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Mechanisms of epigenetic regulation of transcription by lncRNAs in plants

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Abstract

Long noncoding RNAs (lncRNAs) are a ubiquitous feature of eukaryotic genomes, and in recent decades have been shown to be highly abundant and varied. Many prominent examples have been described as having essential roles in regulating the expression of genes in different developmental and environmental contexts. As a result, much work has been done on elucidating the mechanisms by which they modulate the expression of protein coding genes. In this review, we focus on those which have been characterized in plants. We specifically examine common epigenetic mechanisms which regulate gene expression at the level of transcription. In this regard, we focus on the lncRNAs in plants that have primarily been associated with controlling the chromatin environment of genes at the level of modifications, RNA POLYMERASE II (RNAPII) processivity and efficiency of transcription, and mediating the formation of transcriptionally activating and repressive chromatin loops. We discuss open questions in plant lncRNA epigenetic regulation and opportunities for future study of functionally significant lncRNAs with yet-unknown epigenetic mechanisms.

Introduction

Long noncoding RNAs (lncRNAs) are a class of RNAs longer than 200 nucleotides generally lacking in coding potential. LncRNAs are not universally noncoding however, as a small number may produce peptides or have dual functions as lncRNA and peptide (1-5). LncRNAs can share many structural similarities with mRNAs, including being transcribed by RNAPII and containing spliced introns, a 5' cap, and a poly-A tail. LncRNAs are a common genetic feature of eukaryotes, for example numbering in the thousands in yeast (6) and the tens of thousands in human and mouse genomes (7). Efforts at identifying lncRNAs genome-wide in plants suggest they are similarly widespread (8, 9). LncRNAs are classified based on their relative position to nearby protein coding genes. Further classifying them based on their functional potential remains a challenging task, and thus far only a small subset has been experimentally determined to have regulatory importance (10). Already in plants (and primarily in the model system *Arabidopsis thaliana* (Arabidopsis)) lncRNAs have been implicated in having contributing roles to stress responses (11-15), nutrient homeostasis (16-19), and developmental processes (20-46).

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Detection and characterization of lncRNAs still remains a challenge in many species including plants, partly due to their low expression levels compared to protein coding genes. Given their regulatory function, lncRNA molecules may be subject to fast turnover. As a result, lncRNAs may escape detection by standard steady-state RNA sequencing (RNA-seq) methods, requiring more sophisticated nascent RNA profiling methods to achieve de novo annotation of novel long non-coding transcripts. In addition, lncRNAs are remarkably cell- and tissue-specific (47) and thus significantly diluted when sampling organs or whole individuals, a common practice in plant research. With regards to subcellular localization, as shown in animals lncRNAs tend to be retained in the nuclear fraction (48, 49), however they can also be exported to the cytoplasm and other organelles (50). Association of lncRNAs to ribosomes has been observed in Arabidopsis (51), although their potential function in regulating translation requires further exploration in plants.

Unlike mRNA genes, which are under a strong selective pressure to maintain their coding reading frames, lncRNA sequences are generally not conserved across different species (52). lncRNAs do not seem to depend on their sequence to perform their function, but rather conservation of their secondary structure or position in the genome may prevail for propagation of their biological roles as has been demonstrated for the well described Arabidopsis *COLD INDUCED LONG ANTISENSE INTRAGENIC RNA* (*COOLAIR*) (53). The systematic evaluation of secondary and higher-level structures of non-coding RNAs remains a promising field to provide answers on lncRNA function. *In vivo* lncRNA structure dynamics may be a key factor contributing to the formation of biomolecular condensates. In recent years, the formation of membraneless subcellular compartments with distinct biological roles have gained a lot of attention. Many nuclear and cytoplasmic condensates are rich in RNA and RNA-binding proteins (54). Some examples are nucleoli and Cajal bodies in the nucleus, as well as stress granules in the cytoplasm. Recent work depicts lncRNA molecules as facilitators of phase separation and formation of biologically relevant RNA-protein aggregates across cell compartments and species (55), a feature likely explaining functionality of lncRNAs at very low, substoichiometric expression levels in cellular environments (56, 57).

