiation suggests that H2A.B may interfere with DNA repair, whereas the shorter cell cycle may give lymphoma cells a replicative advantage over a normal host.

H3 variants

The role of H3 variants in DNA damage has been investigated primarily in human cells subjected to ultraviolet (UVC) irradiation, which triggers nucleotide excision repair (Figure 2E–G). Transcription is arrested to prevent production of aberrant transcripts and interference of PolII with repair enzymes. Following UVC irradiation, transcription is inhibited for at least 5 h, during which time histone chaperone HIRA (histone regulator A) [41], which deposits the constitutive H3 variant H3.3 during transcription [42], is quickly and transiently recruited to the damage site and incorporates new H3.3 into the chromatin. This recruitment depends not on transcription but on ubiquitylation by the damage detection complex, which ubiquitylates H3 and H4 and is thought to destabilize nucleosomes [43], facilitating assembly of H3.3 into damaged chromatin. HIRA is necessary for transcriptional recovery, but it is gone from the damage site long before transcription resumes at 20–24 h, suggesting that the newly assembled H3.3 may act to 'license' the chromatin for eventual transcription after repair is completed [41].

Following HIRA recruitment, the chaperone CAF-1 (chromatin assembly factor I), which is best known for assembling the canonical histone H3.1 (and H3.2) into chromatin during S-phase replication, is also recruited to damage sites, dependent on its p150 subunit [44], which is known to interact with the DNA polymerase clamp PCNA (proliferating cell nuclear antigen) [45]. CAF-1 then assembles H3.1 at the damage site independently of S-phase. CAF-1-mediated assembly is dispensable for checkpoint activation and DNA synthesis, suggesting that H3.1 packages DNA after repair [44]. Deposition of H3.1 is also affected by HIRA depletion, indicating some dependence of CAF-1-mediated assembly on prior HIRA activity [41].

Centromeric histone variants

Transient recruitment of the centromeric H3 variant cenH3 (CENP-A in vertebrates) to several kinds of DNA damage has also been reported [46], but reports are inconsistent, and cenH3 appears to be actively excluded from DNA damage sites in *Drosophila* by the histone-fold protein CHRAC14 (chromatin accessibility complex 14) (Box 1). Although the role of cenH3 in DNA repair is uncertain, two other histone-fold proteins found at centromeres have a well-established role in the repair of interstrand crosslinks (ICLs) as part of the Fanconi anemia (FA) protein complex. MHF1 (FANCM interacting histone-fold protein 1, also known as CENP-S) and MHF2 (CENP-X), which are essential for ICL repair, dimerize to form a DNA-binding MHF complex, which further complexes with the FA protein M (FANCM) and binds to ICLs [47,48]. MHF1, MHF2, and FANCM are conserved in most eukaryotes, whereas many other FANC proteins are restricted to vertebrates [48]. In both vertebrates and ascomycetes, MHF1 and MHF2 are also found at centromeres as CENP-S and CENP-X, respectively. The roles of MHF1/CENP-S and MHF2/CENP-X in centromeres and DNA repair are distinct (Box 2), involving complexes with different protein partners and with different turnover times in chromatin [49–51].

Box 1. Does cenH3 have a role in DNA repair?

Vertebrate cenH3 (CENP-A) has been reported to be recruited transiently to several kinds of DNA damage [46]. Expression of GFP-linked CENP-A resulted in rapid localization to DSBs in human and mouse cells. By contrast, in a study using SNAP-tag technology, new CENP-A was not observed at DNA damage sites [41], and in another study endogenous CENP-A was not detected at laser damage sites, whereas GFP–CENP-A was only weakly recruited in a limited number of cells [81]. A third study did not detect CENP-A by immunofluorescence at IR damage sites, but an interaction of CENP-A with ATM, dependent on IR and remodeling and spacing factor 1 (RSF1), could be detected after crosslinking. RSF1 has an IR-dependent association with ATM and is required for DSB repair [81,82]. It also affects the establishment of CENP-A at centromeres [83] and interacts with CENP-A independent of IR [82], suggesting that some CENP-A might be recruited to DSBs with RSF1. Alternatively, recruitment might depend on HJURP, a CENP-A chaperone originally reported to be involved in DSB repair through interaction with the MRN complex [84]. The relevance of CENP-A recruitment to DNA damage sites is unclear. Is it mislocalization, or a new function for cenH3 in repair? Overexpression of CENP-A produces heterotypic H3.3/CENP-A particles that mislocalize into sites of high nucleosome turnover on chromosome arms by DAXX-dependent deposition. Interestingly, this improves growth after DNA damage, although it is unclear why [85].

