H2A.Z: a molecular rheostat for transcriptional control

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Abstract

The replacement of nucleosomal H2A with the histone variant H2A.Z is critical for regulating DNA-mediated processes across eukaryotes and for early development of multicellular organisms. How this variant performs these seemingly diverse roles has remained largely enigmatic. Here, we discuss recent mechanistic insights that have begun to reveal how H2A.Z functions as a molecular rheostat for gene control. We focus on specific examples in metazoans as a model for understanding how H2A.Z integrates information from histone post-translational modifications, other histone variants, and transcription factors (TFs) to regulate proper induction of gene expression programs in response to cellular cues. Finally, we propose a general model of how H2A.Z incorporation regulates chromatin states in diverse processes.

Introduction

Regulation of chromatin dynamics and accessibility is critical for proper control of all DNA-mediated processes, particularly in response to cellular cues. The replication-independent incorporation of histone variants by specific ATP-dependent chromatin remodeling complexes has emerged as a critical mechanism for regulating DNA repair, chromosome segregation, genomic stability, and gene expression [1–3]. The highly conserved H2A-type variant H2A.Z has garnered particular interest over the last several years because it has essential but unknown roles in early metazoan development [4,5]. Recent work demonstrating that H2A.Z is necessary for the proper execution of developmental programs during embryonic stem cell (ESC) differentiation [6–8] as well as for regulating the activation of gene expression patterns during muscle differentiation, T cell activation, and hormone-mediated gene activation [9–12] has led to a model whereby H2A.Z functions to mediate gene induction in response to diverse cellular cues.

Both *in vitro* and *in vivo* studies have shown that H2A.Z incorporation can alter nucleosome structure and dynamics, the activity of chromatin remodeling enzymes, and histone modification patterns [2,13]. H2A.Z is enriched at the majority of transcription start sites (TSSs) of RNA polymerase II (RNAPII)-regulated genes across eukaryotes, including mammals as well as regulatory regions, such as enhancers and boundary elements [7,8,14]. Intriguingly, H2A.Z is enriched at both active and silent genes, yet how H2A.Z influences gene expression in a context-dependent manner has remained elusive. In this review, we highlight recent work that reveals new mechanistic insights into how H2A.Z acts as a molecular gatekeeper for RNAPII at promoters and how H2A.Z integrates information from histone post-translational modifications (PTMs), other histone variants, and TFs to affect specific transcriptional outcomes during development, tissue specification, and immune- and hormone-mediated responses. Collectively, these studies suggest that H2A.Z acts as a molecular rheostat for gene control and serve as a paradigm for

understanding its broader roles to regulate chromatin states in response to environmental and genetic perturbations.
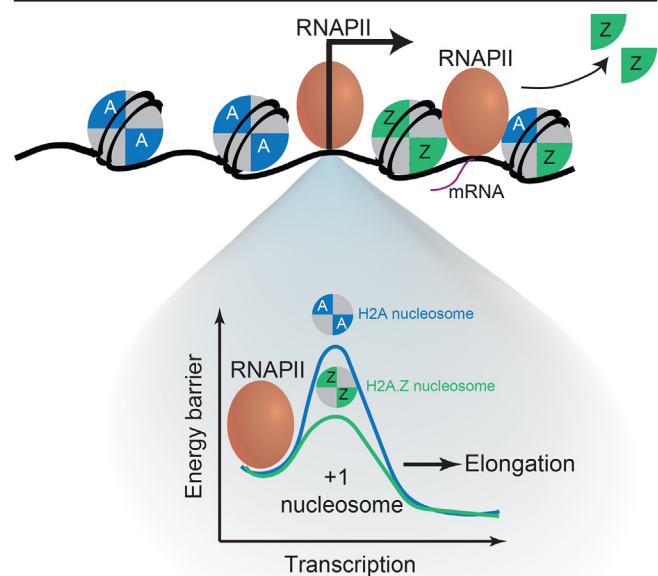
Regulation of +1 nucleosome and RNA polymerase II progression