Epigenetic mechanisms are an integral part of transcriptional regulation in eukaryotes. Chromatin structure and dynamics determine the accessibility of gene regulatory regions for the transcriptional machinery, fine tuning gene expression in responses to stress and development (58). Epigenetic regulation relies on a plethora of proteins and complexes that function as readers, writers, and erasers of different chromatin marks (59), such as posttranslational modifications of histones and DNA methylation. Furthermore, the

expanding field of epitranscriptomics (60) has added RNA editing and non-coding RNA regulation in the list of epigenetic regulators.

Increasing evidence from different eukaryotic systems demonstrate the ability of lncRNAs to act as epigenetic modulators. Seminal work on mammalian *Xist* has provided an excellent example of the role of a lncRNA in developmentally regulated epigenetic silencing. *Xist* lncRNA spreads over the entire length of the X chromosome from which it is transcribed, recruiting chromatin modifiers to induce epigenetic silencing of X-linked genes in cis (61). One remarkable feature of *Xist* is its ability to direct major 3D reorganization of the genome. Over the last decade, multiple efforts to understand the biological function of plant lncRNAs are shedding light into the role of these non-coding molecules in the regulation of epigenetic processes. Recent reviews have covered the challenges of lncRNA classification, the computational tools to detect them, as well as their roles in plant development and stress responses (62-67). In the current work, we plan to highlight examples of plant lncRNAs with demonstrated impact in epigenetic regulation (Table 1), such as mediating the deposition of chromatin marks, modulation of RNAPII dynamics and facilitating the formation and dissociation of chromatin loops.

Influence on histone modifications

LncRNAs have been proposed to function as scaffolds facilitating recruitment of chromatin modifiers. In the case of lncRNAs overlapping a coding gene (intragenic, intronic and antisense lncRNAs) that remain locally associated to the site of transcription, this “recruiter” function of the lncRNA could affect the local chromatin status either promoting or inhibiting transcription at the locus. Several examples of lncRNAs inducing epigenetic silencing by bringing Polycomb Repressive Complexes (PRC) to chromatin have been reported in eukaryotes, including plant species (68). Originally characterized in the fly *Drosophila melanogaster* and later in multiple eukaryotic species including plants, PRC1 and PRC2 complexes carry out silencing of developmental gene programs in a tissue specific and temporally regulated manner. While PRC1 catalyzes mono ubiquitination of histone H2A (H2Aub), PRC2 mediates tri-methylation of the lysine 27 on histone H3 (H3K27me3). These roles can also be overlapping, as for example LHP1 promotes both the spread PRC1 and PRC2 activity in Arabidopsis (69). Deposition of both PRC1 and PRC2 repressive marks has been associated with chromatin compaction and long-term epigenetic silencing (70). Despite their high spatio-temporal target selectivity, PRC expression is ubiquitous, thus relying on “recruiters” for target chromatin binding and gene silencing. The ability of sequence-specific DNA-binding proteins as well as of lncRNAs to guide PRC to chromatin have been documented in several species. In mammals, lncRNAs such as *Xist*, *HOTAIR*, *Braveheart* and *Kcnq1ot1* were shown to recruit PRC2 to chromatin to regulate developmental processes (reviewed in (71)). In line

with this, the first example of PRC2 recruitment by a lncRNA in plants was Arabidopsis *COLDAIR* (28). However, work over the last decade has demonstrated that PRC2 complex components may bind promiscuously to RNA molecules, challenging the "specific recruiter" model, and suggesting that lncRNA-PRC2 interactions would not be as widespread as originally expected. In fact, current models of lncRNA-PRC interactions propose that lncRNAs modulate PRC complex function rather than its recruitment (72).

In Arabidopsis, recruitment of the PRC2 H3K27me3 methyltransferase CURLY LEAF (CLF) by lncRNAs have been proposed for different developmental processes, including the floral transition and flower development (28, 30, 73). This is reminiscent of the recruitment of the mammalian H3K27me3 methyltransferase EZH2 by *Xist* (74). To promote spring flowering in Arabidopsis, the master floral repressor gene *FLOWERING LOCUS C* (*FLC*) needs to be stably repressed during winter. The acceleration of flowering by winter cold temperatures is known as vernalization, which is key to Brassicaceae such as Arabidopsis, as well as for winter cereals. A vernalization specific Plant Homeodomain (PHD) PRC2 (PHD-PRC2) complex assembles at the 5´ end of *FLC* gene during winter to deposit H3K27me3 for long-term gene silencing (75). A series of lncRNA transcripts are upregulated from the *FLC* locus upon exposure to cold, which coincides with transcriptional shutdown of *FLC* gene: the antisense lncRNA *COOLAIR* (22, 25, 32, 34, 37, 38, 45) and two sense lncRNAs, *COLD-ASSISTED INTRONIC NONCODING RNA* (*COLDAIR*) (28) and *COLDWRAP* (30), transcribed from the *FLC* intron 1 and promoter region, respectively. Both sense lncRNAs have been linked to recruitment of CLF to *FLC* during vernalization thus promoting accumulation of H3K27me3 (28, 30). In contrast, *COOLAIR* antisense promotes *FLC* transcriptional downregulation in response to cold but seems not to be required for guiding PHD-PRC2 to *FLC* chromatin.