In *Drosophila* cenH3 mislocalization results in multiple kinetochores and mitotic errors [86]. CHRAC14 and its orthologs are histone-fold proteins that dimerize with other histone-fold proteins to form subunits of the nucleosome remodeling complex CHRAC (chromatin accessibility complex) [87] and of DNA polymerase ε [88]. *Chrac14* mutant embryos are defective in DNA repair and form extra cenH3 spots, whereas CHRAC14 depletion in S2 cells produces extra cenH3 spots that assemble kinetochores, forming dicentric chromosomes and chromosome breaks. *Drosophila* cenH3 was recruited to DNA damage sites in CHRAC14-depleted cells, but not in wild type cells, indicating that CHRAC14 might prevent cenH3 mislocalization at damage sites. The two proteins interact in glutathione *S*-transferase (GST) pulldown assays and in protein extracts from irradiated (but not unirradiated) embryos, suggesting that they form a heterodimer that prevents cenH3 incorporation at DNA damage sites [89]. CHRAC17, the human ortholog of CHRAC14, promotes DSB repair, but whether it interacts with CENP-A is not known [90].

Other variants

H1 linker histones bind to linker DNA between nucleosomes and are believed to contribute to higher-order chromatin structure and gene regulation [52]. Of mutations in the six H1 variants in chicken cells, only H1.R mutants have increased sensitivity to DNA damage by the alkylating agent methyl methanesulfonate [53]. H1.R mutants exhibit impaired sister chromatid exchange and reduced gene targeting, suggesting a role in the HR pathway, possibly through regulating global chromatin structure.

Some variants may have evolved specifically to protect DNA from damage. Bdelloid rotifers, which are highly resistant to IR, lack H2A, H2A.X, and H2A.Z, but have H2A variants with extended C-terminal tails that have been hypothesized to improve survival through dessication and its attendant DNA damage [54]. In sperm, the paternal genome of many animals undergoes an elaborate transition in packaging from histones to protamines as the surrounding cytoplasm is reduced for streamlined motility [55]. One role of protamines, which evolved from H1 [56], may be to protect the paternal genome from damage. In support of this hypothesis, *Drosophila* sperm deficient in protamines are viable but show increased sensitivity to IR [57]. Sea urchin sperm lack protamines but utilize sperm-specific H1 and H2B variants with SPKK motifs in their tails that tightly bind to the minor groove of linker DNA, increasing thermal stability [58].

Box 2. Histone-fold proteins in DNA repair and centromeres

Dimers of MHF1 and MHF2 form a DNA-binding MHF complex with FANCM [47,48]. This anchor complex then recruits a FANC protein core complex that results in mono-ubiquitylation of FANCD2 and FANCI, which in turn signals additional nucleases and repair proteins to correct the ICL (reviewed in [91]). MHF binds DNA synergistically with FANCM and stimulates the replication fork reversal [48] and branch migration activity [47] of FANCM. Crystal structures show that two MHF1/MHF2 dimers form a tetrameric MHF complex resembling an $(H3–H4)_2$ tetramer [92,93], to which the MHF-interacting domain of FANCM binds [50,94]. These structures have suggested models in which MHF–FANCM binds preferentially to branched DNA [50,94].

In addition to their role in repairing ICLs, vertebrate MHF1 and MHF2 are recruited to DSBs, dependent on direct interaction with the C-terminal region of RSF1, which coprecipitates with ATM following IR [81,82]. MFH1 and MFH2 are necessary to recruit and mono-ubiquitylate FANCD2 and FANCI [82], most likely by recruiting FANCM and the FA core complex.

In vertebrates and ascomycetes, MHF1/CENP-S and MHF2/CENP-X are also found at centromeres. CENP-S and CENP-X deletion mutants are viable in chicken DT-40 cells, but have defects in kinetochore function [95]. CENP-S and CENP-X form a heterotetrameric complex with the histone-fold proteins CENP-T and CENP-W [50,93] that can form a nucleosome-like particle that induces positive supercoils in DNA [96].