H2A.Z is highly enriched at the promoter regions of a large subset of genes across cell types, consistent with a role in transcriptional regulation. Although the precise patterns of enrichment differ slightly among eukaryotes, genome-wide studies from yeast to human indicate that H2A.Z-containing nucleosomes flank the nucleosome-depleted region (NDR) at TSSs [9,15,16]. Why is H2A.Z incorporation at the TSS important for transcriptional regulation? *In vitro* studies have demonstrated that the nucleosome immediately downstream of the TSS (denoted +1 nucleosome) poses a sizeable barrier to transcription and can direct the orientation of the pre-initiation complex (PICs) and subsequent transcriptional elongation [17,18]. In *Drosophila*, the +1 nucleosome obstructs RNAPII transit, resulting in the increased stalling and backtracking of the polymerase [19]. Notably, H2A.Z enrichment at the +1 nucleosome correlates with decreased RNAPII stalling, suggesting that its incorporation reduces the high-energy barrier to RNAPII progression. Consistent with this idea, H2A.Z levels anti-correlate with nucleosome turnover, indicating that H2A.Z incorporation at the +1 nucleosome regulates productive elongation by facilitating H2A.Z/H2B dimer loss without depletion of $(H3-H4)_2$ tetramers. These findings suggest that H2A.Z incorporation at the +1 nucleosome regulates transcriptional output by modulating RNAPII kinetics and transcriptional elongation (Figure 1). Given that *Drosophila* H2A.Z has features of both H2A.Z and H2A.X (known as H2AvD) [20,21], whether H2A.Z incorporation at the +1 nucleosome has similar roles in other organisms remains an open question. Moreover, whereas a large number of genes are regulated by polymerase pausing in *Drosophila*, not all H2A.Z target genes in other organisms are regulated in this manner [22]. In mouse ESCs, H2A.Z is present at a large set of genes marked by histone 3 lysine 4 trimethylation (H3K4me3) nucleosomes that include both active genes as well as poised, silent developmental genes [6–8,14]. The poised genes harbor H3K27me3, a repressive mark catalyzed by polycomb repressive complex 2 (PRC2) and dramatically less paused RNAPII [7,8,14,22]. Thus, how H2A.Z functions at the +1 nucleosome to mediate these contrasting transcriptional outcomes will likely be influenced by other factors.

H2A.Z-associated post-translational modifications correlate with transcriptional outcome

H2A.Z is highly conserved among eukaryotes and ubiquitously expressed across cell types and its replication-independent incorporation is critical for regulating diverse
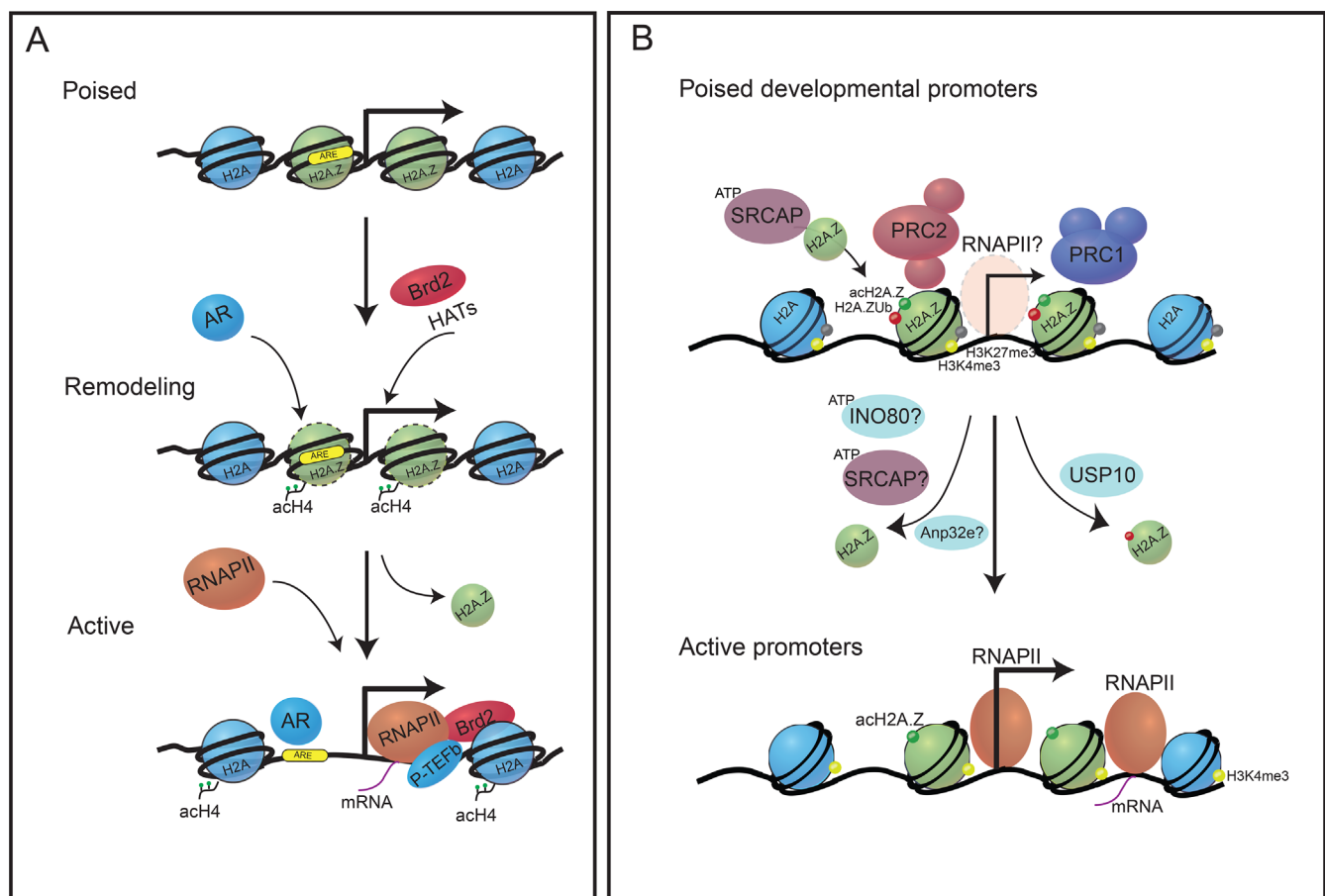
Figure 1. H2A.Z nucleosome composition at promoters influences nucleosome stability and transcriptional state