Parallel and synergistic mechanisms have been proposed for *COOLAIR* transcriptional shutdown of sense *FLC*. First, transcription from *FLC* sense and antisense strands is mutually exclusive, suggesting that the act of *COOLAIR* transcription prevents activation of *FLC* sense promoter (34). Additionally, *COOLAIR* coordinates the switch from active (H3K36me3) to inactive (H3K27me3) chromatin states possibly through the stabilization of the H3K36me3 demethylation activity (22). More recent work has shown that *COOLAIR* promotes cold induction of nuclear condensates that contain the *FLC* transcriptional activator FRIGIDA (FRI) (76). Thus, sequestering away the FRI activator from the *FLC* locus during cold appears as an interesting mechanism for *COOLAIR*-induced *FLC* repression. In a cold-independent context, *FLC* transcription is quantitatively regulated by the co-transcriptional processing of *COOLAIR* antisense transcript which is linked to chromatin regulation. The proximal polyadenylation of *COOLAIR* results in a repressive chromatin environment that reduces *FLC* transcriptional initiation and elongation rates (77). This process requires the action of multiple components including the RNA-binding

protein FCA and the H3K4 demethylase LSD1 FLOWERING LOCUS D (FLD) (25, 78). Interestingly, *COOLAIR* 3′-end processing localize to nuclear bodies (79) suggesting that specialized subnuclear compartments may provide a platform for joint co-transcriptional RNA 3′ processing and histone modification mechanisms.

One additional lncRNA that has been shown to interact with CLF to regulate the plant reproductive transition is the sense intronic lncRNA AG-incRNA4 transcribed from the second intron of the *AGAMOUS* (*AG*) gene (73). *AG* is a MADS BOX transcription factor required for specification of stamens and carpels in Arabidopsis flowers. Given its unique function in development, *AG* expression is highly tissue specific and restricted to reproductive organs. In this context, *AG-incRNA4* lncRNA tightly regulates *AG* expression, securing repression of this floral gene in vegetative tissues.

Apart from the control of master developmental genes, PRC2 has been linked to the regulation of gene clusters in plants (80). In Arabidopsis, biosynthesis of marneral triterpene is carried out by enzymes encoded by genes organized in a small cluster (81). In aerial tissues, marneral cluster is immersed in a repressive chromatin environment covered by H3K27me3 and the PRC2-accessory protein LHP1. The phytohormone Abscisic Acid (ABA) triggers epigenetic reprogramming of the marneral cluster resulting in transcriptional activation of all loci in the cluster. ABA-induced local reduction of H3K27me3 accumulation and binding of LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) protein results in transcriptional activation of the genes in the marneral locus, a process that depends on the intergenic lncRNA *MARneral Silencing* (*MARS*) that maps the marneral cluster (35). Apart from its very well documented function as “reader” of H3K27me3 acting downstream from PRC2 (82), LHP1 associates with RNA molecules in vitro through its RNA-binding hinge domain, a region that is also important in vivo to repress Polycomb target genes (83). In line with this, *MARS* lncRNA has been shown to physically interact with LHP1 to modulate chromatin conformation of the marneral cluster (35). Interestingly, the analysis of *MARS*-LHP1 interaction has revealed a dosage-dependent mechanism, with the levels of *MARS* accumulation affecting LHP1 association to the marneral locus. While basal levels of *MARS* help recruitment of LHP1, low or high levels of *MARS* transcripts may titrate away LHP1 (35). Unlike previously expected, lncRNA activity is not limited to guiding silencing complexes to target regions but likely comprises a much more complex and dynamic mechanism of transient stabilization of chromatin modifiers over a specific site that depends on the local concentration of lncRNA molecules.

