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TITLE

A G(enomic)P(ositioning)S(ystem) for Plant RNAPII Transcription

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ABSTRACT

Post-translational modifications (PTMs) of histone residues shape the landscape of gene expression by modulating the dynamic process of RNAPII transcription. The contribution of particular histone modifications to the definition of distinct RNAPII transcription stages remains poorly characterized in plants. Chromatin Immuno-precipitation combined with next-generation sequencing (ChIP-seq) resolves the genomic distribution of histone modifications. Here, we review histone PTM ChIP-seq data in *Arabidopsis thaliana* and find support for a Genomic Positioning System (GPS) that guides RNAPII transcription. We review the roles of histone PTM “readers”, “writers” and “erasers”, with a focus on the regulation of gene expression and biological functions in plants. The distinct functions of RNAPII transcription during the plant transcription cycle may in part rely on the characteristic histone PTMs profiles that distinguish transcription stages.

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MAIN TEXT

Histones: the coordinates for transcription?

Gene expression relies on different functions of RNA Polymerase II (RNAPII) during transcription. The separation of these functions defines different stages of RNAPII transcription: initiation, elongation and termination. The question how RNAPII recognizes the right time and position to execute a needed function remains an exciting research opportunity. In eukaryotes, genomes are organized in the form of nucleosomes that comprise of two copies of histones H2A, H2B, H3 and H4 [1]. N-terminal histone tail residues undergo extensive post-translational modifications (PTMs), including methylation (me), acetylation (ac) and ubiquitination (ub), which are associated with functional consequences on chromatin organization and gene expression (reviewed in [2, 3]). The establishment and maintenance of histone PTMs requires recognition by “reader” effector enzymes, deposition by “writer” enzymes and removal by “eraser” enzymes. Histone acetylation is mainly read by bromodomain (BRD) proteins, written by histone acetyltransferases (HATs) and erased by histone deacetylases (HDACs). Histone methylation is read by proteins with plant homeodomain (PHD) finger domain and ‘‘Royal Family’’ domains (e.g. Chromodomain and Tudor domain), written by histone methyltransferases (HMTs) and erased by histone demethylases (HDMs) [4, 5]. Similarly, histone ubiquitination is read by proteins with ubiquitin-binding domain (UBD) and modulated by histone ubiquitin ligases (ULs) and deubiquitinases (DUBs) [6]. Trios of “reader-writer-eraser” enzymes define the genomic localization of histone PTMs connected to RNAPII transcription.

Chromatin profiling techniques such as ChIP-chip [7], ChIP-seq [8], CUT&RUN-seq and [9] and CUT&Tag-seq [10] (see Box 1) revealed the genomic distribution of histone PTMs and variants associated with different RNAPII transcription stages. However, the causality of histone PTM and transcriptional consequences is actively debated [11]. On the one hand, histone PTMs can be instructive for RNAPII transcription (reviewed in [12]). On the other hand, the act of RNAPII transcription can shape the chromatin landscape [13-15]. Perhaps these hypotheses can be unified by the idea that the interplay between chromatin state and transcription forms a feedback loop. Here, we focused on how histone PTMs and variants serve as coordinates during RNAPII to identify the current position of transcription and to execute appropriate functions. Our review includes a comprehensive assessment of histone PTM ChIP-seq data in wild type *Arabidopsis*

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thaliana (Col-0). We provide comparable metagene profiles that visualize the interplay between histone PTMs and RNAPII transcription stages (Figure 1A). These ChIP-seq data reflect the localization of histone PTMs in the whole plant under normal growth condition, regardless of cell-, tissue- or condition-specific histone patterns [16-18]. This review covers recent advances in understanding how plant gene expression is underpinned by a histone PTM-based Genomic Positioning System (GPS) that guides RNAPII through transcription stages.

Histone PTMs define transcription stages

RNAPII transcribes the DNA sequence of genes into mRNA. Analyses of the genomic distribution of RNAPII reveals different stages of the transcription process, known to form the RNAPII transcription cycle [19] (Figure 1A). Studies of nascent RNAPII transcription in *Arabidopsis thaliana* have informed events linked to the RNAPII transcription cycle in plants [20, 21]. RNAPII initiates transcription from transcription start sites (TSSs) in promoter regions. After initiation, RNAPII enters the gene bodies and elongates nascent RNA chains (early elongation). RNAPII usually stalls near the 5'-end of genes after initiation, a phenomenon known as promoter proximal RNAPII stalling. RNAPII then enters the productive elongation stage to complete nascent RNA production of the full transcript. When RNAPII passes poly-(A) site (PAS) sequences at 3'-end of genes, RNAPII stalls again to assist transcriptional termination.

RNAPII thus performs different functions in transcription stages that are coordinated with different co-transcriptional molecular events (e.g. capping, splicing and poly-adenylation). These considerations raise the question: what molecular system informs RNAPII of the current transcription stage during transcriptional progression? Chromatin Immuno-precipitation followed by next-generation sequencing (ChIP-seq) resolved the genomic distribution profiles of many histone post-translational modifications (PTMs) and variants. Interestingly, the deposition of different histone PTMs or variants is spatially associated with different stages of transcription (Figure 1A). The profile of histone PTMs may thus be connected to the definition of transcription stages that define distinct RNAPII activities.

Histone PTMs and transcription initiation

Transcription initiation controls the recruitment of RNAPII to promoters, and regulates the polymerase flux into the gene bodies. Transcription initiation relies on the assembly of the pre-initiation complex (PIC) including RNAPII and general transcription factors (GTFs) at promoters.

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In yeast, PIC formation is facilitated by highly conserved general transcription factor II D (TFIID) and Spt-Ada-Gcn5 acetyltransferase (SAGA) complex [22]. TFIID and SAGA complexes both contain subunits with histone acetyltransferase (HAT) activity [23]. Consistently, histone acetylation represents a characteristic genomic signature of transcription initiation. In *Arabidopsis*, a pioneering ChIP-chip study established the enrichment of histone acetylation at histone H3 lysine 9 (H3K9ac) and lysine 27 (H3K27ac) near transcription start sites (TSSs) for many genes [24]. Later, an enrichment of additional histone acetylation modifications at histone H3 lysine 14, 18, 23, 36 and 56 (i.e. H3K14ac, H3K18ac, H3K23ac, H3K36ac and H3K56ac) and at histone H4 lysine 5, 8, 12, 16 and 20 (i.e. H4K5ac, H4K8ac, H4K12ac, H4K16ac and H4K20ac) near TSSs was determined by ChIP-seq in *Arabidopsis* [25-27]. In *Arabidopsis*, *HAF1* and *HAF2* (Histone acetyltransferase of the TAFII250 Family 1 and 2), the homologues to metazoan TFIID largest subunit gene *TAF1* (TATA-binding Protein-Associated Factors 1), promote H3K9ac, H3K27ac and H3K4ac in promoter regions [28]. The acetyltransferase activity of SAGA is derived from its HAT module, represented by the GCN5 (General Control Non-repressed Protein 5) subunit (reviewed in [29]). Loss of function of *Arabidopsis AtGCN5* reduces H3K9ac levels at promoter regions and results in gene repression [30-33]. *Arabidopsis* histone acetylation reader BRAT1 (Bromodomain and ATPase domain-containing protein 1) binds to histone H4 acetylation (H4ac) and presumably facilitates transcription initiation by modulating the chromatin environment in the transcriptionally silenced regions [34]. Similarly, *Arabidopsis* SWR1 (SWI2/SNF2-Related 1) complex subunit MBD9 (Methyl CpG-BINDING DOMAIN 9) and NPX1 (Nuclear Protein X1) read histone H3 acetylation (H3ac) and contribute to histone variant H2A.Z deposition which further recruits DNA demethylation machinery to activate transcription [35]. These results collectively underscore the potential roles of histone acetylation in regulating transcription initiation through events facilitating PIC assembly in plants.