(Top) The transition from homotypic to heterotypic H2A.Z nucleosomes can regulate gene activation through regulation of transcription elongation. (Bottom) H2A.Z nucleosomes can impact the stability of the +1 nucleosome and RNA polymerase II progression.

transcriptional outcomes at a large number of RNAPII-regulated promoters. Promoter nucleosome stability and dynamics can be regulated by histone PTMs. Similar to major type histones, variants are also subject to diverse PTMs which are thought to function to recruit downstream effectors, such as histone writers, readers, or erasers to target genes [23,24]. For example, amino-terminal acetylation of H2A.Z (acH2A.Z) is strongly enriched at the 5′ end of active gene promoters [8,14,25,26]. Whereas in *Saccharomyces cerevisiae*, H2A.Z is acetylated by the ESA1 and NuA4 complexes [27,28], their mammalian counterparts have not been clearly defined. Recent studies showed that the bromodomain-containing protein Brd2 preferentially associates with H2A.Z-containing nucleosomes at active genes and that Brd2 recruitment is necessary for androgen receptor-regulated gene activation [29] (Figure 2A). Brd2 has a higher affinity for H2A.Z nucleosomes compared to canonical H2A, particularly in conjunction with nucleosomes that also harbor H4 amino-terminal acetylation *in vitro*. H4 acetylation has been implicated in decondensing higher-order chromatin structures and in mediating an active chromatin state [30], suggesting that H4 acetylation marks the transition from a repressed state to an active chromatin state. However, whether H4ac is sufficient or whether additional mechanisms are necessary to drive

Figure 2. H2A.Z regulates gene induction

(A) In response to androgen receptor (AR) signaling, H2A.Z allows binding of AR to its response elements, at both promoters and enhancers, and along with acH4 recruits Brd2 and RNA polymerase II to mediate gene activation. (B) H2A.Z is enriched at both active and silent, poised genes in embryonic stem cells. The effect of H2A.Z on transcription depends on the balance of both activating and repressive histone post-translational modifications as well as H2A.Z-specific deposition and removal complexes in response to cellular cues.

Brd2 recruitment to target promoters *in vivo* is not clear. Genome-wide localization studies revealed that acH2A.Z is enriched at activated prostate cancer genes in androgen-sensitive human prostate cancer cells (LNCaP) [26]. Given that bromodomains are recruited to sites of action by recognizing acetylated lysines [31], acH2A.Z may be an additional determinant for Brd2 recruitment at these regions. More broadly, Brd2 is a member of the BET (bromodomain and extra terminal domain) family of proteins that also includes Brd4, which has critical roles in gene activation in ESCs and in cancer [32–36]. Thus, it will be of interest to determine whether acH2A.Z facilitates recruitment of BET proteins during gene activation *in vivo*.

