

H2A.Z at the Core of Transcriptional Regulation in Plants

H2A.Z, an evolutionarily conserved variant form of canonical H2A, has been widely recognized to have decisive roles in multiple cellular processes through its influence on chromatin structure and dynamics in eukaryotes. Many of its effects on regulating chromatin function have been attributed to its role in modulating nucleosome dynamics and DNA methylation. In plants, H2A.Z plays critical roles in multiple processes, such as growth and development, phase transitions, and response to the environment. Despite the progress made in understanding its role in these processes, several aspects of H2A.Z biology, such as the specificity and regulation of its incorporation and action mechanism in transcriptional regulation, remained elusive. Nevertheless, recent discoveries in plants discussed here have provided the much-needed momentum in solving some of the mysteries associated with H2A.Z (Figure 1).

DROP ZONES: SPECIFICITY AND REGULATION OF H2A.Z INCORPORATION INTO NUCLEOSOMES

The evolutionarily conserved SWR1 remodeling complex (SWR1c) catalyzes H2A.Z deposition into the nucleosomes. While the genome-wide role of H2A.Z in chromatin structure and gene regulation is well established, H2A.Z also has defined regulatory roles on specific genes involved in development and adaptation. However, it remained one of the major unanswered question as to how the global and locus-specific functions of SWR1c and H2A.Z could be distinguished. A recent study has provided some insights into locus-specific chromatin remodeling through sequence-specific recruitment of the remodeling machinery to target genes (Gómez-Zambrano et al., 2018). *Arabidopsis* SWC4, a homolog of the yeast SANT-domain protein Swc4/Eaf2, interacts with the core SWR1c subunit SWC6 and influences H2A.Z deposition and consequent changes in growth and development. Interestingly, partial loss of SWC4 function predominantly altered a number of key genes in development such as the floral integrator *FT* but had little effects on others such as *FLC*. The authors showed that SWC4 is a DNA-binding protein with affinity to A/T-rich DNA elements. This offers a mechanism for SWC4 to bind specific genomic loci and recruit the SWR1c to facilitate local nucleosome remodeling through H2A.Z deposition. Providing additional evidence to the specificity of H2A.Z incorporation, a plant-specific phosphorylation of H2A at serine 95 (H2AS95ph) by a MUT9P-LIKE KINASE (MLK4) was recently found to mark nucleosomes for H2A.Z deposition at the *G1* locus (Su et al., 2017). Photoperiod-dependent, target-specific histone modification at *G1* was ensured by CCA1 recruiting MLK4, which also interacted with YAF9, a subunit of the SWR1 and NUA4 complexes responsible for H2A.Z deposition and H4 acetylation, respectively. This targeted recruitment of the remodeling complexes to *G1* regulates the expression of flowering genes. While this provides a specific example of how *G1* is regulated, such paradigms may be in operation at other physio-

logically important loci ensuring the specificity and physiologically meaningful regulatory outcome.