Regarding recruitment of chromatin activation complexes, *the MADS AFFECTING FLOWERING 4* (*MAF4*) *ANTISENSE* (*MAS*) lncRNA has been shown to bind and recruit to chromatin the WD REPEAT DOMAIN 5 (WDR5) component of the Arabidopsis

COMPASS-like complex (44). Both in animals and plants the RNA-binding protein WDR5 promotes H3K4me3 activity. Remarkably, mammalian WDR5 homolog interacts with lncRNAs including *HOTTIP*, *NeST* and *Evx1* (84-86). In Arabidopsis, *MAS* lncRNA localize to the nucleus and is produced in antisense position to the *FLC* homolog *MAF4*. Similar to antisense *COOLAIR* mapping to the *FLC* locus, *MAS* expression peaks in increasing weeks of winter cold. A key difference to *COOLAIR* is that *MAS* induces concordant activation of *MAF4* during cold. *MAS* remains attached to *MAF4* chromatin positively influencing transcriptional dynamics of *MAF4* locus likely by bringing the H3K4me3 activity associated to WDR5 (Figure 1A) (44). In rice, the ncRNA *LRK ANTISENSE INTERGENIC RNA* (*LAIR*) not only binds and recruits to chromatin the rice homolog OsWRD5, but also interacts directly with MALES ABSENT ON THE FIRST (OsMOF) protein, which is involved in H4K16 acetylation (40). The antisense lncRNA *LAIR* is relatively long (1.8 kb, 5´-capped and polyadenylated transcript with 6 exons) and maps at the 5´end of the *LRK* gene cluster in rice chromosome 2. Interestingly, the first gene in the cluster (*LRK1*) is fully immersed in *LAIR* intron 1. Overexpression of *LAIR* leads to transcriptional activation of LRK1 and LRK4 possibly through recruitment of OsMOF and OsWDR5. Thus, *LAIR* provides another example of an antisense lncRNA promoting a permissive chromatin environment thereby activating transcription of its sense coding gene. Moreover, *LAIR* represents another lncRNA capable of coordinating gene expression within a cluster.

In summary, plant lncRNAs are versatile molecules with the ability to recruit and induce local stabilization of different chromatin modifiers resulting in either gene activation or repression. What features of the lncRNAs at the sequence or structural level determine either function remains a very exciting and open question.

Modulation of RNAPII transcriptional activity

Besides modulation of the activity of chromatin modifiers, increasing evidence suggests that the act of lncRNA transcription per ser may exert a regulatory function directly impacting RNAPII activity. RNAPII transcription in eukaryotes proceeds through distinct stages (87-89). The first of these, initiation, involves recognition and opening of promoters by the preinitiation complex (PIC). This is followed by phosphorylation of the Ser5 residue of the carboxy-terminal domain (CTD) of RNAPII by the TFIIH subunit of the PIC. To promote transcriptional elongation, P-TEFb phosphorylates the Ser2 residue of the RNAPII CTD, as well as other factors involved in maintaining RNAPII promoter-proximal pausing. Finally, termination occurs either by its eviction by XRN2 after cleavage of the nascent transcript, or after a conformational change in RNAPII induced by the poly-A signal at the end of the gene. Various lncRNAs in non-plant models have been shown to

regulate different aspects of this process, such as by suppressing transcription initiation of genes (90, 91) or interfering with elongation (92, 93).