In addition to active genes, H2A.Z is enriched at silent genes that are poised for activation in ESCs. In mammals, H2A.Z can be ubiquitinated at K120, K121, and K125 residues by the E3 ligase activity of the PRC1 component

Ring1b, suggesting a repressive role of this H2A.Z PTM [14,37]. Consistent with this idea, H2A.Z monoubiquitination (H2A.Zub) appears to demarcate facultative heterochromatin [37]. Interestingly, loss of the H2A.Z deubiquitinating enzyme USP10 results in a failure of androgen-receptor target gene activation, suggesting that the removal of this modification is necessary for gene induction [38]. Recent reports showed that PRC1-mediated ubiquitination of core H2A (H2Aub) impedes RNAPII recruitment at bivalent genes [39] and may facilitate PRC2 targeting and the establishment of polycomb domain formation [40,41]. Moreover, H2Aub stimulates PRC2 recruitment to chromatinized templates and catalysis of H3K27me3 *in vitro*, suggesting that H2Aub is a critical downstream effector of polycomb silencing [42]. Given that PRC1 catalyzes the ubiquitination of both H2A and H2A.Z [14,39], we speculate that H2A.Zub is also a key determinant for

regulating RNAPII recruitment and polycomb domain formation.

In mouse ESCs, acH2A.Z and H2A.Zub are co-enriched with H3K27me3 nucleosomes, indicating that dually modified H2A.Z (acH2A.Zub) is present at poised developmental genes [14] (Figure 2B). One attractive model is that H2A.Zub contributes to the recruitment of PRC2 and reduces nucleosome accessibility (e.g. dimer loss) at the +1 nucleosome, whereas acH2A.Z promotes RNAPII progression perhaps through recruitment of BET family members, known regulators of polymerase pause release [43,44]. Notably, H2A.Z facilitates access to both active and repressive complexes to chromatin in ESCs to regulate the balance between self-renewal and differentiation [8]. Thus, the balance of acH2A.Z and H2A.Zub at promoters may be critical for regulating the induction of gene expression programs in response to developmental cues.

Regulation of H2A.Z incorporation is critical for gene regulation

Although histone PTMs provide context-dependent signals to regulate H2A.Z function at promoters, dissecting how this variant is targeted to discrete genomic sites is also important for understanding its regulatory roles. The exchange of H2A for H2A.Z is catalyzed by ATP-dependent complexes in a replication-independent manner in all eukaryotes, namely the SWR1 complex (SRCAP in mouse and human) as well as by p400/Tip60 in higher eukaryotes [45,46]. Notably, INO80, an ATP-dependent remodeler known for its role in DNA repair, has been implicated in transcription-dependent removal of H2A.Z in yeast [47,48]. Whether INO80 functions similarly in higher eukaryotes is yet to be determined. Histone chaperones are additional factors that regulate histone variant incorporation [49]. For example, the metazoan-specific histone chaperone, ANP32E (acidic nuclear phosphoprotein 32 kDa E), which is a member of the p400 complex, regulates variant incorporation by facilitating the removal of H2A.Z dimers as evidenced by increased accumulation of H2A.Z at promoters and enhancers in ANP32E knockout cells [50,51]. Together, these studies suggest that the interplay between H2A.Z-specific ATP-dependent remodelers and histone chaperones is crucial for regulating H2A.Z incorporation.

Until recently, how H2A.Z exchange complexes are targeted to specific sites remained largely unknown. Evidence now suggests that SWR1 and INO80 complexes may recognize NDRs to remodel H2A.Z at the +1 nucleosome by selective positioning of distinct components of the remodeling complex around the NDR [52]. Biochemical analyses suggest that SRCAP and ANP32E

recognize specific features of H2A.Z that are divergent from core H2A, such as the C-terminal α-helix to facilitate the exchange reaction [50,53], providing an additional mechanism for how remodelers distinguish between the two histones. Recent work also highlights roles for histone PTMs in proper histone exchange [46,48]. For example, in contrast to its well-documented role in H2A.Z deposition, yeast SRCAP (SWR-C) displays altered substrate specificity in the presence of H3K56ac, leading to the removal of H2A.Z dimers from chromatin [48]. In human ESCs, H3K56ac is enriched at both active and inactive genes that largely overlap targets of the core pluripotency TFs OCT4, SOX2, and NANOG [54]. Notably, H3K56ac is redistributed to developmental genes that are activated in response to retinoic acid during ESC differentiation. Thus, H3K56ac and H2A.Z may cross-talk to regulate H2A.Z dynamics and gene expression across eukaryotes.

