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Histone modifications and dynamic regulation of genome accessibility in plants

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Summary

In all eukaryotes chromatin physically restricts the accessibility of the genome to regulatory proteins such as transcription factors. Plant model systems have been instrumental in demonstrating that this restriction is dynamic and changes during development and in response to exogenous cues. Among the multiple epigenetic mechanisms that alter chromatin to regulate gene expression, histone modifications play a major role. Recent studies in *Arabidopsis* have provided the first genome-wide histone modification maps, revealed important biological roles for histone modifications, and advanced our understanding of stimulus-dependent changes in histone modifications.

Introduction

Within each cell, the genetic information encoded by DNA is compacted into chromatin. The fundamental unit of chromatin is the nucleosome, which is formed by wrapping 147 bp of DNA around a histone octamer (two copies each of histones H2A, H2B, H3 and H4). Nucleosomal DNA presents a barrier for proteins that need to contact the DNA, including those regulating gene expression. Constitutively expressed genes in plants and other organisms often have nucleosome free regions in their promoters[1,2]. Expression of many other genes is regulated by altering these chromatin constraints in response to endogenous and exogenous cues, which ultimately creates a cell- or stimulus-specific accessible genome. How is this achieved? Within the context of chromatin, three processes act in concert to regulate gene expression and thus should be considered together (Table 1). First, histone modifiers covalently alter amino acids primarily in the exposed N-terminal tails of histones, which changes the histone-DNA interaction and creates or blocks protein binding sites (Table 1)[3]. Second, chromatin remodeling ATPases use the energy derived from ATP hydrolysis to alter position or composition of nucleosomes (Table 1)[2]. Finally, methylation of cytosine residues in the DNA interferes with binding of some proteins (including transcription factors) and recruits other proteins (Table 1)[4,5].

Here we focus on the role of covalent histone modifications in regulation of gene expression in euchromatin. These modifications can activate or repress transcription by generating more ‘open’ or ‘closed’ chromatin configurations, respectively. Generally, open chromatin increases the accessibility of the genome to transcription factors and/or the general transcription machinery, thereby activating transcription, while closed chromatin represses transcription by limiting the accessibility of the genome to these proteins (Table 2). Within a histone, the amino acid modified, the type of modification and the degree of modification (for example mono-,

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di- or trimethylation) all affect whether a given modification is activating or repressive[3] (Table 2). The roles of the individual histone modifications are conserved between the plant and animal kingdoms.

In this review we will discuss genome-wide elucidation of the distribution of histone modifications in plants, identification of the processes regulated by histone modifications, and how histone modifiers are recruited to their targets in plants. A unique advantage of studying chromatin regulation in plants is that functional and molecular studies are usually conducted in context of the multicellular organism, not in cell lines. While this can be technically challenging (see conclusions), it facilitates identification of biologically relevant changes in histone modifications. That very few chromatin regulators are essential in plants is a significant advantage for elucidating the in vivo role of these regulators.

Genome-wide distribution of covalent histone modifications

In addition to the type of histone modification present, the effect on gene expression depends on the spatial distribution of a given modification across a gene region (the histone modification landscape[6]) and the combinatorial presence of other modifications[3,7,8]. Two examples from yeast and mammalian cell lines highlight this point. Tri-methylation of histone H3 on lysines 9 and 36 (H3K9me3 and H3K36me3) is repressive when found in the promoter region but activating when found in gene proper (reviewed in[3,7,8]). These modifications close chromatin to prevent transcription initiation. Thus, when present within genes, this stimulates full-length transcript production by preventing internal initiation from cryptic start sites. Genome-wide binding and expression studies showed that presence of an activating histone modification such as H3K4me at the promoter alone is not sufficient to trigger transcriptional activation, additional histone modifications present at the same locus affect gene expression in a combinatorial fashion (reviewed in [3,6,7], see also[9,10]).

Recent studies in plants also address these topics. Genome-wide analyses of H3K27me3 were recently published for *Arabidopsis*[1,11]. This is a repressive histone modification established by SET-domain containing histone methyl transferases of the Polycomb Repressive Complex 2 (PRC2). H3K27me3 is required for proper expression of several important transcriptional regulators[12–18]. Chromatin immunoprecipitation studies with H3K27me3 antibodies followed by hybridization of the precipitated genomic DNA to whole genome *Arabidopsis* tiling arrays (ChIP on chip) revealed that H3K27me3 is associated with many individual genes (18% of all genes), especially transcription factors and developmental regulators that are not expressed at the developmental stage examined (ten-day-old seedlings)[1]. The modification is limited to the transcribed regions of genes, with a slight bias towards the 5′ end and the proximal promoter[1,11](Figure 1), suggesting that regulatory sequences in the DNA may be the primary targets of H3K27me3 repression. In metazoans H3K27me3 also represses expression of transcription factors and developmental regulators, but is generally associated with large multigene chromatin domains instead of single genes[19,20]. The reduced tendency of plants to form large compacted chromatin domains may be the basis for their high developmental plasticity and the ease with plant cells can change developmental programs and even de-differentiate.

H3K27me3 was found to be the in vivo binding site for the only *Arabidopsis thaliana* Heterochromatin Protein 1 (HP1) homolog, LHP1/TFL2[11], which plays a role in repressing gene expression in euchromatin[21,22]. This contrasts with the situation in metazoans, where instead of HP1, Polycomb Repressive Complex 1 (PRC1) recognizes H3K27me3[19,20] and generates large multigene silenced domains. Intriguingly, a homolog of PRC1 is not present in plants [18], thus LHP1/TFL2 may functionally substitute for PRC1-mediated repression in

euchromatin[11]. The strong, pleiotropic phenotypes of *lhp1/tfl2* mutants are consistent with a role for LHP1/TFL2 as an important repressor of gene expression [23].