Tri-methylation on histone H3 lysine 4 (H3K4me3) characterizes a well-studied chromatin modification associated with transcription initiation (Figure 1A). In *Arabidopsis*, H3K4me3 density across transcription units peaks at 5'-end of genes, and high levels of H3K4me3 are often correlated with gene expression [36, 37]. Intriguingly, *Arabidopsis* H3K4me3 readers EBS (EARLY BOLTING IN SHORT DAY) and SHL (SHORT LIFE) can both read active H3K4me3 and repressive tri-methylation on histone H3 lysine 27 (H3K27me3) [38, 39]; and the H3K27me3 reader PRC1 (Polycomb Repressive Complex 1) is also shown to have H3K4me3 binding property. The dual specificity may facilitate chromatin state switching to control transcription activity [40]. In metazoans, H3K4me3 facilitates the formation of PICs through the interaction with the TFIID subunit TAF3 [41]. In *Arabidopsis*, PIC formation correlates with H3K4me3, yet a direct role in PIC recruitment remains unclear. Deposition of H3K4me3 requires the function of ATX1 (ARABIDOPSIS TRITHORAX 1)/COMPASS-like complex in *Arabidopsis*. While PIC formation is dependent on the ATX1/COMPASS-like complex, it is independent of the H3K4me3 level [42-45]. Interestingly, a catalytically inactive ATX1 mutant that distinguishes the effect of the ATX1/COMPASS-like complex and H3K4me3 reveals defects in RNAPII elongation rather than initiation, arguing for a role of H3K4me3 in transcription elongation instead of initiation [42]. These results support an indirect molecular connection between transcription initiation and H3K4me3 in plants. Perhaps this connection depends on the genomic context, since the SWI/SNF (Switch/Sucrose Non-Fermentable) chromatin remodeler complex controls the activation and repression of sense gene transcription and anti-sense non-coding transcription through PIC formation correlating with H3K4me3 levels at both gene ends [46]. In summary, H3K4me3 may promote PIC formation and RNAPII initiation in plants, yet the precise molecular mechanisms remain to be elucidated.

Histone PTMs and early transcriptional elongation

We sub-divide transcription elongation by RNAPII into early elongation and productive elongation [47]. Promoters coincide with nucleosome-depleted region (NDR) with resulting low levels of histone PTMs. In contrast, the first (i.e. +1) nucleosomes fall within the early elongation zone during RNAPII transcription. These nucleosomes dominate the genomic distribution of histone PTMs. In metazoans, early elongation refers the stage of RNAPII between transcription initiation and productive elongation linked to well-defined RNAPII promoter proximal pausing sites regulated by pausing factors such as negative elongation factor NELF (Negative Elongation Factor) [48]. Even though NELF is conspicuously absent in plants, RNAPII tends to stall at the position of the first nucleosome in gene bodies [20]. In addition, the distribution of RNAPII in plant promoter proximal regions is wider compared to metazoans, perhaps indicating an extension of the RNAPII early elongation stage in plants compared to metazoans. Di-methylation on histone H3 lysine 4 (H3K4me2) and tri-methylation on histone H3 lysine 36 (H3K36me3) peak slightly downstream of histone PTMs for transcription initiation [37, 49-52], thus could be associated with RNAPII early elongation and RNAPII stalling (Figure 1A)[20]. However, the mechanistic connections between chromatin during early RNAPII elongation and RNAPII stalling are yet to be firmly established.

It is plausible to imagine a cross talk between transcription initiation and productive elongation that occurs during early elongation to facilitate progression further into the gene. In *Arabidopsis*, increased H3K4me2 levels by mutations in the H3K4me2/me3 demethylase FLD (FLOWERING LOCUS D) are associated with elevated H3K4me3 and H3ac levels near the 5'-end of the genes, and increased H3K36me3 level over the gene bodies [15]. In addition, reduced H3K4me2/me3 levels are associated with decreased levels of H3ac, H3K36me3 and a drop of RNAPII occupancy near promoters in *Arabidopsis* [53, 54]. Moreover, repression of plant transposable elements (TE) requires coordinated modulation of histone acetylation (i.e. H3ac and H4ac) and histone methylation (e.g. H3K4me2 and H3K4me3) [55]. However, H3K4me2 in rice and *Arabidopsis* may exhibit negative correlations with transcription activity [56]. This phenomenon could be attributed to the dynamic removal of H3K4me3 that may recruit H3K4me2 readers to facilitate repression. Interestingly, characterizations of histone PTMs during circadian oscillations revealed a sequential enrichment of H3ac, H3K4me3 and H3K4me2 [57]. These data may reflect an orchestrated progression through RNAPII transcription stages from initiation to early elongation. In conclusion, H3K4me2 during early RNAPII elongation might represent a molecular hub that coordinates the transition from transcription initiation to elongation through the interaction with histone acetylation.

During early transcription elongation, H3K36me3 often correlates with H3K4me2 at positions just downstream of H3K4me3 (Figure 1). Roles of H3K4me3 and H3K36me3 in transcription initiation and elongation characterize these histone PTMs as excellent predictors for gene expression in plants [58]. In *Arabidopsis*, H3K36me3 acts in concert with other histone PTMs for active transcription (e.g. H3K4me3 and histone acetylation) to promote gene expression [59-62]. Moreover, H3K36me3 is highly enriched at temperature-regulated alternatively spliced genes, and a reduction of H3K36me3 affects alternative splicing outcomes in *Arabidopsis* [63]. Likewise, retained introns in the *Arabidopsis* spliceosome mutant *brra2* often exhibit low H3K36me3 profiles [64]. These data link chromatin features during RNAPII transcription to pre-mRNA processing. *Arabidopsis* MRG (MORF Related Gene) proteins read H3K36me3 as well as H3K4me3 and mediate transcription activation by directing H4ac deposition near 5'-end of target genes [65, 66]. The genomic distributions of H3K36ac and H3K36me3 overlap downstream of TSSs, albeit with antagonizing effects even though both are associated with active transcription [25]. Combinatorial effects on gene expression of histone PTMs of the same residue as suggested for H3K36 may increase the resolution to differentiate stages of RNAPII transcription. In summary, the H3K36me3 peak during early RNAPII elongation is linked to chromatin features ahead of the peak, and to pre-mRNA processing after the peak, supporting a role in bridging RNAPII initiation and elongation.