Transcriptional interference is a widely prevalent biological phenomenon whereby active transcription from one promoter can suppress in *cis* the transcription from a neighboring promoter. Efficient termination of RNAPII transcription of an upstream gene is one of the key processes to avoid transcription read-through, and hence transcriptional interference of a downstream locus (94). Intergenic lncRNAs can be a source of transcriptional interference when located upstream of a coding gene (95). An example of such a lncRNA in plants has yet been described, though examples of transcription interference derived from T-DNA insertions have been observed. In the last decades, Arabidopsis reverse genetic analyses have heavily relied on T-DNA insertion mutants. While T-DNA insertions over gene bodies often result in reduction of gene expression, promoter T-DNA insertions can either ectopically induce or disrupt expression of coding genes. One way of gene inactivation by T-DNA insertions is transcriptional interference: aberrant transcripts generated from T-DNA insertions upstream of the Arabidopsis *RFD1* gene (*T-DNA-RFD* lncRNA) and *QUA1* gene (*T-DNA-QUA1* lncRNA) inhibit transcription initiation from the *RFD1* and *QUA1* TSSs, respectively, likely through transcriptional interference (96, 97). These T-DNA driven lncRNAs are chimeras of T-DNA plus promoter (intergenic) sequences followed by the downstream mRNA. Interestingly, in the case of *RFD1* gene, T-DNA insertions located over the 5´UTR do not inhibit transcription. Instead, transcription from the upstream T-DNA inactivates the *RFD1* TSS (96). This mechanism of T-DNA-lncRNA-dependent downstream promoter repression may be driven by co-transcriptional histone modifications (97). Each RNAPII stage is characterized by the co-transcriptional recruitment of factors involved in nascent RNA processing and specific chromatin modifications (98). For example, methylation of H3K4 exhibits a distinct profile that correlates with the different phases of transcription, with enrichment of H3K4me3 at the TSS (initiation), H3K4me2 over the gene body (elongation) and H3K4me1 towards the 3´end (termination). The proposed mechanism for silencing of *QUA1* and *RFD1* by upstream lncRNAs is the switch from promoter- to elongation-type co-transcriptional histone marks (97). RNAPII initiates transcription from the upstream T-DNA insertions, elongating through the promoter and coding sequences of *QUA1* and *RFD1,* thus suppressing the TSSs of both genes at least partly via the activity of the FACT complex.

Convergent antisense transcription can likewise interfere with RNAPII transcription of genes. A prominent case is the light-dependent transcription of the antisense lncRNA *qrf* leads to early transcriptional termination of the clock gene *rfq* in the fungus *Neurospora* (93). Specific examples of lncRNAs involved in such processes have also been characterized in Arabidopsis, including the lncRNA *SVALKA* (*SVK*). Exposure to cold triggers rapid activation of the C-REPEAT/DEHYDRATION-RESPONSIVE ELEMENT

BINDING FACTORS (CBFs) transcription factors which promote tolerance to cold temperatures by activating downstream cold-responsive genes (99). *SVK* is a cold-inducible intergenic lncRNA located downstream and head-to-head to the *CBF1* gene (Figure 1B) (11). *CBF* transcripts are only transiently activated, reaching high levels of expression a few hours after start of cold treatment and then returning to basal, almost undetectable levels. This time-dependent regulation of *CBF* expression is partly due to the production of lncRNAs. Transcriptional read-through of *SVK* results in the expression of a cryptic antisense *CBF1* lncRNA (*asCBF1*) from the 3´UTR of *CBF1*. *asCBF1* transcription results in RNAPII collision thereby repressing the expression of full-length *CBF1*. Thus, production of two antisense lncRNAs during cold limit the production of maximal *CBF1* mRNA levels by an RNAPII collision mechanism over the *CBF*1 locus.

Several other plant antisense lncRNAs with functional importance (but for which their mechanisms remain to be fully understood) share similar patterns of anticorrelation with their sense genes which may in part be as a result of RNAPII interference. Examples include, *CDF5 LONG NONCODING RNA* (*FLORE*), which is antisense to the circadian clock gene *CYCLING DOF FACTOR 5* (*CDF5*) and regulates its phasic expression pattern (27); the seed-maturation *DELAY OF GERMINATION 1* (*DOG1*) gene antisense *1GOD*, which represses *DOG1* expression during seed maturation (26, 43); a convergent isoform of *COOLAIR*, transcribed from the first intron of *FLC* (45); and *SUF*, an antisense lncRNA expressed in male tissues to suppress the female sexual differentiation regulator *MpFGMYB* in *Marchantia polymorpha* (29). Additional work is needed to clarify their modes of regulation, as well as to characterize the large numbers of discovered antisense lncRNAs in plants (44, 100)

Mediating chromosomal looping dynamics

3D genome organization plays a significant role in regulating gene expression. In metazoan genomes, chromosomal loci tens of kilobases apart come into contact and form topologically associated domains (TADs) and constrain epigenetic marks to specific parts of the genome (101). TAD looping (and additionally sub-TAD chromatin looping) events driven by pioneer transcription factors also bring distal regulatory elements near promoters and subsequently activate gene expression (102, 103). Seminal studies in human disease also demonstrated the importance of lncRNAs in this area, revealing them to be essential for the formation and maintenance of specific chromatin loops involved in activating gene expression (104, 105). In plants, HiC analyses of crop species including maize, tomato, sorghum, foxtail millet, rice and wheat confirmed the presence of TAD-like features within their genomes, though sub-TAD chromatin loops could only be confirmed in the plants with the largest genomes: maize, rice, tomato and wheat (106-

109). Currently little is known about the possible role of lncRNAs in regulating chromatin loops in these plants, in part owing to the limited availability of molecular techniques.