A comprehensive analysis of multiple histone modifications was recently reported for H3K9me2, H3K9me3, and H3K27me3 using a chromosome 4 tiling array[11]. As previously described, H3K9me2 was exclusively associated with repetitive heterochromatic regions[24]. Both H3K27me3 and H3K9me3 were present in small dispersed but largely non-overlapping regions in euchromatin with a similar 5′ concentrated distribution over genes[11](Figure 1). The mutually exclusive presence of these two histone modifications suggests that they are part of distinct repressive mechanisms and perhaps regulate different groups of genes or respond to different types of cues.

Role of histone modifications in the organism

Histone modifications in plants regulate gene expression in response to diverse exogenous stimuli including stress, pathogen attack, temperature, and light[25]. For example, the histone acetyltransferase (HAT) HAC1 is required for transcriptional upregulation of the heat shock gene *HSP17* and accumulation of HSP17 after heat shock[26]. The histone deacetylase (HDAC) HDA19 regulates expression of pathogenesis related genes and promotes resistance to a fungal pathogen[27]. Prolonged exposure to cold silences the flowering time repressor *FLOWERING LOCUS C* (*FLC*) in *Arabidopsis* winter annuals by histone deacetylation and repressive histone methylation (reviewed in [28,29]). HDA19 and two HATs (HAF2 and GCN5) have negative and positive roles, respectively, in light-responsive gene expression and in photomorphogenesis[30]. Thus, histone modifications control expression of important regulators in response to environmental signals in plants.

Histone modifications also play a central role in developmental regulation (reviewed in [17, 18,25,28,31]). In a recent study, three-dimensional fluorescence in situ hybridization of the locus encoding the homeodomain transcription factor GLABRA2 (GL2) revealed cell-type-specific hybridization of a *GL2* probe in atrichoblast cells (which express *GL2* and do not form root hairs) but not in root-hair forming trichoblast cells[32]. This hybridization and hence accessibility of the locus changes dynamically when a cell perceives positional cues that cause a switch from trichoblast to atrichoblast fate, apparently by resetting of the responsible epigenetic marks during the cell cycle[32]. A second study showed that certain histone modifications (high levels of H3K9me2, low levels of H3K9me3, and low H3 acetylation) at the proximal promoter of *GL2* are required for repression of this gene[33]. Because these histone modifications can also be reset during the cell cycle[33], it is possible that they cause the differential *GL2* locus accessibility. It is intriguing that the H3K9me2 modification is readily reversible upon perception of altered positional cues. As described above, H3K9me2 is nearly exclusively heterochromatic[11,24], and thus likely associated with tightly compacted chromatin.

Recruitment and regulation of histone modifying activities

An important, largely unanswered, question is how histone-modifying activities are recruited to their targets. One mechanism is recruitment by transcription factors. As discussed below, transcription factors can directly bind and recruit histone-modifying complexes or recruit them indirectly, via co-regulators. In addition, existing histone modifications can affect recruitment of histone modifiers. Several plant transcription factors are known to interact with histone modifying complexes. The cold-inducible C-REPEAT BINDING FACTOR (CBF1) is proposed to recruit the GCN5 HAT complex via direct interaction with the HAT complex subunits ADA2a and ADA2b[34]. The C2H2 zinc-finger transcription factor SUPPRESSOR OF FRIGIDA4 (SUF4) is thought to recruit the histone methyl transferase EARLY

FLOWERING IN SHORT DAYS (EFS) to the *FLC* promoter[35,36]. The APETALA2/EREBP-type transcription factor AtERF7, which mediates ABA responses, likely recruits HDA19 via its interaction with the HDAC complex subunit SIN3 [37]. The seed specific ABI3-like transcription factor ALF appears to recruit a HAT activity to the bean *phaseolin* promoter [38]. A steroid inducible system revealed that ALF binding causes promoter potentiation through increased histone acetylation. Full promoter activation after subsequent exposure to the hormone ABA correlates with altered histone acetylation, and increased H3K4 levels[38], indicating that a step-wise sequence of histone modifications is necessary for *phaseolin* activation. Although more recruiting factors need to be identified to better understand what types of transcription factors play this role, the available data suggests that expression and/or activity of the recruiting transcription factors is cell- or stimulus-specific.

The LEUNIG (LUG) co-repressor complex bridges interactions between transcription factors and chromatin regulators. LUG is recruited by certain MADS-domain transcription factors to the promoter of the floral homeotic regulator *AGAMOUS(AG)* and represses *AG* expression in the outer whorls of flowers via recruitment of the HDA19 HDAC[39–41]. TOPLESS (TPL), a protein with limited similarity to LUG, was recently identified as pivotal in embryo patterning in *Arabidopsis*. Among the earliest patterning events in the embryo are establishment of an apical (shoot) pole and a basal (root) pole. Dominant negative mutations in TPL inactivate all five TPL family members in *Arabidopsis* and convert the apical pole into a basal pole, which can lead to a dramatic double root phenotype[42]. Elegant genetic interaction studies indicate that the TPL family of proteins likely works in conjunction with HDA19 to repress genes required for basal fate in the apical half of transition stage embryos[42].

Once a histone modification has been added, it can serve to recruit or inhibit recruitment of additional histone modifying complexes. For example, GENERAL TRANCRIPTION FACTOR 6 (GTE6) contains a domain that binds acetylated lysines on histone tails. GTE6 is recruited to the promoter of the MYB transcription factor *ASYMMETRIC LEAVES 2* (*AS2*) and is required for histone acetylation of the *AS2* promoter via positive feedback, perhaps by recruiting a HAT complex[43].