Histone PTMs/variants and productive transcriptional elongation

Eukaryotic transcription elongation, processivity and co-transcriptional histone PTMs are regulated by transcription elongation factors such as pTEF-b (positive transcription elongation factor b), PAF1-C (polymerase-associated factor 1 complex) and SPT4/5 (suppressor of Ty 4/5) [67-71]. In *Arabidopsis*, both PAF1-C and pTEF-b are part of RNAPII elongation complex [72]. The *Arabidopsis* pTEF-b subunit CDKC;2 regulates the global level elongating RNAPII (RNAPII-Ser2 Phosphorylation) transcription [73]. In addition, *Arabidopsis* SPT5 can be phosphorylated by CDKC;2, interact with PAF1C subunit VIP5 (VERNALIZATION INDEPENDENCE 5) and further influence H3K4me3 deposition on target loci [74]. During the productive transcriptional elongation stage, RNAPII translocates along the DNA template to synthesize a growing nascent RNA chain. In eukaryotes, the activity of elongating RNAPII is modulated by various elongation factors, including histone modifiers and splicing regulators [75]. RNAPII encounters few nucleosome barriers during transcription initiation and early elongation, while many nucleosomes need to be navigated during the productive elongation stage. The chromatin landscape shaped by histone PTMs on these intragenic nucleosomes thus provides the opportunity to regulate RNAPII elongation. In plants, a variety of histone PTMs localize to this stage and display nuanced distribution patterns. Histone PTMs that peaked at early elongation stage (i.e. H3K4me2 and H3K36me3) decline gradually towards 3'-end of genes. Mono-ubiquitination of histone H2B (H2Bub) and mono-methylation on histone H3 lysine 4 (H3K4me1) prominently cover most of the gene body without a clear peak. Di-methylation on histone H3 lysine 36 (H3K36me2) is gradually enriched towards the 3'-end of genes where it peaks, representing a histone PTM characterizing late stages of productive transcriptional elongation in plants (Figure 1A).

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In *Arabidopsis*, H2Bub is deposited by E3 ubiquitin ligases for example HUB1 and HUB2 (HISTONE MONO-UBIQUITINATION 1 AND 2) [76]. Chromatin profiling determined a H2Bub profile covering gene bodies [52, 77]. In *Arabidopsis*, HUB1 genetically interacts with transcription elongation factor ELONGATOR complex and the FACT (Facilitates Chromatin Transcription) complex with synergistic effects on plant development [77, 78]. In addition, HUB2-mediated H2Bub functions with histone methyl-transferase SDG8 (SET DOMAIN GROUP 8)-mediated H3K36me3 to reinforce transcription activity at selected loci [79]. Furthermore, H2Bub is associated with rapid gene induction during environmental changes [80]. In rice, defects in H2Bub are associated with reduced global H3K4me2, suggesting a potential role of H2Bub promoting other histone elongation PTMs [81]. H2Bub and H3K4me3 both correlate with active transcription. In yeast and human cells, the deposition of H3K4me3 is mediated by H2Bub, suggesting a crosstalk between histone PTMs associated with elongation to this controlling transcription initiation [82, 83]. However, an equivalent mechanistic crosstalk awaits discovery in plants. In *Arabidopsis*, a reduction of H3K4me3 level has been observed at target genes in H2Bub defective mutants, but not globally [77, 84, 85]. Recent advances suggested that the establishment of H3K4me3 is largely independent of H2Bub [80, 86], arguing against the histone crosstalk model in plants. H2Bub is removed by the histone deubiquitination module (DUBm), which is part of the SAGA complex in yeast, but may be uncoupled from SAGA in plants [49, 87]. Interestingly, plant DUBm co-purifies with RNAPII subunits, mediator, histone chaperons and RNA processing factors, while HUB1 also co-purifies with transcription elongation factors. These data suggest a strong association of H2Bub biology and productive transcriptional elongation in plants [72, 87].

H3K4me1 shows a similar distribution profile over gene bodies to H2Bub, but with a trend to increase towards 3'-gene ends (Figure 1A). Interestingly, H3K4me1 may negatively correlate with initiation, potentially due to the dynamic conversion to the higher-order methylation states H3K4me2/me3 [37]. In gene bodies, H3K4me1 is enriched at cryptic intragenic TSSs that are repressed by the activity of the histone chaperone FACT complex in *Arabidopsis* [88]. The repressive effect of H3K4me1 on intragenic initiation appears to be distinct from H3K36 methylation, although SDG8, a H3K36 methyltransferase, has been proposed to read H3K4me1 as well as deposits H3K36me2/me3 [89]. In metazoans, H3K4me1 is classically associated with enhancers, whereas in plants, H3K4me1 is largely associated with RNAPII elongation and antagonizes the repressing effect of di-methylation on histone H3 lysine 9 (H3K9me2) [90]. Intriguingly, the individual methylation states of H3K4 are associated with transcriptional initiation (H3K4me3), early elongation (H3K4me2) and productive elongation (H3K4me1) in *Arabidopsis*. It is tempting to speculate that the dynamics of H3K4 methylation-state conversion could guide the progression of plant RNAPII transcription.

H3K36me2 represents an additional key histone PTM for productive transcriptional elongation [88]. In *Arabidopsis*, the distribution of H3K36me2 spreads over gene bodies and peaks towards the 3’-end of genes [25, 91]. The distribution of H3K36me2 shifts further towards 3’-ends of genes in RNAPII elongation factor mutants [92]. Although direct evidence for a role of H3K36me2 in promoting RNAPII elongation is lacking, there is evidence to implicate H3K36 methylation in alternative splicing. In rice, the distributions of H3K36me2 or H3K36me3 correlate with differences in intron retention [93]. In addition, H3K36me2 showed a possible interaction with mRNA m^{6}A modification in *Arabidopsis* [94]. Collectively, these studies support the co-transcriptional roles of H3K36me2 in modulating transcriptional elongation and RNA processing events such as splicing and RNA modification in *Arabidopsis*.

The histone variant H2A.Z is linked to transcriptional regulation in plants. On the one hand, H2A.Z is enriched near TSSs and anti-correlates with repressive DNA methylation [95]. On the other hand, H2A.Z is also enriched over gene bodies of lowly expressed genes [96]. H2A.Z may have the ability to activate or to repress transcription, depending on its genomic deposition. Curiously, H2A.Z can also regulate transcription by balancing the gene accessibility of +1 and -1 nucleosomes in *Arabidopsis* [97]. H2A and H2A.Z carry PTMs, for example ubiquitination. H2Aub co-localizes with repressive chromatin marks (e.g. H3K27me3), showing a profile peaking towards 5’-end of repressed genes [98] (Figure 1). In *Arabidopsis*, Polycomb Repressive Complex 1 (PRC1) and PRC2 mediates gene repression. Although the sequence of PRC1 and PRC2 recruitment during gene repression is actively debated, H2A.Zub deposition by PRC1 shows a strong correlation with PRC2-independent gene repression genome-wide in *Arabidopsis* [99, 100]. Interestingly, the *Arabidopsis* histone H3 reader YAF9 (YEAST ALL1-FUSED GENE FROM CHROMOSOME 9) proteins mediate the acetylation of H2A.Z and H4 at target loci potentially through the interaction with histone acetyltransferase HAM1 in *Arabidopsis* (HISTONE ACETYLTRANSFERASE OF THE MYST FAMILY 1) [101]. Collectively, the diversity of PTMs on H2A.Z may help to explain the its dual roles in transcription regulation suggested for bulk H2A.Z. In conclusion, chromatin-based signatures linked to RNAPII elongation interact with pre-mRNA processing and transcriptional initiation, highlighting important aspects of plant gene expression.