THE KINGPIN: ROLE OF H2A.Z IN DEVELOPMENT AND ENVIRONMENTAL RESPONSES

H2A.Z has previously been shown to play critical roles in growth and development and environmental responses. While the well-established role of H2A.Z in reproductive transition was re-validated in a recent study (Gómez-Zambrano et al., 2018), it has also been extended to the modulation of vegetative phase change through the control of key microRNA genes *MIR156A* and *MIR156C* (Xu et al., 2018). In addition, it has been shown that SWR1c/H2A.Z control inflorescence architecture by activating the expression of *PRE1* family genes and promoting pedicel elongation by cooperatively with *ERECTA* signaling (Cai et al., 2017). H2A.Z has also been shown to be key in controlling cell fate determination in germline development in *Arabidopsis*. In the developing ovules, the cytochrome P450 (CYP78A5) *KLU* promotes the incorporation of H2A.Z at *WRKY28*. This ensures tissue-specific expression of *WRKY28* to maintain the fidelity of megasporocyte specification by suppressing cells surrounding the megaspore mother cell from acquiring germline identity (Zhao et al., 2018). One of the well-studied roles of H2A.Z in environmental responses is its involvement in regulating the response to temperature (Kumar and Wigge, 2010), providing a molecular framework for environmental modulation of development and physiological responses underlying adaptation. In line with this, H2A.Z was recently shown to be involved in controlling seed dispersal in Brassicaceae, particularly in response to changes in ambient temperature (Li et al., 2018). H2A.Z nucleosomes contribute to transcriptional regulation of genes such as *INDEHISCENCE* (*IND*) through temperature-responsive chromatin dynamics to control tissue specification underlying fruit dehiscence. This additional layer of environmental control through chromatin modification on the well-established developmental program offers a mechanism to ensure phenotypic plasticity. A recent study has explored the genome-wide role of H2A.Z nucleosome dynamics in regulating the expression of temperature-responsive genes (Cortijo et al., 2017), and found that H2A.Z acts as a repressor of environmentally regulated gene expression. While the mechanism of thermosensory H2A.Z dynamics remains elusive, a new study has implicated histone deacetylation in the process (Tasset et al., 2018). This study discovered that the interaction of POWERDRESS (PWR) with the histone deacetylase HDAC9 is critical for thermomorphogenesis and is associated with H2A.Z eviction. Despite these advances, our

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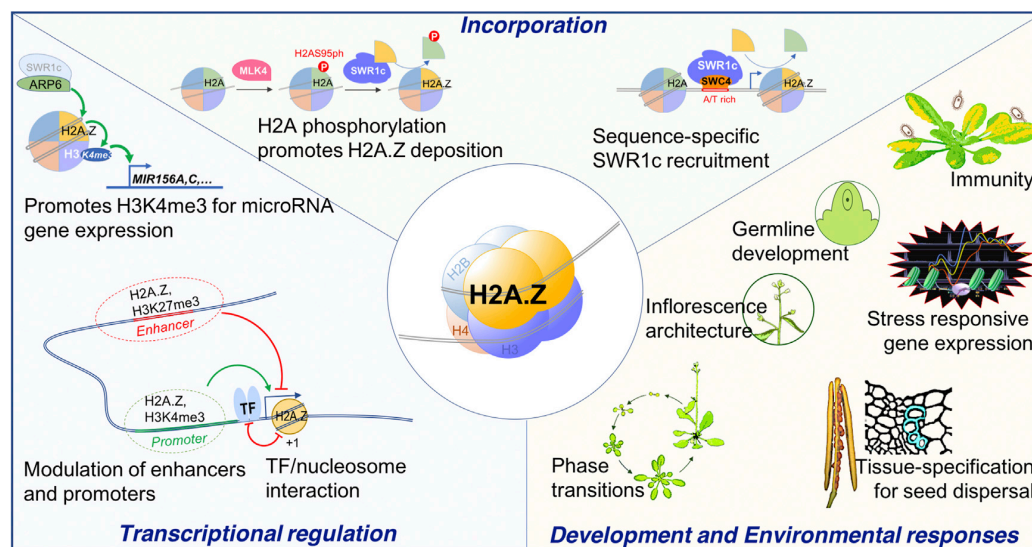


Figure 1. Recent Advances in Understanding the Role of H2A.Z in Plants.

Summary of recent advances in elucidating H2A.Z incorporation, its role in environmental responses, and the mechanisms of transcriptional regulation by H2A.Z in plants.

understanding of the roles of H2A.Z in stress responses is still limited, because it appears that H2A.Z plays much more complex roles in response to diverse stresses. Genome-wide analysis of H2A.Z distribution and gene expression under stress conditions exposed regulatory complexities of stress-responsive gene expression (Sura et al., 2017). Responsiveness to drought stress was associated with H2A.Z enrichment at gene bodies, where it generally assumed a repressive role offering tight regulation under control conditions. In addition, H2A.Z at the +1 nucleosome displayed a dual role with both repressive and activating effects. This also echoes the recent update on the role of SWR1c/H2A.Z in plant immunity, where they have been found to be positive regulators (Berriri et al., 2016), albeit with some non-redundant roles between the complex subunits. H2A.Z was required for resistance to pathogens. Although mutants affecting H2A.Z incorporation showed high basal expression of key defense genes, upon pathogen challenge they showed reduced/defective activation, suggesting that while H2A.Z has a general repressive role in basal transcription, it is required for full inducibility. How these two regulatory roles can be mechanistically uncoupled remains to be investigated. As the evidence for H2A.Z in environmental responses grows, it becomes immediately apparent that the regulatory principles are far more complex than assumed and should be interpreted with caution. More studies are required to clarify the regulatory principles underlying the role of H2A.Z in stress adaptation.