Specific recruitment of histone modifying complexes enables them to regulate particular target genes in response to endogenous and exogenous cues. Formation of multiple histone modifier complexes also contributes to their target specificity. For example three distinct PRC2 complexes exist in *Arabidopsis* that regulate unique target genes through H3K27 trimethylation [18]. Specificity of histone modifiers can also be regulated posttranslationally, the maize HDAC Hda1 is activated by proteolytic cleavage during seed germination [44].

Conclusion

Ultimately many histone modifications are directed at coordinating gene expression. As summarized above the recent genome-wide studies in plants have provided tantalizing glimpses into how this coordination is actually achieved. These studies have revealed that in *Arabidopsis* repressive histone modifications (such as H3K27 me3) occupy smaller domains than in metazoans, likely making these modifications more readily reversible. The observation that factors binding this histone modification may also be distinct further underscores that fundamental differences may exist in chromatin-mediated regulation between the two kingdoms. These differences may reflect the higher developmental plasticity of plants and allow these sessile organisms to respond rapidly to fluctuating environmental conditions. It is the expectation that the coordinated gene expression enabled through these histone modifications patterns would be target of environmental or developmental cues. Indeed, as discussed above, histone modifications change rapidly in response to diverse exogenous cues and during patterning and differentiation to control expression of important regulators of the

processes triggered by these cues. The precise patterns of histone modifications observed genome-wide as well as the dynamic changes in histone modifications observed at the single gene level suggest presence of an accurate recruitment mechanism for histone modifiers. As evidenced from the studies described here, this likely involves a combination of sequence specific DNA binding proteins, co-regulator complexes, feedback mechanisms as well as regulation of histone modifier activity.

The challenge for the future is to detect the global, dynamic changes in multiple histone modifications in the organism that occur within subpopulations of cells, to further delineate how these modifications are triggered by exogenous and endogenous cues at specific genes, and to determine how they correlate with gene expression. This will require new approaches and techniques, including inducible systems to dissect the responses to endogenous cues, better cell fractionation methods, and increased sensitivity of techniques such as ChIP. Genetic analyses will continue to be instrumental in deciphering the biological significance of the observed alterations in histone modifications.

Understanding chromatin-mediated regulation of genome accessibility will ultimately require expanding these studies to include other chromatin regulatory mechanisms such as chromatin remodeling and DNA methylation. The intricate epigenetic regulation of *FLC* [28,31] clearly demonstrates that histone modifications and chromatin remodeling act together to control expression of this master regulator. Other chromatin remodeling complexes play important roles in chromatin-mediated control of gene expression[45,46]. Similarly, genome-wide DNA methylation maps[47–51] suggest that in addition to its role in heterochromatin, DNA methylation may affect transcription in plants. Because of the viability of null mutants, plants are ideally suited to sort out how these processes coordinately regulate genome accessibility.

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References

1. Zhang X, Clarenz O, Cokus S, Bernatavichute YV, Pellegrini M, Goodrich J, Jacobsen SE. Whole-genome analysis of histone H3 lysine 27 trimethylation in Arabidopsis. PLoS Biol 2007;5:e129. [PubMed: 17439305]** The first genome-wide analysis of a histone modification in plants. Trimethylation of lysine 27 of histone H3 (H3K27 me3) is localized throughout individual genes that are repressed at the developmental stage examined. A large fraction of the 4,400 genes marked by this histone modification are transcription factors and developmental regulators.
2. Rando OJ, Ahmad K. Rules and regulation in the primary structure of chromatin. Curr Opin Cell Biol. 2007
3. Berger SL. The complex language of chromatin regulation during transcription. Nature 2007;447:407–412. [PubMed: 17522673]
4. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 2006;31:89–97. [PubMed: 16403636]
5. Zemach A, Grafi G. Methyl-CpG-binding domain proteins in plants: interpreters of DNA methylation. Trends Plant Sci 2007;12:80–85. [PubMed: 17208509]
6. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell 2007;128:669–681. [PubMed: 17320505]
7. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007;128:707–719. [PubMed: 17320508]

8. Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693–705. [PubMed: 17320507]
9. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. Cell 2007;129:823–837. [PubMed: 17512414]
10. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA. A chromatin landmark and transcription initiation at most promoters in human cells. Cell 2007;130:77–88. [PubMed: 17632057]
11. Turck F, Roudier F, Farrona S, Martin-Magniette ML, Guillaume E, Buisine N, Gagnot S, Martienssen RA, Coupland G, Colot V. Arabidopsis TFL2/LHP1 Specifically Associates with Genes Marked by Trimethylation of Histone H3 Lysine 27. PLoS Genet 2007;3:e86. [PubMed: 17542647]** Comprehensive chromosome 4 binding study for the only *Arabidopsis* Heterochromatin Protein 1 homolog LHP1/TFL2 as well as for three repressive histone modifications. The study showed that in vivo LHP1 binds to one of these, H3K27me3. In addition, the repressive euchromatic histone modifications H3K9me3 and H3K27me3 are found in different genes and present throughout each gene.
12. Makarevich G, Leroy O, Akinci U, Schubert D, Clarenz O, Goodrich J, Grossniklaus U, Kohler C. Different Polycomb group complexes regulate common target genes in Arabidopsis. EMBO Rep 2006;7:947–952. [PubMed: 16878125]
13. Schubert D, Primavesi L, Bishopp A, Roberts G, Doonan J, Jenuwein T, Goodrich J. Silencing by plant Polycomb-group genes requires dispersed trimethylation of histone H3 at lysine 27. Embo J 2006;25:4638–4649. [PubMed: 16957776]
14. Sung S, Amasino RM. Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. Nature 2004;427:159–164. [PubMed: 14712276]
15. Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 2004;427:164–167. [PubMed: 14712277]
16. Schonrock N, Bouveret R, Leroy O, Borghi L, Kohler C, Gruissem W, Hennig L. Polycomb-group proteins repress the floral activator AGL19 in the FLC-independent vernalization pathway. Genes Dev 2006;20:1667–1678. [PubMed: 16778081]
17. Kohler C, Makarevich G. Epigenetic mechanisms governing seed development in plants. EMBO Rep 2006;7:1223–1227. [PubMed: 17139298]
18. Pien S, Grossniklaus U. Polycomb group and trithorax group proteins in Arabidopsis. Biochim Biophys Acta. 2007
19. Schuettengruber B, Chourrout D, Vervoort M, Leblanc B, Cavalli G. Genome regulation by polycomb and trithorax proteins. Cell 2007;128:735–745. [PubMed: 17320510]
20. Schwartz YB, Pirrotta V. Polycomb silencing mechanisms and the management of genomic programmes. Nat Rev Genet 2007;8:9–22. [PubMed: 17173055]
21. Libault M, Tessadori F, Germann S, Snijder B, Fransz P, Gaudin V. The Arabidopsis LHP1 protein is a component of euchromatin. Planta 2005;222:910–925. [PubMed: 16244868]
22. Nakahigashi K, Jasencakova Z, Schubert I, Goto K. The Arabidopsis heterochromatin protein1 homolog (TERMINAL FLOWER2) silences genes within the euchromatic region but not genes positioned in heterochromatin. Plant Cell Physiol 2005;46:1747–1756. [PubMed: 16131496]
23. Kotake T, Takada S, Nakahigashi K, Ohto M, Goto K. Arabidopsis TERMINAL FLOWER 2 gene encodes a heterochromatin protein 1 homolog and represses both FLOWERING LOCUS T to regulate flowering time and several floral homeotic genes. Plant Cell Physiol 2003;44:555–564. [PubMed: 12826620]
24. Fuchs J, Demidov D, Houben A, Schubert I. Chromosomal histone modification patterns--from conservation to diversity. Trends Plant Sci 2006;11:199–208. [PubMed: 16546438]
25. Chen ZJ, Tian L. Roles of dynamic and reversible histone acetylation in plant development and polyploidy. Biochim Biophys Acta 2007;1769:295–307. [PubMed: 17556080]
26. Bharti K, Von Koskull-Doring P, Bharti S, Kumar P, Tintschl-Korbitzer A, Treuter E, Nover L. Tomato heat stress transcription factor HsfB1 represents a novel type of general transcription coactivator with a histone-like motif interacting with the plant CREB binding protein ortholog HAC1. Plant Cell 2004;16:1521–1535. [PubMed: 15131252]

27. Zhou C, Zhang L, Duan J, Miki B, Wu K. HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. Plant Cell 2005;17:1196–1204. [PubMed: 15749761]

28. Schmitz RJ, Amasino RM. Vernalization: A model for investigating epigenetics and eukaryotic gene regulation in plants. Biochim Biophys Acta. 2007

29. Sung S, Amasino RM. Molecular genetic studies of the memory of winter. J Exp Bot 2006;57:3369–3377. [PubMed: 16980591]

30. Benhamed M, Bertrand C, Servet C, Zhou DX. Arabidopsis GCN5, HD1, and TAF1/HAF2 interact to regulate histone acetylation required for light-responsive gene expression. Plant Cell 2006;18:2893–2903. [PubMed: 17085686]

31. Reyes JC. Chromatin modifiers that control plant development. Curr Opin Plant Biol 2006;9:21–27. [PubMed: 16337828]

32. Costa S, Shaw P. Chromatin organization and cell fate switch respond to positional information in Arabidopsis. Nature 2006;439:493–496. [PubMed: 16362059]** Using 3D fluorescence in situ hybridization, the locus for *GLABRA 2*, a regulator of root epidermal cell fate, was shown to be accessible only in non hair-forming cells. Altered positional cues cause cell cycle linked resetting of this differential chromatin-mediated locus accessibility and altered cell fate.

33. Caro E, Castellano MM, Gutierrez C. A chromatin link that couples cell division to root epidermis patterning in Arabidopsis. Nature 2007;447:213–217. [PubMed: 17450124]** GEM, a new regulator of *GLABRA 2(GL2)* expression, represses *GL2* by maintaining repressive H3 lysine 9 methylation at the *GL2* promoter. The histone modifications and *GL2* expression are cell-cycle regulated, providing a link between cell division and cell fate.

34. Mao Y, Pavangadkar KA, Thomashow MF, Triezenberg SJ. Physical and functional interactions of Arabidopsis ADA2 transcriptional coactivator proteins with the acetyltransferase GCN5 and with the cold-induced transcription factor CBF1. Biochim Biophys Acta 2006;1759:69–79. [PubMed: 16603259]

35. Kim S, Choi K, Park C, Hwang HJ, Lee I. SUPPRESSOR OF FRIGIDA4, encoding a C2H2-Type zinc finger protein, represses flowering by transcriptional activation of Arabidopsis FLOWERING LOCUS C. Plant Cell 2006;18:2985–2998. [PubMed: 17138694]

36. Kim SY, Michaels SD. SUPPRESSOR OF FRI 4 encodes a nuclear-localized protein that is required for delayed flowering in winter-annual Arabidopsis. Development 2006;133:4699–4707. [PubMed: 17079264]

37. Song CP, Agarwal M, Ohta M, Guo Y, Halfter U, Wang P, Zhu JK. Role of an Arabidopsis AP2/EREBP-type transcriptional repressor in abscisic acid and drought stress responses. Plant Cell 2005;17:2384–2396. [PubMed: 15994908]

38. Ng DW, Chandrasekharan MB, Hall TC. Ordered histone modifications are associated with transcriptional poising and activation of the phaseolin promoter. Plant Cell 2006;18:119–132. [PubMed: 16326929]* Steroid-inducible recruitment of the seed specific transcription factor ALF to its target promoter allowed identification of distinct promoter states that are correlated with unique chromatin modifications.