Genomic information for transcriptional termination

The final stage of the transcription cycle represents transcriptional termination. RNAPII transcribes through the poly (A) sites (PAS) at 3'-end of genes. Here, RNAPII decelerates and stalls downstream of PASs, presumably to facilitate nascent RNA cleavage and poly-adenylation by CF/CPFs (Cleavage/Cleavage and Polyadenylation Factors) [102-104]. RNAPII elongates beyond PASs until the process of transcriptional termination releases RNAPII from the DNA template. RNAPII dissociation from the DNA template is presumably triggered when 5'-3' exonucleases acting on the non-capped 5'-end reach RNAPII [20, 21, 105].

In plants, H3K36me2 marks late transcriptional elongation and peaks upstream of PASs. This profile may indicate a role for H3K36me2 in transcriptional termination. ChIP-seq analyses of the *Arabidopsis* histone H3 variant H3.3 revealed a positive correlation with transcription level [106-108]. Moreover, H3.3 density is low in gene bodies and enriched at gene boundaries, in promoter regions and close to PASs in *Arabidopsis*. The spatial correlation between PASs and H3.3 levels would be consistent with a role of H3.3 in transcriptional termination. Interestingly, the histone H3 variant H3.1, which differs from H3.3 by only 4 amino acid residues, preferentially marks heterochromatin represented by H3K9me2 and H3K27me3 [109, 110]. In conclusion, meta-genomic associations spatially connect chromatin marks to transcriptional termination, but experimental evidence testing these correlations are currently missing.

Transcriptional termination involves RNAPII stalling near PAS of genes [20, 21]. In mammals, PAS-associated RNAPII pausing can be achieved through local heterochromatin formation marked by H3K9me2 and the formation of R-loops [111]. R-loops are chromatin structures formed by DNA:RNA hybrids, which often correlate with RNAPII pausing during transcriptional termination in mammals [112]. Interestingly, another non-canonical DNA secondary structure G-quadruplex (G4) may interact with R-loops and facilitate transcriptional termination in human cells [113, 114]. However, in plants, genome-wide mapping of R-loops revealed a strong correlation with repressive histone PTMs (e.g. H3K9me2), whereas R-loop levels are low in

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transcriptional termination regions, suggesting distinct regulatory mechanisms of R-loops in transcriptional termination in plants [115]. Curiously, *Arabidopsis* anti-sense R-loops and maize anti-sense G4s localize to 5'-UTR of genes [115, 116], perhaps indicating a role in transcriptional initiation in sense direction or transcriptional termination of anti-sense RNAPII transcription in plants. *Arabidopsis* BORDER proteins contribute to transcriptional termination, however the links to chromatin states remain elusive [117]. In summary, while transcription initiation and termination share key genomics features of RNAPII transcription, such as NDRs and RNAPII-stalling, our understanding of chromatin-based mechanisms affecting initiation greatly exceed those connected to termination. Whether this represents an opportunity for discovery, or whether RNAPII termination may rely less on chromatin-based signals awaits to be resolved in future studies.

Mis-specification of the PTM-based GPS.

Genome-wide analyses of histone PTMs reveal characteristic patterns indicative of histone-based regulatory mechanisms in plants. The integration of these epigenomic data yielded a database of histone states during gene expression for *Arabidopsis*, rice and maize [118]. The access for scientists to genome-wide experiments interrogating chromatin structures is improving through such databases (Box 1). The reproducible patterns of histone modifications over transcription units in various tissues raises the question of how they assist RNAPII transcription and gene expression [32]. Moreover, it is possible to identity genes deviating from this consensus pattern, perhaps highlighting gene regulation through mis-specification of the PTM GPS.

Chromatin-based effects during transcription elongation ensure the fidelity of gene expression [52, 72, 119]. In *Arabidopsis*, the H3K4me1, and H3K36me2 PTMs associate with productive elongation of transcription [52]. These two features of transcription elongation were recently associated with chromatin-based “repressive transcription” [47]. The *qua1-1* T-DNA allele represents an insertion upstream of the *QUASIMODO1* (*QUA1*) promoter. In this allele, RNAPII elongation over the *QUA1* gene promoter results in a recessive loss-of-function phenotype. Transcription units within the T-DNA extend together with elongation PTMs into the genome and interferes with the consensus PTM profile at the downstream *QUA1* promoter. The *QUA1* promoter DNA sequence displays elevated elongation (H3K36me2) signatures, and consistently

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reduced initiation and early elongation-associated histone PTMs H3K4me3 and H3K36me3. The underlying molecular mechanism is consistent with “transcriptional interference”, where the act of RNAPII transcription changes the chromatin state at gene promoters to repress functional transcriptional initiation (Figure 1B) [120]. In *Arabidopsis*, Genome-wide data support the idea that H3K36me2, when localized to promoters, correlates with negative gene expression. Perhaps through a similar mechanism to transcription repression associated with enrichment of H3K4me1 at human promoters [121, 122]. It will be instrumental to clarify the consequences and mechanisms that trigger mis-enrichment of elongation marks such as H3K36me2 and H3K4me1 at TSSs. The FACT complex, a known facilitator of chromatin-based transcriptional elongation [123], is required for transcriptional interference in *qua1-1,* as the mutant phenotype was partially restored in mutants of the FACT components *SSRP1* and *SPT16*. Thus, in *Arabidopsis*, FACT can both facilitate RNAPII elongation by recycling nucleosomes and repress transcription initiation associated with H3K4 methylation dynamics [88]. More generally, FACT restricts the usage of TSSs located in intragenic positions in yeast, human and plants [88, 124-126]. In plants, the intragenic TSSs repressed by FACT displayed elevated H3K4me1, but the role of H3K4me1 and its connection to the FACT complex in transcriptional repression in plants awaits clarification. It appears that H3K4me1 role in transcription elongation is of interest to understand transcription elongation in plants. The H3K4 specific methyltransferase FAD family of FLD; LDL1,LDL2 and LDL3 (Table 1) appear to be important for erasing H3K4 methylation at highly regulated genes involved in flowering time and root elongation [127]. LDL2, one of the FAD family H3K4 methyltransferase is important for the maintenance of transcription-associated H3K4me1 in gene bodies and prevents deposition of the silencing H3K9me2 modification [90]. Interestingly, the LDL1 histone demethylase forms a complex with the deacetylation factor HDA6 (Table1) to repress transcription [128]. Characterizations of the chromatin-based gene repression through the act of RNAPII transcription in the *qua1-1* mutant provided important first insights into the functional significance of histone PTM mis-specification of across gene bodies. The identification and characterization of additional examples where shifted histone GPS signals mediate transcriptional interference represent an exciting future research area.