In addition to histone PTMs, H2A.Z incorporation appears to be influenced by DNA methylation levels. Studies in both plants and mammals suggest that H2A.Z and DNA methylation are mutually antagonistic at promoters and within gene bodies [55,56]. Consistent with this idea, 5-aza 2′-deoxycytidine-induced DNA demethylation stimulates SRCAP-mediated H2A.Z incorporation, facilitating nucleosome depletion and gene activation in cancer cells [57]. Although these studies point to a model whereby H2A.Z incorporation is targeted to discrete regions of the genome by a variety of mechanisms, evidence also suggests that H2A.Z can be randomly incorporated at low levels and that the removal of H2A.Z is a key event for regulating chromatin states [58,59]. Thus, a balance between the targeted deposition and removal of H2A.Z is likely critical for maintaining proper chromatin states.

H2A.Z nucleosome composition affects chromatin dynamics

Modulating the number of H2A.Z copies in a nucleosome can also have consequences on nucleosome structure and function. Early structural studies suggested that, owing to steric clashes between the L1 loops of H2A.Z and H2A, formation of a heterotypic H2A.Z nucleosome is unlikely [60], but *in vitro* and *in vivo* evidence now indicates that H2A.Z can form both heterotypic and homotypic nucleosomes [16,61]. *In vitro* biochemical analyses showed that two copies of H2A.Z (homotypic) result in a more stable nucleosome that is refractory to RNAPII progression [62,63]. However, homotypic H2A.Z is enriched at the +1 nucleosome in *Drosophila*, which is thought to decrease the energy barrier to RNAPII progression, and is depleted downstream of paused polymerase [16]. In mouse trophoblast cells, H2A.Z appears to be redistributed to

heterochromatin (for example, telomeres) during G_2/M, resulting in a shift from homotypic to heterotypic H2A.Z nucleosomes and to an expanded NDR at the TSS of active H2A.Z genes [64]. Surprisingly, this transition from homotypic to heterotypic H2A.Z nucleosomes during the cell cycle does not appear to correlate with cell cycle-dependent transcriptional changes. Thus, the consequence of H2A.Z composition on transcription is highly complex and likely depends on its levels at the TSS relative to gene bodies as well as the presence of histone modifications or other histone variants or both.

The incorporation of other histone variants with H2A.Z can alter the functional properties of nucleosomes. H3.3 differs from major type H3 by only four or five amino acids in metazoans yet displays distinct regulation and biochemical properties. In mammalian cells, double-variant nucleosomes containing H2A.Z and H3.3 mark regions of dynamic chromatin regulation and are highly salt-labile [65–67]. Moreover, in yeast, where non-centromeric H3 is most similar to vertebrate H3.3, *in vitro* reconstituted nucleosomes display release of H2A.Z dimers in low salt [68,69]. Together, these findings suggest a conserved mechanism in which H3.3 further regulates the stability of the H2A.Z nucleosome. Notably, whereas H2A.Z facilitates intranucleosomal folding, H3.3 counteracts the H2A.Z-mediated compaction of nucleosomal arrays *in vitro* [70,71] and can relieve the transcription repression caused by H2A.Z-containing chromatin [71]. In ESCs, H3.3 depletion results in reduced nucleosome turnover and an increase in PRC2 enrichment [6,7,72,73]. Conversely, mutation of the C-terminal H2A.Z acidic patch to resemble core H2A leads to an increase in H2A.Z dynamics, de-repression of poised developmental genes, and increased H3.3 enrichment in ESCs [7]. Together, these studies indicate that H3.3 cooperates with H2A.Z to regulate nucleosome stability, chromatin accessibility, and transcriptional output. Future investigations to determine how the balance between H3.3 and H2A.Z is regulated at specific genomic locations are needed to understand how these variants regulate global gene expression programs.