39. Sridhar VV, Surendrarao A, Gonzalez D, Conlan RS, Liu Z. Transcriptional repression of target genes by LEUNIG and SEUSS, two interacting regulatory proteins for Arabidopsis flower development. Proc Natl Acad Sci U S A 2004;101:11494–11499. [PubMed: 15277686]

40. Sridhar VV, Surendrarao A, Liu Z. APETALA1 and SEPALLATA3 interact with SEUSS to mediate transcription repression during flower development. Development 2006;133:3159–3166. [PubMed: 16854969]

41. Gonzalez D, Bowen AJ, Carroll TS, Conlan RS. The transcription corepressor LEUNIG interacts with the Histone Deacetylase HDA19 and Mediator components MED14 (SWP) and CDK8 (HEN3) to repress transcription. Mol Cell Biol. 2007

42. Long JA, Ohno C, Smith ZR, Meyerowitz EM. TOPLESS regulates apical embryonic fate in Arabidopsis. Science 2006;312:1520–1523. [PubMed: 16763149]** Genetic characterization of the TOPLESS family as important regulators of apical-basal patterning in the embryo. TPL proteins act as transcriptional co-repressors together with histone deacetylases to repress basal fate in the apical embryo.

43. Chua YL, Channeliere S, Mott E, Gray JC. The bromodomain protein GTE6 controls leaf development in Arabidopsis by histone acetylation at ASYMMETRIC LEAVES1. Genes Dev 2005;19:2245–2254. [PubMed: 16166385]
44. Pipal A, Goralik-Schramel M, Lusser A, Lanzanova C, Sarg B, Loidl A, Lindner H, Rossi V, Loidl P. Regulation and processing of maize histone deacetylase Hda1 by limited proteolysis. Plant Cell 2003;15:1904–1917. [PubMed: 12897261]
45. Kwon CS, Wagner D. Unwinding chromatin for development and growth: a few genes at a time. Trends Genet. 2007
46. Jerzmanowski A. SWI/SNF chromatin remodeling and linker histones in plants. Biochim Biophys Acta. 2007
47. Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, Chen H, Henderson IR, Shinn P, Pellegrini M, Jacobsen SE, et al. Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis. Cell 2006;126:1189–1201. [PubMed: 16949657]** Genome-wide DNA methylation analysis revealed a possible role for DNA methylation in regulation transcription, in addition to its role in silencing repetetive DNA and transposons. Ubiquitously expressed genes are characterized by extensive CG methylation throughout the genes, while a small number of genes with a tissue specific expression pattern have methylated promoters.
48. Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S. Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription. Nat Genet 2007;39:61–69. [PubMed: 17128275]** Genome-wide DNA methylation study revealed extensive CG methylation throughout moderately expressed genes with less methylation at the 5′ and 3′ ends. This methylation may be triggered by aberrant transcription initiation within the gene and may dampen transcription elongation.
49. Gehring M, Henikoff S. DNA methylation dynamics in plant genomes. Biochim Biophys Acta. 2007
50. Penterman J, Zilberman D, Huh JH, Ballinger T, Henikoff S, Fischer RL. DNA demethylation in the Arabidopsis genome. Proc Natl Acad Sci U S A 2007;104:6752–6757. [PubMed: 17409185]
51. Henderson IR, Jacobsen SE. Epigenetic inheritance in plants. Nature 2007;447:418–424. [PubMed: 17522675]
52. Liu Y, Koornneef M, Soppe WJ. The absence of histone H2B monoubiquitination in the Arabidopsis hub1 (rdo4) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell 2007;19:433–444. [PubMed: 17329563]* The enzyme required for monoubiquitination of lysine 143 of H2B (HUB1) was identified in a genetic screen for seed dormancy mutants.
53. Fleury D, Himanen K, Cnops G, Nelissen H, Boccardi TM, Maere S, Beemster GT, Neyt P, Anami S, Robles P, et al. The Arabidopsis thaliana homolog of yeast BRE1 has a function in cell cycle regulation during early leaf and root growth. Plant Cell 2007;19:417–432. [PubMed: 17329565]* The HUB1 E3 ubiquitin ligase monoubiquitinates lysine 143 of H2B in vitro. This study links H2BK143ub to activation of expression of cell cycle regulators.
54. Sridhar VV, Kapoor A, Zhang K, Zhu J, Zhou T, Hasegawa PM, Bressan RA, Zhu JK. Control of DNA methylation and heterochromatic silencing by histone H2B deubiquitination. Nature 2007;447:735–738. [PubMed: 17554311]** *Arabidopsis* H2B was found to be ubiquitinated on lysine 143. A T-DNA mutagenesis in a sensitized background allowed identification of the H2BK143 deubiquitinase. This activity is required for silencing of transposons by promoting histone H3 lysine 9 dimethylation and DNA methylation.
55. Ng DW, Wang T, Chandrasekharan MB, Aramayo R, Kertbundit S, Hall TC. Plant SET domain-containing proteins: Structure, function and regulation. Biochim Biophys Acta. 2007
56. Alvarez-Venegas R, Avramova Z. Methylation patterns of histone H3 Lys 4, Lys 9 and Lys 27 in transcriptionally active and inactive Arabidopsis genes and in atx1 mutants. Nucleic Acids Res 2005;33:5199–5207. [PubMed: 16157865]
57. Kim SY, He Y, Jacob Y, Noh YS, Michaels S, Amasino R. Establishment of the vernalization-responsive, winter-annual habit in Arabidopsis requires a putative histone H3 methyl transferase. Plant Cell 2005;17:3301–3310. [PubMed: 16258034]
58. Naumann K, Fischer A, Hofmann I, Krauss V, Phalke S, Irmler K, Hause G, Aurich AC, Dorn R, Jenuwein T, et al. Pivotal role of AtSUVH2 in heterochromatic histone methylation and gene silencing in Arabidopsis. Embo J 2005;24:1418–1429. [PubMed: 15775980]