CAUGHT IN ACTION: MECHANISM OF TRANSCRIPTIONAL REGULATION BY H2A.Z

Despite the wide roles of H2A.Z in transcriptional regulation in development and environmental responses, the mechanism by which H2A.Z brings about global and locus-specific regulatory

outcomes is not well understood. One of the well-studied roles of H2A.Z found to date is to regulate transcriptional response to ambient temperature through temperature-responsive nucleosome dynamics (Kumar and Wigge, 2010), which has been shown to control key transcription factors such as PIF4 in modulating growth and developmental transitions (Kumar et al., 2012). A recent study further showed that the antagonistic interactions between the transcription factor HSF1 and H2A.Z nucleosomes provide a framework for transcriptional regulation of a subset of genes in response to temperature changes (Cortijo et al., 2017). Such a mechanism offers tight control of gene expression and robust regulatory dynamics desirable for environmental and developmental responses. While the majority of such studies addressing the role of H2A.Z in gene regulation has focused on nucleosome structure and dynamics proximal to the transcription start site or on the gene bodies, a recent study has also provided some insights into the modulation of enhancer and promoter functions by H2A.Z (Dai et al., 2017). It was found that H2A.Z represses gene expression through stabilizing +1 nucleosome and reducing gene accessibility. In addition, genome-wide analysis revealed that at enhancers and promoters, H2A.Z regulates gene expression through cooperative action with histone modifications. While its association with the H3K4me3 mark at promoters activated gene expression, H2A.Z repressed enhancer function by promoting the repressive H3K27me3 marks and inhibiting H3K4me3. Supporting this, an epigenetic pathway that links H2A.Z and H3K27me3 was recently proposed (Carter et al., 2018). In this model, PIE1, the catalytic subunit of SWR1c, was shown to cooperatively work together with PICKLE and the histone methyltransferase CURLY LEAF to promote H3K27me3-mediated chromatin repression. The role of H2A.Z in transcriptional regulation cooperatively through histone marks, particularly H3K4me3, has been further supported by the findings of a study addressing the mechanism of transcriptional regulation

by SWR1c/H2A.Z of two key microRNA genes *MIR156A* and *MIR156C*, which are involved in vegetative phase change (Xu et al., 2018). Modulation of nucleosome occupancy has been regarded as one of the major mechanisms by which H2A.Z imparts its role in gene regulation. Contrary to this view, Xu et al. (2018) showed that rather than changes in nucleosome occupancy, H2A.Z promotes gene expression of *MIR156A* and *MIR156C* by facilitating H3K4me3 marks. This further points to the emerging view that while H2A.Z and other chromatin elements regulate global gene expression through general regulatory principles, the gene-specific, physiologically relevant local interactions likely bring the specificity to their role in a variety of processes.

Recent discoveries discussed above have reinforced the role of H2A.Z as a key regulator of multiple biological processes and at least partially answer some major outstanding questions (Figure 1). The roles of specific molecules and mechanisms that drive the specificity of H2A.Z deposition at particular loci rather than as a general replacement histone are becoming increasingly clear. This will add to the functional dissection of H2A.Z in the context of specific traits/loci in future. These studies have also exposed some major questions that still remain to be answered. Particularly, it remains elusive as to how H2A.Z brings about global and locus-specific regulatory outcomes. Uncoupling the function of H2A.Z in its global role in controlling basal transcription from its specific role in gene induction in response to external and intrinsic cues is required to understand this question. Beyond fundamental biology, elucidating the roles of H2A.Z and the underlying mechanisms will be crucial in exploiting the potential of H2A.Z for improving environmental adaptation of crop plants particularly in the context of global climate change.

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