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Functional implications of plant-specific co-transcriptional chromatin profiles.

Plants and metazoans display an overall similar profile of histone PTMs across transcription units. However, some histone PTMs adopt deviant roles in the plant kingdom. In budding yeast, drosophila and human cells, H3K36me3 localizes to the 3'-end of gene bodies of expressed genes, and H3K36me2 to the 5' region [7, 129-132]. The marks are deposited at chromatin during RNAPII transcription. In yeast, interactions of the RNAPII complex, histone chaperones, such as Spt6p FACT, and subunits of the PAF-I complex can maintain the characteristic pattern of H3K36me3/me2 during transcription. Depletion of the PAF-I complex shifted H3K36me3 towards the 5'-end of yeast genes [133]. In contrast, H3K36me3 localizes to the early transcriptional elongation zone in rice and *Arabidopsis,* while H3K36me2 peaks at the 3'-end of genes [25, 134-136]. These H3K36me2/me3 profiles thus characterize an intriguing difference between plants and metazoans, with implications for the RNAPII GPS, whereby plants progressively erase methyl groups from H3K36me3 to H3K36me1 during RNAPII transcription elongation (Box 2). The functional implications are presently unclear, yet it may indicate evolutionary adjustments of the chromatin-based GPS for RNAPII during the plant lineage. In mammals, H3K36m3 represses cryptic initiation through recruitment of histone deacetylases during elongation, whereas the repression of intragenic TSSs in *Arabidopsis* is linked to H3K4me1 [137]. In *Arabidopsis*, H3K36 methylation may repress natural antisense transcripts (NATs) at a subset of transcriptionally active genes [50]. In human, H3K36me3 mis-localization to gene promoters during RNAPII elongation of non-coding antisense transcription represses transcription initiation of the sense mRNA [138]. Finally, the role of the H3K36 methyltransferase SDG8 in splicing and capping of pre-mRNAs appears shared with the budding yeast homolog Set2p [139, 140]. However, several other transcription-associated chromatin PTMs appear under-studied in plants, for instance H4K20me3 is widely studied in animals and represents a heterochromatin-associated histone modification, whereas it would associated with transcription activation in *Arabidopsis*. [52, 141] It remains unclear which factors are involved in deposition, reading and erasing of the modification. Extending analyses to poorly explored PTMs in plants could improve our understanding of how the specifications of the chromatin-based RNAPII GPS in plants offer advantages, perhaps linked to a sessile life cycle.

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Histone-based GPS service guides plant development and environmental responses

The chromatin states are dynamically regulated by the trios of histone "reader-writer-eraser" enzymes. Mutants defective in a particular histone modifying enzyme often profoundly affect gene expression and are associated with defects in plant growth, development and environmental response. We summarize the recent advances in understanding how histone PTM enzymes regulate biological processes in plants (Table 1 and Figure 2).

In plants, H2Aub is deposited by the "writer" enzyme ubiquitin E3 ligase (e.g. RING1A/RING1B and BMI1A/BMI1B) [142-145] and removed by "eraser" deubiquitinases (DUBs) (e.g. UBP12/UBP13) [146]. Writing and erasing H2Bub requires equivalent enzymatic activities. The E3 ubiquitin ligases HUB1/HUB2 and E2 ubiquitin-conjugating (UBC) enzymes (UBC1/UBC2) are involved in H2Bub deposition on target genes that control plant development and environmental responses [77, 81, 84, 147-149]. In plants, removal of H2Bub requires DUB activity for example from the DUB module (DUBm) of SAGA complex [49, 87] or other histone deubiquitinases [150, 151].

In *Arabidopsis*, methylation on histone lysine residues (i.e. H3K4 and H3K36) are added by the "writer" enzymes, SDG (SET Domain Group) proteins [152]. The histone methyltransferases (HMTs) SDG4 [153, 154], SDG8 [155, 156], SDG25 [157-162] and SDG26 [156, 159] can methylate both H3K4 and H3K36. SDG2 [57, 158, 163-165], SDG27 [42, 166, 167] and SDG30 [167] are specific writers for H3K4 methylation. SDG14, SDG16 and SDG19 represent additional putative HMTs for H3K4 methylation [168]. *Arabidopsis* histone lysine methylation is largely erased by enzymes harboring JmjC domains [169]. For example, JMJ14 [164, 170, 171], JMJ15 [172, 173], JMJ16 [174], JMJ17 [175] and JMJ18 [176] are responsible for removing H3K4 methylation states, while JMJ30 is involved in H3K36 demethylation [177, 178]. Additionally, H3K4 methylation can also be removed by cofactor FAD-dependent lysine-specific demethylases including FLD [179, 180] and LDL1/2/3 (LYSINE-SPECIFIC DEMETHYLASE 1-LIKE HISTONE DEMETHYLASES 1/2/3) [127, 128, 181, 182].

The dynamics of histone acetylation is maintained by histone acetyltransferases (HATs) and histone deacetylases (HDACs). *Arabidopsis* HATs classify into four families: the GNAT (GCN5-

RELATED ACETYL TRANSFERASE) family, the MYST (MOZ-YBF2/SAS3-SAS2/TIP60) family, the CREB-binding protein (CBP) family and the TAFII250 family [183, 184]. The GNAT family includes H3K14ac-spcific HAT GCN5/HAG1 [30, 33, 185-187], H4K12ac-specific HAT HAG2 (HISTONE ACETYLTRANSFERASE OF THE GNAT FAMILY 2) [188] and HAG3 [189-192]. *Arabidopsis* HAM1 and HAM2 (HISTONE ACETYLTRANSFERASE OF THE MYST FAMILY 1 and 2) of the MYST family catalyze H4K5ac [188, 193]. The CPB family HATs, for example HAC (HISTONE ACETYLTRANSFERASE OF THE CBP FAMILY) 1, 2, 4, 5 and 12 are involved in general histone H3 and H4 acetylation [194-198]. The TAFII250 family includes HAF1 (HISTONE ACETYLTRANSFERASE OF THE TAFII250 FAMILY 1) and HAF2, which are the homologues to TAF1, the largest subunit of transcription initiation factor TFIID in metazoans [195, 199-201]. *Arabidopsis* HDACs are largely from Reduced Potassium Dependency 3 (RPD3)/Histone Deacetylase 1 (HDA1) family, that can be further divided into Class I and Class II [184]. The RPD3/HDA1 family Class I HATs include HDA6 [128, 202-205], HDA7 [206], HDA9 [207-211] and HDA19 [204, 212-217]. Class II contains HDA5 [218], HDA14 [219], HDA15 [212, 220-223] and HDA18 [200, 224]. Interestingly, Class I and Class II enzymes can have opposite roles in regulating particular plant biological functions [225, 226], suggesting antagonistic regulation by the same type of histone modifying enzymes. Another HDAC family in plant is the Silent Information Regulator 2 (SIR2) family including SRT1 and SRT2 (SIRTUIN 1 and 2) [227-229]. Additionally, the Histone Deacetylase 2 (HD2) family contains the plant-specific histone deacetylases HD2A, HD2B, HD2C and HD2D, which are also involved in various plant development processes [230-232] and environmental response [233-235].