59. Jackson JP, Johnson L, Jasencakova Z, Zhang X, PerezBurgos L, Singh PB, Cheng X, Schubert I, Jenuwein T, Jacobsen SE. Dimethylation of histone H3 lysine 9 is a critical mark for DNA methylation and gene silencing in Arabidopsis thaliana. Chromosoma 2004;112:308–315. [PubMed: 15014946]
60. Ebbs ML, Bender J. Locus-specific control of DNA methylation by the Arabidopsis SUVH5 histone methyltransferase. Plant Cell 2006;18:1166–1176. [PubMed: 16582009]
61. Thorstensen T, Fischer A, Sandvik SV, Johnsen SS, Grini PE, Reuter G, Aalen RB. The Arabidopsis SUVR4 protein is a nucleolar histone methyltransferase with preference for monomethylated H3K9. Nucleic Acids Res 2006;34:5461–5470. [PubMed: 17020925]
62. Noh B, Lee SH, Kim HJ, Yi G, Shin EA, Lee M, Jung KJ, Doyle MR, Amasino RM, Noh YS. Divergent roles of a pair of homologous jumonji/zinc-finger-class transcription factor proteins in the regulation of Arabidopsis flowering time. Plant Cell 2004;16:2601–2613. [PubMed: 15377760]
63. Zhao Z, Yu Y, Meyer D, Wu C, Shen WH. Prevention of early flowering by expression of FLOWERING LOCUS C requires methylation of histone H3 K36. Nat Cell Biol 2005;7:1256–1260. [PubMed: 16299497]
64. Wang X, Zhang Y, Ma Q, Zhang Z, Xue Y, Bao S, Chong K. SKB1-mediated symmetric dimethylation of histone H4R3 controls flowering time in Arabidopsis. Embo J 2007;26:1934–1941. [PubMed: 17363895]* SKB1, a novel histone modifier, represses *FLC* expression via symmetric dimethylation of arginine 3 on histone H4 (H4R3sme2). By contrast, this modification activates gene expression in metazoans.
65. Pei Y, Niu L, Lu F, Liu C, Zhai J, Kong X, Cao X. Mutations in the Type II Protein Arginine Methyltransferase AtPRMT5 Result in Pleiotropic Developmental Defects in Arabidopsis thaliana. Plant Physiol. 2007
66. Guyomarc'h S, Benhamed M, Lemonnier G, Renou JP, Zhou DX, Delarue M. MGOUN3: evidence for chromatin-mediated regulation of FLC expression. J Exp Bot 2006;57:2111–2119. [PubMed: 16728410]
67. Deng W, Liu C, Pei Y, Deng X, Niu L, Cao X. Involvement of the Histone Acetyltransferase AtHAC1 in the Regulation of Flowering Time via Repression of FLC in Arabidopsis. Plant Physiol. 2007
68. Han SK, Song JD, Noh YS, Noh B. Role of plant CBP/p300-like genes in the regulation of flowering time. Plant J 2007;49:103–114. [PubMed: 17144897]
69. Tian L, Fong MP, Wang JJ, Wei NE, Jiang H, Doerge RW, Chen ZJ. Reversible histone acetylation and deacetylation mediate genome-wide, promoter-dependent and locus-specific changes in gene expression during plant development. Genetics 2005;169:337–345. [PubMed: 15371352]
70. Earley K, Lawrence RJ, Pontes O, Reuther R, Enciso AJ, Silva M, Neves N, Gross M, Viegas W, Pikaard CS. Erasure of histone acetylation by Arabidopsis HDA6 mediates large-scale gene silencing in nucleolar dominance. Genes Dev 2006;20:1283–1293. [PubMed: 16648464]
71. Xu CR, Liu C, Wang YL, Li LC, Chen WQ, Xu ZH, Bai SN. Histone acetylation affects expression of cellular patterning genes in the Arabidopsis root epidermis. Proc Natl Acad Sci U S A 2005;102:14469–14474. [PubMed: 16176989]
72. Ueno Y, Ishikawa T, Watanabe K, Terakura S, Iwakawa H, Okada K, Machida C, Machida Y. Histone deacetylases and ASYMMETRIC LEAVES2 are involved in the establishment of polarity in leaves of Arabidopsis. Plant Cell 2007;19:445–457. [PubMed: 17293570]
73. Sridha S, Wu K. Identification of AtHD2C as a novel regulator of abscisic acid responses in Arabidopsis. Plant J 2006;46:124–133. [PubMed: 16553900]
74. Houben A, Demidov D, Caperta AD, Karimi R, Agueci F, Vlasenko L. Phosphorylation of histone H3 in plants-A dynamic affair. Biochim Biophys Acta. 2007
75. Choi K, Park C, Lee J, Oh M, Noh B, Lee I. Arabidopsis homologs of components of the SWR1 complex regulate flowering and plant development. Development 2007;134:1931–1941. [PubMed: 17470967]
76. Deal RB, Topp CN, McKinney EC, Meagher RB. Repression of flowering in Arabidopsis requires activation of FLOWERING LOCUS C expression by the histone variant H2A.Z. Plant Cell 2007;19:74–83. [PubMed: 17220196]