Comparatively, the plant Arabidopsis has been much more extensively studied. Likely owing to its smaller genome, early studies utilizing 4C and HiC techniques revealed that the bulk of chromatin interactions occur primarily at pericentromeric heterochromatin and telomeres and failed to identify clear TAD-like structures (110-112). Regardless, chromatin loops were found to be abundant between heterochromatin islands containing H3K27me3 or H3K9me2 within euchromatin, which was supported by subsequent HiC experiments in Arabidopsis (113-115). H3K27me3-HiChIP experiments later revealed in greater resolution how this mark plays a significant role in mediating chromatin loop formation and primarily producing tissue-specific Polycomb-dependent compartments leading to gene repression (116). This class of chromatin interaction was also observed in maize, rice, and wheat (106, 108, 117). In parallel with this research, several studies were published which revealed the role of lncRNAs in mediating the formation and maintenance of individual instances of these H3K27me3-dependent loops in Arabidopsis.

The first of these was the lncRNA *APOLO*. After observing that it was coregulated with the approximately 5 kilobase upstream auxin transport regulator gene *PINOID* (*PID*) during auxin treatment, 3C experiments revealed the existence of a chromatin loop between the *APOLO* locus and the 5' region of *PID* (20). These contact regions were marked with H3K27me3 and DNA methylation, the former of which decreased alongside contact frequency in *lhp1* mutants. ChIRP-qPCR experiments revealed that *APOLO* was bound to both contact sites, and RIP-qPCR experiments further showed it interacted with LHP1. Studying loop dynamics during auxin treatment and in various mutant and transgenic lines revealed a mechanism whereby an initial repressive loop was quickly relaxed via loss of DNA methylation and LHP1 binding in a RNAPII-transcribed *APOLO*-dependent manner, leading to elevated RNAPII levels at the *PID* locus and increased transcription, followed by *APOLO*-dependent closing of the loop once again at 24h by an increase in DNA methylation (via RNA POLYMERASE V (RNAPV)-transcribed *APOLO*) and LHP1 binding (Figure 1C) (20). A follow-up study provided further details on the mechanism by showing that an essential TTCTTC element within the *APOLO* sequence allowed for the formation of R-loops at contact sites via complementary binding to GAAGAA sequences in the genome, and furthermore revealed using overexpression lines of *APOLO* that increases in *APOLO* levels led to a decrease in LHP1 binding by decoying it away from its targets (118). In addition, the authors found several additional auxin-related putative target genes and confirmed this for *WAVY ROOT GROWTH* (*WAG2*) and *AZA-GUANINE RESISTANT 2* (*AZG2*), suggesting a wider role for *APOLO* in regulating auxin response (118). This role was further expanded to root hair elongation, as some of its putative targets included root hair development related genes (118).

Additional studies confirmed that two such genes had similar *APOLO* target dynamics (this time in cold-treated plants): *ROOT HAIR DEFECTIVE 6* (*RHD6*) and *EXTENSIN 3* (*EXT3*), though now it involved an interaction with the transcription factor WRKY42, suggesting these two may be working together alongside the target transcription factor *RHD6* to regulate many root hair elongation processes (119, 120). *APOLO* functional characterization was also extended to show that in the case of the auxin biosynthesis gene *YUCCA2*, it can interact with the DNA methylation regulator VARIANT IN METHYLATION 1 (VIM1) in addition to LHP1 and modulate both DNA methylation and H3K27me3 deposition at the *YUCCA2* promoter (121).

Another important example of a lncRNA mediating a repressive chromatin loop was discovered by (30), who characterized *COLDWRAP* as being transcribed in the sense direction 225 base pairs upstream of *FLC* using RT-qPCR and interacting with CLF using RIP-RT-PCR during vernalization. RNA binding assays demonstrated its interaction with CLF occurred at the 5' end and was dependent on its secondary structure, and decreased H3K27me3 accumulation over *FLC* in *COLDWRAP* RNAi lines suggested this interaction was necessary for proper CLF activity (30). Importantly, the authors found a *COLDWRAP*-dependent chromatin loop occurred the *FLC* promoter and the 3' end of its first intron, and that this loop (as well as its interaction with CLF) could be maintained by *COLDWRAP* in trans using transgenic *COLDWRAP* and FLC lines (30). This suggests a similar mechanism to the loop reforming dynamics of *APOLO*, whereby the loop is closed in part by *APOLO* interacting with LHP1 and increasing H3K27me3 levels at the contact loci (20, 118).