Histone readers also contribute to the regulatory functions of histone PTMs. For example, *Arabidopsis* H3K4me3 reader EBS and SHL can read both H3K4me3 and H3K27me3 [38, 39]. The H3K36me3 reader MRG1 and MRG2 (MORF RELATED GENE 1 and 2) read H3K36me3 and interact with histone acetyltransferases (HATs) for the deposition of histone H4 acetylation (H4ac) [65, 66]. Histone acetylation readers MBD9 and NPX1 mediate the deposition of histone variant H2A.Z and further lead to DNA demethylation to activate gene transcription [35]. Thus, histone readers and effectors not only recognize particular histone PTMs, but also mediate the downstream regulation and contribute to the crosstalk between different histone PTMs. Trios of “reader-writer-eraser” enzymes collectively regulate gene expression through the dynamics of histone PTMs/variants. In general, loss-of-function mutant of a particular “writer”, “eraser” or

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"reader" enzyme will directly or indirectly affect the local or global histone PTM/variant levels, which may further impairs the RNAPII functions associated with transcription stages, reflected by changes of RNAPII occupancy or expression level of target genes. Even though the chromatin state profiles in many of the mutants listed in Table 1 is incomplete, it may be interesting to interpret the resulting phenotypic defects through the GPS model presented in this review. This view may reveal defects resulting in the mis-specification of transcription stages and associated defects in pre-mRNA processing. Future progress in this area would help to appreciate the biological significance of diverse spatially resolved effects chromatin modifications may have on gene isoform expression.

Conclusion Remarks and Future Directions

The roles of histone PTMs and associated factors in plant RNAPII transcription cycles remain largely uncharacterized. In this review, we summarized the current information on how histone PTMs appear to serve as coordinates to guide RNAPII transcription. Thus, histone PTMs can regulate progression through plant RNAPII transcription cycles. The act of transcription activity deposits histone PTMs throughout the cycle. It remains unclear for most histone modification and histone variants whether they affect transcription, are the result of transcription, or form a positive feedback loop. The number of possible combinations of histone PTMs and variants may build a complex chromatin-based regulation system for RNAPII transcription. Considering that some histone PTMs differ in their basic genomic distribution and functions from metazoans to plants, the epigenetic regulation of RNAPII transcription is largely unclear. The histone-based GPS associates the common histone PTM profiles at most expressed genes and aids the discovery of potentially regulatory "repressive transcription" events through dynamic mis-specification of histone PTMs. The combination of histone ChIP-seq experiments and emerging transcriptomics methods promises to stimulate future research to understand chromatin-based effects exerted through the act of RNAPII transcription in plant genomes (see Outstanding Questions).

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FIGURE LEGENDS

Figure 1 Key Figure. Histone-based genomic positioning system (GPS) for RNAPII transcription.

(A). (Top) A combined metagene plot illustrating the profiles of histone post-translational modifications (PTMs) during RNAPII transcription of *Arabidopsis thaliana* genes. The metagene plots are derived from reanalysis of published ChIP-seq data (Summarized in Table S1).The Y-axis represents the normalized ChIP-seq signal of histone PTMs. The raw values to plot these data are provided in (Table S2). The X-axis indicates the relative position across a gene (grey), from transcription start site (TSS) to polyadenylation site (PAS) with flanking regions. Colored curves represent the genomic distributions of indicated histone PTMs. mono-, di- and tri-methylation (me1/me2/me3) at histone H3 residues lysine 4 (K4) and lysine 36 (K36); ubiquitination of H2A and H2B (H2Aub and H2Bub); grouped profile of all lysine acetylation modifications on H3 and H4 (H3ac/H4ac). (Bottom) Schematic illustration of plant transcription cycle by RNAPII includes transcriptional initiation (light red), early elongation (light green), productive elongation (light blue) and termination (light purple) across a gene. PIC: preinitiation complex; GTFs: general transcription factors; P-TEFb: positive transcription elongation factor b; PAF1-C: polymerase-associated factor 1 complex; FACT: facilitates chromatin transcription; SPT4/5: suppressor of Ty 4/5; CFs/CPFs: cleavage factors/cleavage and poly-adenylation factors; XRNs: exoribonuclease [72, 102-104]. All histone PTMs are positively correlated with RNAPII transcription, yet their localization to different positions has functional implications for RNAPII.

(B). Mis-specification of histone-PTM-based GPS indicates potential genomic transcription conflicts. In plant genome, most of transcription events are restrained within their own genomic territories. In the case of tandem transcription interference (TI), where upstream transcription represses the transcription initiation from downstream promoter through the function of FACT complex, thus setting transcription elongation associated histone PTMs in transcription initiation region.

Figure 2 Histone-based GPS guides gene expression in various biological process in plants.

A histone octamer consists of 2 copies of histone H2A (green cycles), H2B (yellow circles), H3 (blue circles) and H4 (purple circles), wrapped by DNA (black curve). Lysine residues (grey circles, numbers denote the residue positions from C-terminals) on histone tails are subject to intensive post-translational modifications (PTMs), for example ubiquitination (ub, green rectangles), methylation (me, blue rectangles) and acetylation (ac, red rectangles). Histone residues can be mono-, di- and tri-methylated (double and triple blue rectangles). Histone PTMs regulate gene expression that control various biological processes in plants, including plant growth, germination, flowering, defense, environmental responses and circadian rhythm.

Figure Box 1

In chromatin immunoprecipitation (ChIP, left), plant tissues are first cross-linked to stabilize the histone-DNA interaction. Then, the cross-linked chromatin is fragmented by sonication or MNase digestion. Specific antibodies are used to target certain histone PTM or variants (red triangle) and for further precipitation. DNA that binds to histones is released by reverse cross-linking. Purified DNA can be used in microarray (ChIP-chip) or library construction and followed by next-generation sequencing (NGS, ChIP-seq). When using CUT&RUN technology (right), antibodies control the digestion *in situ* by linking to protein A-MNase fusion. Chromatin fragments generated after MNase digestion can be used for further DNA purification. Purified DNA can be used for library construction and followed by next-generation sequencing (NGS, CUT&RUN-seq). CUT&tag represents an updated version of CUT&RUN. Instead of using protein A-MNase fusion, CUT&tag uses protein A-Transposase Tn5 fusion which mediates the tagmentation of NGS sequencing adapters to the targeted loci. Purified DNA can be PCR-amplified and directly used in NGS sequencing.

TABLE

Table 1 Summary of Histone PTM readers/writers/erasers and related biological functions in *Arabidopsis*.