77. March-Diaz R, Garcia-Dominguez M, Florencio FJ, Reyes JC. SEF, a new protein required for flowering repression in Arabidopsis, interacts with PIE1 and ARP6. Plant Physiol 2007;143:893–901. [PubMed: 17142478]

Abbreviations

ac
: acetylation

ChIP
: chromatin immunoprecipitation

H3K9
: histone H3 Lysine 9

HAT
: histone acetyl transferase

HDAC
: histone deacetylase

HMT
: histone methyl transferase

HMD
: histone demethylase

HP1
: Heterochromatin Protein 1

me
: methylation

me2
: demethylation

me3
: trimethylation

LHP1
: LIKE HETEROCHROMATIN PROTEIN 1 (also called TFL2); *Arabidopsis* HP1 ortholog

ph
: phosphorylation

PRC1
: Polycomb Repressive Complex 1

PRC2
: Polycomb Repressive Complex 2

sme2
: symmetric dimethylation

SWR1
: chromatin remodeling complex that mediates histone exchange of the histone variant H2A.Z for the canonical histone H2A

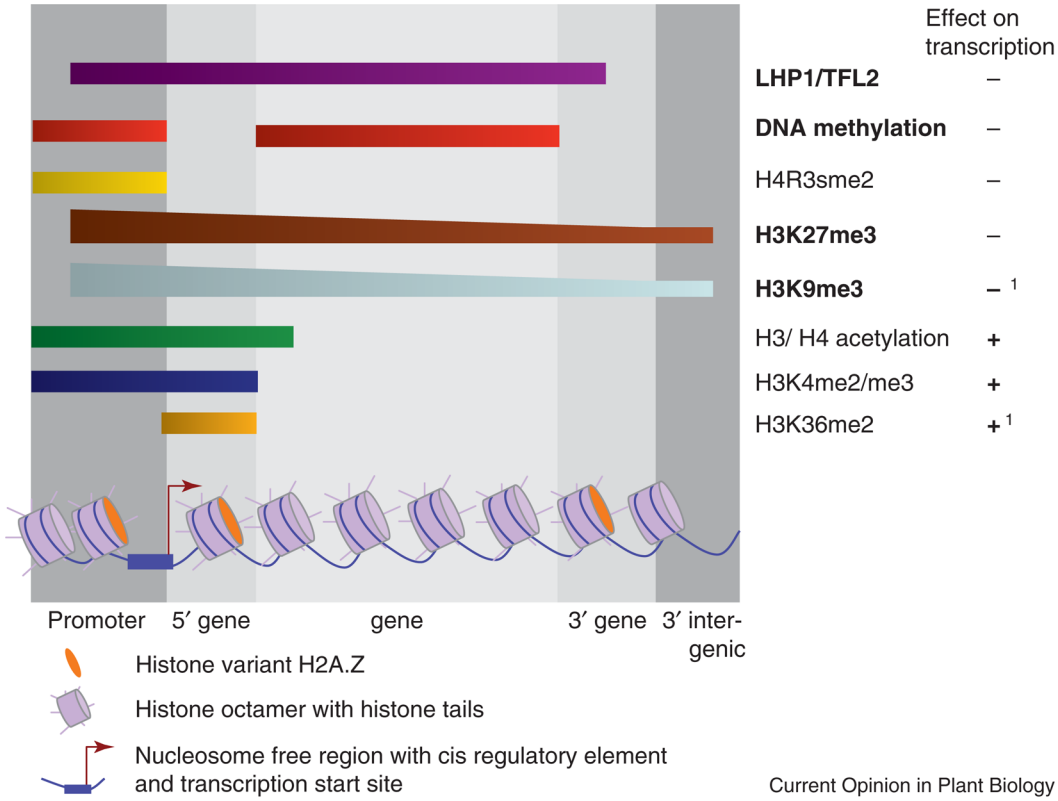

Figure 1. Spatial arrangement of chromatin modifications important for gene expression

Shown is a generic euchromatic *Arabidopsis thaliana* gene in the context of chromatin modifications known to affect gene expression. Relevant gene regions are indicated. Histone octamers are shown as purple cylinders. The histone variant H2A.Z incorporated by the SWR1 chromatin remodeling complex[75–77] is shown as a green ellipse. The promoter is partially depleted of nucleosomes, which is observed in many constitutively expressed genes and in inducible genes after the combined activity of chromatin remodeling ATPases and histone modifiers. Above the graphic grey shading indicates the promoter region, the 5′ end of the gene, the central gene region, the 3′ end of the gene, and the 3′ intergenic region. The spatial distribution of covalent histone modifications is indicated relative to these five regions. Modifications for which distribution was determined by global binding studies are indicated in bold[1,11,47,48,50], to distinguish them from those for which information is only available from studies of a limited number of genes (see Table 2 for examples). Note that H3K27me3 and H3K9me3 do not overlap, genes repressed by H3K27me3 are devoid of H3K9me3 and vice versa[11]. Spatial distribution of DNA methylation and of the Heterochromatin Protein 1 homolog LHP1/TFL2, two other chromatin regulators whose distribution was mapped genome-wide, is also indicated.