The heat-shock response

Heat shock is a highly conserved transcriptional response to an environmental stimulus. In heat shock, most of the genome is transcriptionally repressed whereas heat-shock protein (*Hsp*) genes become highly transcribed. Under heat shock and other stresses, the heat-shock transcription factor HSF trimerizes from an inactive monomer and binds to the promoters of *Hsp* genes, releasing paused PolIIs, resulting in rapid incorporation of H3.3 (reviewed in [59]). This activation has been extensively studied in *Drosophila*, where it can be visualized in polytene chromosomes (Box 3). In mammalian cells, as in *Drosophila*, heat-shock-inducible genes undergo rapid PARylation, nucleosome loss, and H3.3 deposition [60]. Unlike *Drosophila*, which has no macroH2A, macroH2A.1.1 is enriched on the promoters of inducible *HSP70* genes, but not on a constitutive paralog. At 5 min after heat shock, macroH2A.1.1 and H3 are reduced at the promoter as PARylation increases, then macroH2A.1.1, PARP1, and ADPR all decrease as H3.3 increases. This suggests a model in which macroH2A.1.1 sequesters PARP1 at the promoter and releases it upon heat shock, whereupon it PARylates histones and associated proteins to open the chromatin.

Seasonal responses

Many organisms undergo profound changes in gene expression with the seasons. In the common carp, *Cyprinus carpio*, a notable seasonal morphological change in the nucleolus of hepatocytes correlates with changes in rRNA transcription, which is highest in the summer. During

Box 3. Histone replacement during heat shock in *Drosophila*

In *Drosophila* polytene chromosomes, the two *Hsp70* loci expand as large puffs following heat shock, dependent on PARP activity [97]. Within 30 seconds after instantaneous heat shock, nucleosomes are lost throughout the *Hsp70* gene, faster than RNA PolII can transit the gene. More loss occurs by 2 min, the time by which PolII reaches the 3′ end of the gene. The initial rapid loss is dependent on HSF, the GAGA transcription factor, and the catalytic activity of PARP, whereas the later loss is transcription-dependent and is affected by a variety of elongation factors and chromatin remodelers [98].

Drosophila heat-shock puffs become dramatically enriched in H3.3, dependent on RNA PolII transcription elongation [42]. Upon heat shock in wild type cells, H3.3 chaperones HirA and XNP/ATRX rapidly accumulate on the nucleosome-depleted puffs, and then leave after heat shock ceases, but in H3.3-depleted cells they persist even after loss of PolII from the loci and cessation of transcription, suggesting that they bind to exposed DNA and are only displaced by H3.3 nucleosome deposition. Single mutants of *Xnp* and *HirA* are viable, and the proteins bind to puffs independently of each other, but double mutants are lethal as larvae and are unable to assemble H3.3 into chromatin [99].

Insight into global transcriptional repression during heat shock comes from a study of nucleosome turnover and low-salt-soluble 'active' chromatin in *Drosophila* S2 cells [100]. At heat-shock-induced genes, nucleosome turnover was increased as expected, and subnucleosomal particles were reduced at promoters and increased in the *Hsp* gene bodies, suggesting that these particles represent the progressing transcriptional machinery. By contrast, genome-wide loss of stalled PolII at active genes during heat shock correlates with reduced nucleosome turnover and resembles reduced turnover after inhibition of transcription elongation, suggesting transcription can evict nucleosomes, and that loss of stalled PolII mediates global transcription inhibition. Retention of subnucleosomal particles that probably represent transcription factors at the transcription start-site may allow efficient recovery after heat shock.

winter, downregulation of rRNA is accompanied by hypermethylation of the ribosomal cistron [61]. H2A.Z levels are increased overall in these cells during winter, but at the ribosomal cistron H2A.Z is increased during summer. Ubiquitylation of H2A.Z, which is usually associated with gene silencing [3,4], was also enriched at the ribosomal cistron during summer. This suggests multiple layers of seasonal regulation [62].

Similarly to other vertebrates, carp has two macroH2A genes. MacroH2A.1 is enriched at the ribosomal cistron and at the promoter of the *L41* ribosomal protein gene during winter [61,63]. Enrichment of macroH2A.1 at these sites colocalizes with enrichment for H3K27 methylation, a mark of repressed chromatin. Consistent with this, macroH2A.1 represses rDNA transcription in human cells [64].