Histone PTMs	**Role**	**Enzyme (Gene locus)**	**Functions**
H2Aub	Writer	RING1A (AT5G44280) RING1B (AT1G03770)	Part of PRC1, double mutant exhibits globally reduced H2AUb, curly leaves and late flowering [144, 145].
		BMI1A (AT2G30580) BMI1B (AT1G06770)	Part of PRC1, mutants regulates and drought responses Part of PRC1, mutants exhibits globally reduced H2AUb, regulates cotyledon and root development [143] and drought responses [142].
	Eraser	UBP12 (AT5G06600) UBP13 (AT3G11910)	Interact with polycomb protein LHP1, prevent autonomous endosperm development during seed development [146].
H2Bub	Reader/ Effector	DET1 (AT4G10180)	Mutant exhibits reduced bulk H2Aub, DET1 regulates the light-dependent degradation of DUBm [49], represses light-induced seed germination [236] and light induced photomorphogenesis [237].
	Writer	HUB1 (AT2G44950) HUB2 (AT1G55250)	Mutants exhibit reduced seed dormancy [147], early flowering Mutants exhibit reduced seed dormancy [147], early flowering [84], changes in plant defense [149] and circadian clock gene expression , changes in plant defense [149] and circadian clock gene expression [77].
	Eraser	SGF11 (AT5G58575)	Part of DUBm of *Arabidopsis* SAGA-like complex [87].
		UBP22 (AT5G10790)	Part of DUBm of *Arabidopsis* SAGA-like complex, function as a major H2Bub deubiquitinase [49, 87].
		ENY2 (AT3G27100)	Part of DUBm of *Arabidopsis* SAGA-like complex [87].
		UBP26 (AT3G49600)	Mutant exhibits early flowing phenotype and high rate of seed abortion [150].
		OTLD1 (AT2G27350)	Erases H2Bub, work with KDM1C to repress gene expression [151].
H3K4me1	Reader/	EBS (AT4G22140)	Reads H3K4me3 and H3K27me3, mutant exhibits early flowering phenotype [38].

H3K4me2 H3K4me3	Effector	SHL (AT4G39100)	Reads H3K4me3 and H3K27me3, mutant exhibits early flowering phenotype [39].
	Writer	SDG2/ATXR3 (AT4G15180)	Writes H3K4me3, mutant exhibits global development defects, early flowering phenotype [158, 163], changes in circadian clock gene expression [57, 164] and defects in gametogenesis [165].
		SDG8/ASHH2 (AT1G77300)	Writes H3K4me3 (can also write H3K36 methylation, listed below), mutant exhibits growth defects [155] and early flowering phenotype [156].
		SDG25/ATXR7 (AT5G42400)	Writes H3K4me1/me2/me3, mutant exhibits early flowering phenotype [157-159], reduced seed dormancy [160] and changes in plant defense [161, 162].
		SDG26/ASHH1 (AT1G76710)	Writes H3K4me3, mutant exhibits late flowering phenotype [156, 159].
		SDG4/ASHR3 (AT4G30860)	Writes H3K4me2/me3 (can also write H3K36 methylation, listed below), mutant exhibits reproductive defects in ovules [153] and unsynchronized DNA replication and cell division, further leading to defects in root development [154].
		SDG27/ATX1 (AT2G31650)	Writes H3K4me3 [42], mutant exhibits defects in root developments [166] and early botting [167].
		SDG30/ATX2 (AT1G05830)	Writes H3K4me2, mutant displays no clear phenotypes [167],
		SDG14/ATX3 (AT3G61740) SDG16/ATX4 (AT4G27910) SDG29/ATX5 (AT5G53430)	Write H3K4me2/me3, triple mutants display drastic defects in seed development and plant growth [168]. *atx4/5* double mutant showed drought tolerance in seed development [168].
	Eraser	FLD (AT3G10390)	Erases H3K4me2, mutant exhibits late flowering [238] and defects in plant systemic acquired resistance [179, 180].
		LDL1 (AT1G62830) LDL2 (AT3G13682) LDL3 (AT4G16310)	Erase H3K4me2/me3, mutants exhibit late flowering phenotype [127, 181], increased seed dormancy [182] and changes in circadian clock gene expression [128].
		JMJ14 (AT4G20400)	Erases H3K4me1/me2/me3, mutant exhibits early flowering phenotype [170], changes in RNA-directed DNA methylation [171] and circadian clock gene expression [164].

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		JMJ15 (AT2G34880)	Erases H3K4me3, overexpression leads to early flowering [172] and increased salt tolerance [173].
		JMJ16 (AT1G08620)	Erases H3K4me1/me2/me3, mutants display leaf senescence phenotype [174].
		JMJ17 (AT1G08620)	Erases H3K4me1/me2/me3, mutants exhibit enhanced resistance to dehydration [175].
		JMJ18 (AT1G30810)	Erases H3K4me2/me3, mutant shows late flowering phenotype while overexpression leads to early flowering phenotype [176].
H3K36me1 H3K36me2 H3K36me3	Reader/ Effector	MRG1 (AT4G37280) MRG2 (AT1G02740)	Bind H3K36me3 and interact with HATs to mediate H4 acetylation. Double mutant is late flowering under long day condition [65, 66].
	Writer	SDG4/ASHR3 (AT4G30860)	Writes H3K36me1/me2, mutant displays unsynchronized DNA replication and cell division, further leading to defects in root development [154].
		SDG8/ASHH2 (AT1G77300)	Writes H3K36me2/me3 [239].
		SDG25/ATXR7 (AT5G42400)	Writes H3K36me2 [240].
		SDG26/ASHH1 (AT1G76710)	Writes H3K36me3 [159].
	Eraser	JMJ30 (AT3G20810)	Erases H3K36me2/me3, regulates circadian clock related flowering gene expression [177], root development and response to auxin [178].
H3ac	Reader/ Effector	YAF9A (AT5G45600) YAF9B (AT2G18000)	Read acetylated and unmodified H3, regulates H4 and H2A.Z acetylation. Mutant shows late flowering phenotype [101].
		MBD9 (AT3G01460)	Binds to acetylated histone H3 and H4, mutants exhibit enhanced shoot branching and early flowering phenotype [241] . MBD9 also regulates SWR1 complex mediated H2A.Z deposition and DNA demethylation Binds to acetylated histone H3 and H4, mutants exhibit enhanced shoot branching and early flowering phenotype [241]. MBD9 also regulates SWR1 complex mediated H2A.Z deposition and DNA demethylation [35, 242].
		NPX1 (AT5G63320)	Binds acetylated histone H3 and regulates SWR1 complex mediated H2A.Z deposition and DNA demethylation [35].