[1] H3K9me3 and H3K36me2 may have different roles on transcription dependent on their location[3].

Table 1

Types of chromatin alterations that regulate gene expression

Chromatin alteration[1]	Subtype	Acts upon	Mechanism	Outcome for transcription
Histone Modifications	Ubiquitination	Lysine (K)	distances histones from DNA (bulky moiety)	Activation (H2B) or repression (H2A)[2]
	Methylation	Lysine, Arginine (R)	Recruitment of other chromatin regulators	Activation (H3K4, H3K36[3]) or repression (H3K9[3], H3K27, H4K20, H4R3)
	Acetylation	Lysine	Charge neutralization[4], recruitment of other chromatin regulators	Activation
	Phosphoryation	Serine (S), Threonine (T)	Charge neutralization[4], recruitment of other chromatin regulators	Activation
Chromatin remodeling[5]	SWI/SNF	Nucleosome position and occupancy[2]	Sliding to new position, histone octamer eviction[2]	Actvation and repression
	SWR1	Histone exchange	H2A.Z histone variant incorporation	Activation
DNA methylation	CG and non-CG	Promoter	Inhibition of transcription factor binding	Repression
	CG	Gene (less at 5′ and 3′ ends)	May reduce transcription elongation	Repression

[1] These and additional chromatin alterations also play a role in heterochromatic silencing of repetitive DNA and transposons, genome integrity, and chromosome stability, which are reviewed elsewhere [24,49,51].

[2] Not yet demonstrated for plants.

[3] May activate transcription when localized in transcribed region, but repress transcription when localized in the promoter region.

[4] Charge neutralization decreases the affinity of positively charged histones for negatively charged DNA.

[5] For simplicity, only a subset of the chromatin remodeling complexes that regulate transcription are listed here.

Table 2

Histone modifications in *Arabidopsis*

Modification	Location	Effect	Transcription	Established by	Removed by
Ubiquitination					
H2BK143ub1	Eu-chromatin	open	activation	Ring-Type E3 ligases **HUB1, HUB2**[52,53]	Deubiquitinases **SUP32/UBP26**[54]
Methylation					
H3K4me*	Eu-chromatin	open	activation	trxG class of histone methyltransferases (HMTs)[55]	LSD1-type of histone demethylases (HDMs)[3,8] **FLD**[28]
H3K4me1	Eu-chromatin	open			
H3K4me2	Eu-chromatin	open	activation intron[1][38]	**ATX1**[56]	
H3K4me3	Eu-chromatin	open	activation proximal promoter [55]; 5′end gene[57]	**ATX1**[56] **EFS/SDG8**[57]	
H3K9me*	Both	closed		Su(var) class of HMTs[55]	JmjC-domain and LSD1-type HDMs[3,8]
H3K9me1	Hetero-chromatin	closed		**SUVH2**[58] **SUVH4, SUVH6**[59] **SUVH5**[60]	
H3K9me2	Hetero-chromatin	closed		**SUVH2**[58] **SUVH4, SUVH6**[59] **SUVR4**[61]	
H3K9me3	Eu-hromatin	closed	repression proximal promoter, 5′ end gene, gene[11]		
H3K27me*	Both	closed	repression	E(Z) class of HMTs (PRC2 complex)[55]	
H3K27me1	Hetero-chromatin	closed			
H3K27me2	Hetero-chromatin	closed			
H3K27me3	Eu-chromatin	closed	repression promoter, 5′end gene, gene[11-13]	**CLF, SWN, MEA** [12,13]	
H3K36me*	Eu-chromatin	open	activation	Set domain HMTs	JmjC-domain HDMs. Possibly **REF6**[28,62]
H3K36me1	Eu-chromatin	open			
H3K36me2	Both	open	activation intron[1][63]	**EFS/SDG8**[63]	
H3K36me3	Eu-chromatin	open			
H4K20me*		closed			JmjC domain HDMs
H4K20me1	Hetero-chromatin			**SUVH2**[58]	
H4K20me2	Eu-chromatin				
H4K20me3	Eu-chromatin				
H4R3sme2	Eu-chromatin	closed	repression promoter[64]	Arginine methyltransferases **SKB1/AtPRMT5**[64,65]	Deimination[8]
Acetylation					
H3K ac/H4K**	Both	open	activation promoter, 5′end gene [33,38,66]	Histone acetyltransferases (HATs)[25] GNAT family: **GCN5/HAG1**[30,34,42] CBP/p300 family: **HAC1, HAC5, HAC12**[26,67, 68] TAFII family: **HAF2/TAF1**[30]	Histone Deacetylases (HDACs)[25] RDP3 family: **HDA19**[27,30,37,42,69] **HDA6**[70] HDA1 family: **HDA18**[71] HD2 family: **HD2A, HD2B**[72], **HD2C** [73]
Phosphorylation[2]					
H3S10ph H3S28ph H2T11ph	Both	open	activation	Kinases[74]	Phosphatases[74]

Histone modifications, their locations in the genome and their effects on chromatin (open/closed) as well as on transcription (where known) are listed. Also listed are the enzymes known to add and remove each modification. Plant histone modifiers are listed in bold type.

denotes all modifications of a certain type, for example, H3K9me denotes mono, di, and trimethylation of lysine nine of histone H3, while H3*ac denotes general acetylation of lysines in histone H3.

[1] These introns contain cis regulatory elements important for transcriptional regulation.

[2] The role of histone phosphorylation in the cell cycle is described elsewhere[24,74].