H2A.Z is a critical binding platform for pioneer TFs
Given that TF binding is influenced by nucleosome density and histone PTMs [69,74] and that H2A.Z exhibits strong genetic interactions with TFs in yeast [74], it is likely that H2A.Z also coordinates transcriptional responses by modulating TF accessibility. Several recent studies in mammals demonstrated that H2A.Z nucleosomes are necessary for the recruitment of pioneer TFs (Figure 3). Unlike classic TFs, pioneer TFs bind nucleosomal DNA and remain bound to their sites
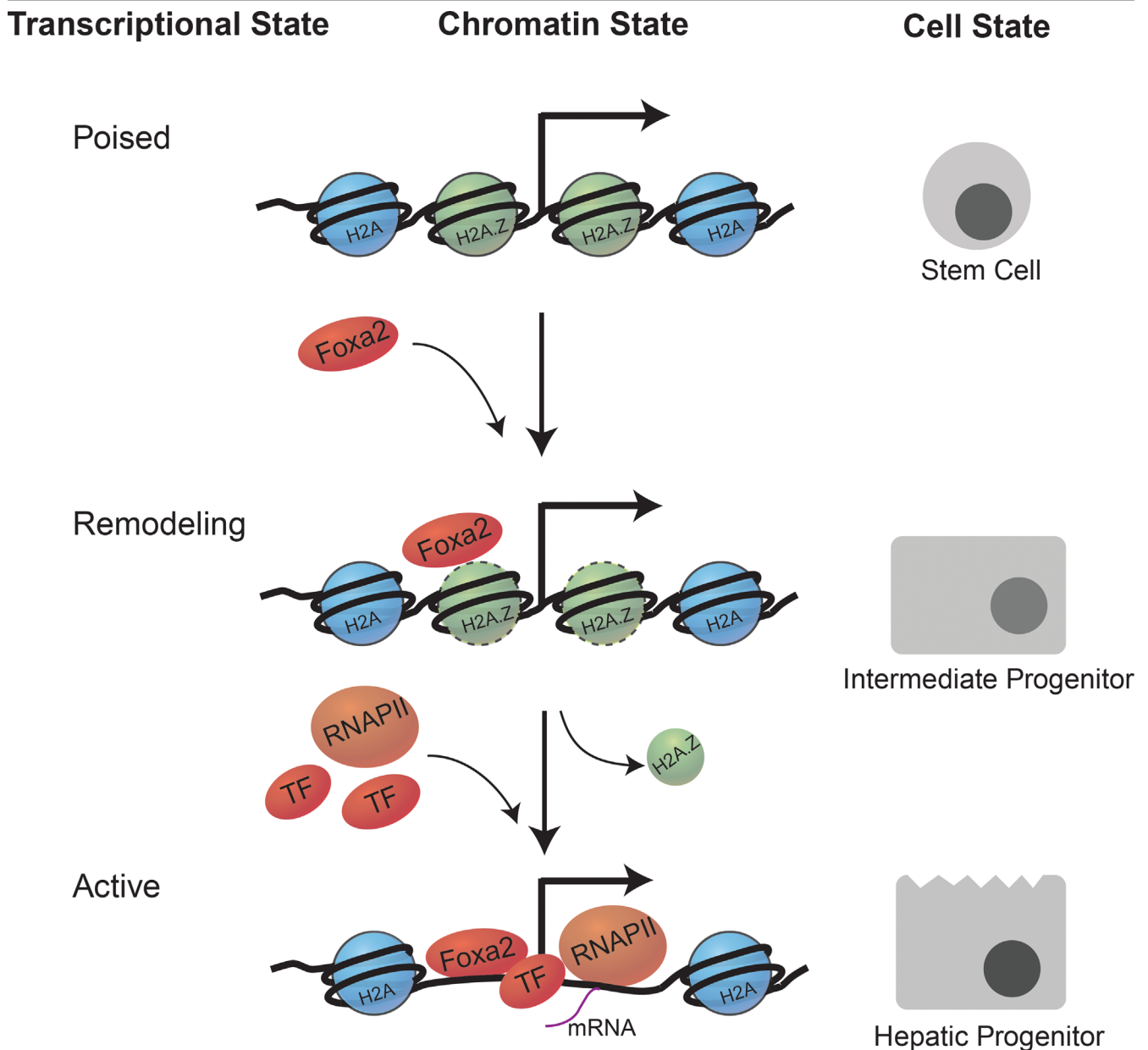
through mitosis, providing a level of epigenetic memory [75]. Foxa2 is an example of a pioneer TF that has key roles in development and transcriptional activation. In ESCs, H2A.Z is required for the recruitment of Foxa2 to promoters of genes activated during early endoderm differentiation [76]. Moreover, the binding of Foxa2 to H2A.Z nucleosomes promotes the recruitment of SWI/SNF and INO80 complexes, resulting in H2A.Z removal and nucleosome depletion, establishing a platform for the binding of other TFs. On the other hand, loss of either H2A.Z or Foxa2 impairs gene induction and differentiation. This work adds another layer to how H2A.Z mechanistically regulates gene expression.