	Writer	GCN5/HAG1 (AT3G54610)	Writes H3K14ac, globally associated with growth and developmental defects [33, 185, 186]. Regulates plant heat tolerance [30], plant regeneration [187]
H4ac	Reader/ Effector	BRAT1 (AT1G05910)	Reads H4K5/K8/K12ac. Functions as an anti-silencing factor that prevents gene silencing at methylated loci [34].
	Writer	HAG2 (AT5G56740)	Writes H4K12ac [188].
		HAM1 (AT5G64610) HAM2 (AT5G09740)	Write H4K5ac [188] and is involved in gametophyte development [193].
Histone Acetylation		HAG3/ELO3 (AT5G50320)	Involved in plant response to UV [189], leaf growth [190], plant defense [191] and associated with RNAPII elongation [192].
		HAC1 (AT1G79000) HAC2 (AT1G67220) HAC4 (AT1G55970) HAC5 (AT3G12980) HAC12 (AT1G16710)	Regulate leaf senescence [194], response to UV [195] and ethylene [196], plant defense [197] and global plant growth and development [198].
		HAF1 (AT1G32750) HAF2 (AT3G19040)	Involved in circadian clock gene expression [199], response to UV [195], root development [200] and male gamete development [201],
	Eraser	HDA5 (AT5G61060)	Interacts with other histone modifying enzymes, such as HDA6 and FLD. Mutant displays late flowering phenotype [218]. Quadruple mutant *hda5/14/15/18* exhibits hypersensitivity towards salt [225].
		HDA6 (AT5G63110)	Involved in circadian clock gene expression [128], plant defense [202], transposon silencing [203], flowering [204] and seed dormancy [205].
		HDA7 (AT5G35600)	Involved in the development of female gametophyte and embryo [206].
		HDA9 (AT3G44680)	Mediates the H3K27 deacetylation and further leads to *FLC* gene repression by H3K27me3 [207]. Mutants also display differential responses to salt and drought [208]; enhanced pathogen resistance by activating NLR genes [209]. HDA9 also involves the regulation of flowering [210] and leaf development [211].
		HDA14 (AT4G33470)	Potentially links to protein acetylation to phosphorylation [219].

		HDA15 (AT3G18520)	Regulates temperature sensing [212], cell elongation [220, 221], response to auxin [222] and light [223] .
		HDA18 (AT5G61070)	Involved in cell fate control in root epidermis [200, 224].
		HDA19 (AT4G38130)	Regulates photoperiod-dependent flowering time [204], temperature sensing [212], root development [213], germination [214, 215], plant defense [216] and plant stress responses [217]. Quintuple mutant *hda5/14/15/18/19* exhibits salt tolerance [226].
		HD2A (AT3G44750) HD2B (AT5G22650) HD2C (AT5G03740) HD2D (AT2G27840)	Plant-specific histone deacetylases, involved in stem vascular development [230], root meristem development [231], flowering [232], plant defense [233], heat and cold stress response [234, 235].
		SRT1 (AT5G55760) SRT2 (AT5G09230)	Involved in mitochondrial metabolite transport [227, 228] and ethylene signaling pathway [229].

Table 1. Summary of Histone PTM readers/writers/erasers and related biological functions in *Arabidopsis*. Enzymes are clustered by their roles (column 2) as histone PTM (column 1) readers, writers and erasers. Gene name and ID are shown in column 3. The biological functions of corresponding enzymes are shown in column 4.

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TEXT BOX

Box 1

ChIP-chip: Chromatin immunoprecipitation (ChIP) followed by microarray (chip) to determine the protein-DNA interaction in genome-wide,

ChIP-seq: ChIP sequencing (ChIP-seq) is the combination of chromatin immuno-precipitation (ChIP) and next-generation sequencing (NGS). The DNA-bound protein is immune-precipitated by antibody and the associated DNA is then fragmented, purified and sequenced. ChIP-seq analyzes the protein-DNA interaction and reveals the genomic distribution of a particular DNA-bound protein by NGS.

CUT&RUN-seq: CUT & RUN (Cleavage Under Targets and Release Using Nuclease) followed by next-generation sequencing is a technology to study DNA-protein interaction. DNA-protein complex is separated by the antibody-targeted *in situ* MNase cleavage and the DNA is subsequently purified and sequenced.

PCSD: a Plant Chromatin State Database providing information of chromatin states across the genomes of Arabidopsis thaliana, Oryza sativa and Zea mays based on various epigenomic sequencing data sets [118]. Based on Hidden Markov model, the genomes where clustered in relation with their chromatin environment. It is possible to download genome browser files for various ChIP-Seq experiments (i.e transcription factors, histones PTMs, or chromatin associated factors).

PlantDHS: a Plant DNase I Hypersensitive Site database also including the genomic information of histone PTMs, nucleosome positioning and transcription factor binding sites in plants [243]. The database provides downloadable ChIP-Seq files to load into a genome browser for several nucleosome related sequencing methods.

ReMap: A large database including integrative analysis of Arabidopsis ChIP-seq and DAP-seq data sets, providing the distribution information of histone PTM/variants, transcription regulators and factors [244].

WERAM: a database for writers, erasers and readers of histone acetylation and methylation in eukaryotes including many plant species [245].

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PlantPAN3.0: A database exploring transcription factors from ChIP-Seq experiments from 78 plant species [246].

Box 2

In plants, the intragenic distribution profiles of H3K36me3 and H3K36me2 differs from many other higher organisms. Perhaps, H3K36me3 is progressively de-methylated in plants, where H3K36 is thought to be progressively methylated during RNAPII elongation in animals. These differences may result in plant-specific chromatin states [247]. The molecular mechanism responsible for the specific positioning of H3K36me3 and H3K36me2 along genes bodies in plants remains unclear. In yeast, a single H3K36 methyltransferase (Set2p) is responsible for all methylation states of H3K36. There are around eight H3K36 methyltransferases in mammals, but many more histone methyltransferases in *Arabidopsis*. H3K36-specific histone methyltransferase in plants, such as *Arabidopsis* SDG8 and SDG26 or SDG725 in rice, currently represent the main candidates responsible for these H3K36me patterns [134]. Since the H3K36me3 and H3K36me2 distributions along gens are opposite in plants compared to the distribution in animals, it remains to be investigated how this effects H3K36 methylation-associated events such as splicing and intragenic transcription initiation. A focus on the plant equivalents of H3K36me3, H3K36me2 and rarely studied H3K36me1 (peaking at the extreme 3' end of genes in rice) in association with transcription elongation, termination and gene regulation promises to instruct intriguing lessons about plant-specific transcription regulation mechanisms.

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GLOSSARY

Histone

A histone is a basic protein component of eukaryotic chromosomes, packaging DNA into ordered structures named nucleosomes. Core histones include histone H2A, H2B, H3 and H4.

Histone PTM

A histone post-translational modification (PTM) describes the chemical modification on histone residues, such as acetylation of histone H3 lysine 4 (H3K4ac). The common histone PTMs include methylation (me), phosphorylation (ph), acetylation (ac) and ubiquitination (ub). A histone residue can be modified in different states. For example, lysine residues can be mono-, di- and tri-methylated (me1, me2 and me3), expanding the diversity and functionality of histone PTMs.

RNAPII

DNA dependent RNA polymerase II (RNAPII or Pol II) is a multiprotein complex including 12 subunits. It is responsible for the transcription of messenger RNA (mRNA), long non-coding RNA, snRNA and microRNA.

PIC

A transcription preinitiation complex (PIC) is an assembly of RNAPII, co-factors and transcription factors in gene promoter region that facilitates RNAPII transcription initiation.