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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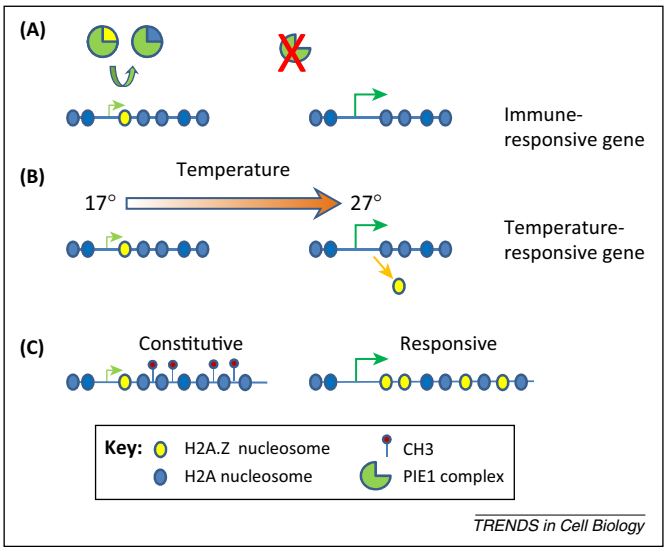

Figure 1. H2A.Z in *Arabidopsis*. **(A)** H2A.Z/H2B dimers are exchanged for H2A/H2B dimers by the PIE1 complex. Mutations (red X) in subunits (PIE1 or ARP6) of this complex result in misregulation of immunity response genes. **(B)** Increasing temperature results in reduced occupancy of H2A.Z at the +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