In addition to its requirement for regulating developmental transitions, H2A.Z is necessary for the expression of circadian-regulated genes by modulating TF binding. CLOCK and BMAL1 are pioneer TFs that bind to circadian clock-regulated genes to induce their expression in a temporally regulated manner [77]. Like binding of Foxa2, binding of these TFs promotes gene activation and the recruitment of RNAPII. Interestingly, CLOCK: BMAL1 binding strongly correlates with cyclical changes in H2A.Z occupancy and nucleosome depletion [78]. Using mouse livers at different time points in the light/dark cycle, investigators demonstrated that H2A.Z-enriched nucleosomes flank CLOCK-binding sites at both promoters and intergenic regulatory regions. Notably, H2A.Z levels oscillate on the basis of CLOCK: BMAL1 binding. The cyclical binding of CLOCK:BMAL1 induces nucleosome depletion at promoters, allowing additional TFs to gain access to DNA. Loss of BMAL1 in mice results in a decreased H2A.Z levels and a higher nucleosome occupancy at CLOCK-binding sites as well as loss of circadian regulation, suggesting that H2A.Z is necessary to maintain these sites in a highly plastic state. Collectively, these studies reveal that H2A.Z provides a crucial binding platform for pioneer TFs to facilitate precise regulation of gene expression programs.

Concluding remarks
Since H2A.Z was first identified over 30 years ago, studies have begun to reveal the many layers of regulation and complex functions of H2A.Z in modulating chromatin dynamics and gene expression. Recent studies have uncovered many players involved in H2A.Z-mediated transcriptional regulation, but how H2A.Z translates upstream signals into diverse transcriptional outcomes is still largely unknown. Although we have focused largely on the roles of H2A.Z at promoters, H2A.Z also appears to be enriched at a small subset of enhancers; however, its roles in enhancer regulation are poorly understood [7,8,14]. Overall levels of H2A.Z are lower at enhancers compared with promoters, whereas H3.3

Figure 3. H2A.Z acts a scaffold for binding of chromatin remodelers and pioneer transcription factors

During endodermal differentiation, the pioneer transcription factor Foxa2 binds to H2A.Z nucleosomes, leading to the recruitment of chromatin remodelers, nucleosome depletion, transcription factor binding, and subsequent gene activation.

displays the opposite trend [79]. Thus, the dynamic interplay between H2A.Z and H3.3 at enhancers and promoters may facilitate gene regulation. Consistent with this idea, recent evidence supports the role of H2A.Z in mediating higher-order chromatin interactions between promoters and enhancers [80]. Additional mechanistic studies are needed to understand how histone variants contribute to these critical regulatory interactions.

Insights into the function of H2A.Z in gene induction are critical for understanding how H2A.Z functions in other processes, such as DNA repair and genomic stability. For example, p400-mediated H2A.Z incorporation during double-strand break (DSB) repair stimulates an open chromatin conformation, resulting in the formation of an efficient chromatin template for DSB repair, albeit through different effectors [81]. Interestingly, H2A.Z acetylation and ubiquitylation are also hallmarks of

chromatin at DSBs, suggesting a general model by which H2A.Z modulates chromatin in diverse DNA-mediated processes. Given that H2A.Z has been implicated in the activation of estrogen- and androgen-responsive genes in models of breast and prostate cancer [11,38,82,83], continued mechanistic studies into H2A.Z function will be critical to fully understand how H2A.Z contributes to development and disease.

Abbreviations

ANP32E, acidic nuclear phosphoprotein 32 kDa E; BET, bromodomain and extra terminal domain; DSB, double-strand break; ESC, embryonic stem cell; H2Aub, H2A ubiquitination; H2A.Zub, H2A.Z ubiquitination; acH2A.Z, acetylated H2A.Z; NDR, nucleosome-depleted region; PRC, polycomb repressive complex; PTM, post-translational modification; RNAPII, RNA polymerase II; TF, transcription factor; TSS, transcription start site.

Disclosures

The authors declare that they have no disclosures.

Acknowledgments

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