In summer the ribosomal cistron and *L41* are instead enriched for macroH2A.2 and H3K4me3, a mark of active chromatin, consistent with the increased transcription of both loci. By contrast, no seasonal change is seen in macroH2A.1 or macroH2A.2 at the prolactin gene promoter. Although the roles of macroH2A.1 and macroH2A.2 are not well understood, these observations suggest that they may have opposing or complementary roles in gene expression [61,63].

Drought response

Many plants have a structurally distinct H1 histone that is specifically induced by drought. In *Arabidopsis*, H1.3 is significantly shorter than variants H1.1 and H1.2, and is induced by water stress or abcissic acid [65]. Under well-watered conditions it is expressed primarily in the secondary roots, but under drought stress it becomes active in the root meristem. Although changes in expression level of H1.3 have little phenotypic effect, H1.3 immunoprecipitates the drought-inducible gene *RAB18* (RESPONSIVE TO ABA 18) under drought stress but not under well-watered conditions, whereas no change is seen in immunoprecipitation of *RAB18* by H1.1 [66,67]. Antisense constructs of the orthologous H1.S in tomato exhibit increased stomatal conductance and transpiration rates, indicating a role in the regulation of stomata [68]. Differences in the drought-inducible transcription level of the orthologous H1.S gene in two cotton genotypes appear to underlie their differences in drought tolerance [69]. In tobacco, however, orthologous H1 variants show no evidence of induction by prolonged drought, and antisense constructs knocking down their expression have no phenotype [70]. Thus 'drought-inducible' H1s might have additional functions, and accumulation of *Arabidopsis* H1.3 in older non-dividing leaf tissue points to this same conclusion [52].

Histones as weapons

Histones are most commonly found in chromatin, but histones and histone fragments enriched in basic residues are surprisingly common as extracellular antimicrobial peptides that function as part of the innate immune system in both vertebrates and invertebrates (Box 4). Although such secreted histones protect against external and ingested bacteria, anti-microbial histones are also found internally in hemocytes of the Pacific white shrimp (*Litopenaeus vannamei*) [71] and in lipid droplets in *Drosophila melanogaster* embryos. Excess cytoplasmic histones are commonly deleterious to cells [72], but the *Drosophila* histones are stably bound to the lipid droplets, and are released by bacterial envelope components [73]. Mutations in the *Drosophila* histone receptor *Jabba* fail to localize histones to lipid droplets, resulting in increased sensitivity to killing by bacteria, suggesting that the histones in lipid droplets contribute to the intracellular antibacterial response [73]. Histones are also found in lipid droplets in mammals, suggesting a conserved mechanism of antibacterial defense [74].

Some viruses also make use of histones to subdue opponents. The *Cotesia plutellae* bracovirus (CpBV) is a polydnavirus found in the braconid wasp *Cotesia plutella*, which is an endoparasitoid of the diamondback moth *Plutella xylostella*. CpBV has a segmented genome of some 27 parts integrated as a provirus into the genome of *C. plutella*. Viral particles containing an episomal form of the genome are coinjected into a *Plutella* larva along with a *Cotesia* egg, and viral genes help to suppress the host immune response [75,76]. CpBV encodes a viral H4 that differs from typical insect H4 by the addition of a long acetylated tail. This H4 is expressed in the nuclei of hemocytes, and inhibits their spreading [76]. It also reduces the expression of the host H4 and transferrin genes [77,78]. The viral H4 can be assembled into nucleosomes *in vitro*. Its transient expression in the beetle *Tribolium casteneum* leads to numerous changes in gene expression

Box 4. Histone fragments as antimicrobial peptides

Several histone fragments with antimicrobial activity are derived from canonical H2A (Figure I). The potent antimicrobial peptide buforin I is produced in the stomach of the Korean toad *Bufo bufo gargarizans* by cleavage of unacetylated canonical H2A by pepsin isozymes, releasing a 39 amino acid N-terminal fragment [101,102]. This forms a protective coating on the surface of the stomach and inhibits bacterial growth. Buforin II is a 21 amino acid antimicrobial peptide derived from buforin I that is twice as potent as buforin I [102]. Buforin II can penetrate bacterial cells without permeabilizing them and is hypothesized to bind to DNA and RNA based on its ability to do so *in vitro* [103]. Peptide analogs of buforin II have DNA-binding affinities that correlate with their antimicrobial activity [104].

Unacetylated H2A is found in the cytoplasm of epithelial mucous cells of the catfish *Parasilurus asotus*. In wounds, it is cleaved to form the 19 amino acid antimicrobial peptide parasin I by the protease cathepsin D, which derives from a proenzyme that is itself cleaved by a metalloprotease upon wounding of the mucosa [105]. The N-terminal lysine of parasin I is necessary to bind to the cell membrane of *Escherichia coli*, whereas a region that can form an α helix (residues 9–19) is necessary to permeabilize and kill the cell [106].

The Atlantic halibut (*Hippoglossus hippoglossus*) produces hipposin, a 51 amino acid N-terminal H2A fragment that encompasses the homologous sequences found in parasin I and the buforins [107]. Similarly to parasin I, it renders bacterial membranes permeable and kills cells. The region identical to buforin II and a C-terminal region can translocate into cells [108], and although only the buforin II region seems to have antimicrobial activity, this is enhanced by the additions of the parasin and C-terminal regions [108,109].

Not all antimicrobial histones are H2A fragments. Schlegel's green tree frog, *Rhacophorus schlegelii*, secretes intact H2B [110], which becomes cleaved by *E. coli* outer-membrane protease OmpT to generate a fragment that penetrates bacterial cells, and is thought to act similarly to buforin II [111]. H2B has also been implicated in antimicrobial activity in catfish [112] and in oyster gills [113]. In the human placenta, H2A and H2B are found on the extracellular surfaces of amnion epithelial cells, where they exhibit antibacterial activity [114]. H1 has been implicated as an antimicrobial protein in salmon [115], rainbow trout [116], and in human gastrointestinal epithelial villi [117].

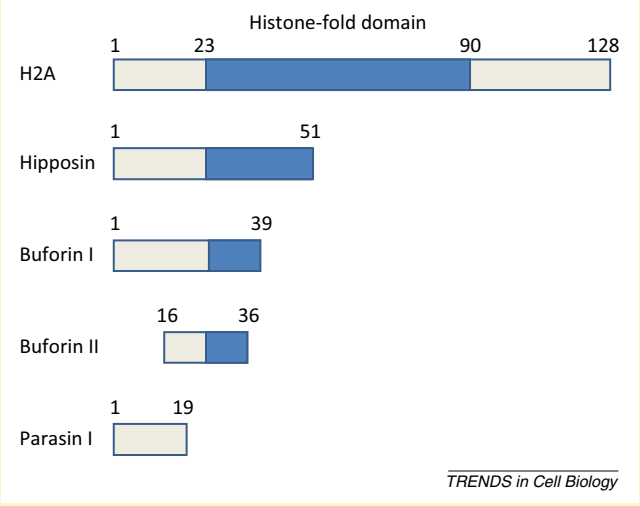

Figure I. Antimicrobial peptides derived from H2A. Peptides derived from H2A are used by several organisms to combat bacteria as part of the innate immune response. Numerals indicate the number of residues in each peptide.

that depend on the long tail [79]. Although the mechanism by which viral H4 inhibits spreading of hemocytes in *Cotesia* remains unclear, its potential for altering host gene expression and immune response is apparent.

Concluding remarks and future directions

Nearly all histone variants seem to be involved in environmental adaptation through mechanisms as diverse as affecting nucleosome dynamics, carrying variant-specific modifications, binding to PAR, mediating rearrangement of chromosomes, promoting transcriptional competence or silencing, attracting specific repair proteins, poisoning bacteria, altering cell mobility, and probably others. Variants substitute for their corresponding homologs in canonical nucleosomes, but there are a growing number of combinations of histone proteins, as seen in interactions of cenH3/CENP-A with H3.3 and CHRAC14, and even more obviously in histone-fold proteins such as MHF1/CENP-S, MHF2/CENP-X, and the CHRAC proteins.

Histones are not only chromatin proteins but are also weapons in interorganismal conflict that can even be acquired and deployed by viruses. The use of viral H4 against a host organism invites comparison to the relationship of cancer cells to their hosts. Overexpression or mutation of several histone variants and their chaperones has been implicated in cancers (reviewed in [80]), suggesting that cancer cells have deployed histone variants to gain an advantage over their host cells.

Although we still have much to learn about the biology of histone variants in such well-studied processes as DNA repair and heat shock, investigation of responses to seasonal variation and to pathogens or parasites suggests a future in which the roles of histones in a broad range of environmental responses will be better understood.

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