Finally, in an example of mediating a chromatin loop instead associated with gene activation, the lncRNA *MARS* was recently characterized as facilitating the formation of a loop between the *MRN1* gene and a distal enhancer (35). As mentioned above, *MRN1* is part of the marneral gene cluster including *CYP705A12* and *MRO*; the *MARS* locus is also found within it. Plant biosynthetic gene clusters generally share a common transcriptionally active or inactive environment via 3D compartmentalization (122). Accordingly, (35) found that upon ABA treatment, a loop formed between *MRN1* and an upstream intergenic region in the middle of the gene cluster, specifically between *MRO* and *MARS*. *MARS* RNAi lines demonstrated that the formation of this loop was dependent on increased transcription of *MARS* which then decoys away LHP1, leading to loss of H3K27me3, decompaction of the chromatin and loop formation (35). The formation of this loop is accompanied with increased transcription of the cluster genes, suggesting the intergenic is acting as an enhancer; indeed, cloning this region into a minimal 35S promoter system demonstrated it was sufficient to increase expression of the associated GUS reporter (35). Interestingly, the authors also showed that under normal conditions basal levels of *MARS* instead promote LHP1 binding to the gene cluster and subsequent

H3K27me3 deposition, thus compacting the chromatin and preventing loop formation, suggesting a dual mechanism of loop control similar to what was described for *APOLO* (20, 35, 118).

As our knowledge of RNA-DNA interactions and chromatin loops increases, opportunities for future studies to explore specific lncRNA-chromatin loop dynamics increase. Many recent large-scale studies in plants of R-loops (123, 124), RNA-DNA interactions (125) chromatin looping as well as PRC2-mediated chromatin looping (69, 106, 116) are revealing many new possible candidate lncRNA-loop regulatory dynamics for investigation.

Conclusions

While the number of mechanistic studies on plant lncRNAs have significantly increased over recent years, we are still at the early days of fully understanding the potential of these non-coding transcripts in regulating biological processes. Some clear examples as the ones described above argue against the idea of lncRNAs being just transcriptional noise. Yet, the many thousands of natural antisense lncRNAs detected in plant genomes (44, 100) open the question of how many actually affect the expression of their sense genes. Additional lncRNAs will have to be characterized in depth to define conserved mechanisms of epigenetic regulation of transcription by lncRNAs in plants.

The capacity of lncRNAs to modulate chromatin structure has been recognized for several years. However, the original view of lncRNAs as recruiters of chromatin modifiers could now be upgraded to more dynamic mechanisms that involve the transient stabilization of epigenetic modulators over specific regions in the genome. Strikingly, this role as stabilizers of chromatin complexes depends not only on the local accumulation of lncRNAs but also on the concentration of lncRNA molecules. While small amounts of lncRNA transcripts may transiently retain protein complexes at specific sites, over-production of these molecules act as molecular barriers sequestering away chromatin modifiers. This dual regulatory role characterized for *MARS* lncRNA in Arabidopsis (35) had previously been proposed for mammalian lncRNAs interacting with PRC2 complexes (126). RNA invasion of DNA via formation of R-loops both locally (37) or even at distant loci in the genome (118) appears as another common mechanism that may help to stabilize or decoy away epigenetic regulators. As research on biomolecular condensates continues to expand, we will be learning about new examples of lncRNAs participating in formation of membraneless subcellular components. In plants, *COOLAIR* antisense RNA stands out in this field by being able to induce formation of nuclear condensates that sequester *FLC* sense positive regulators thus triggering transcriptional silencing (76). Additionally, our understanding of 3D chromatin organization in plant genomes is steadily

growing and the role of lncRNAs in mediating chromatin looping remains an area of research with great potential for additional discovery. Prominent examples such as *APOLO* and *MARS* have demonstrated the essential role of lncRNAs in controlling the formation of repressive and activating chromatin loops (35, 118).

Regardless of the mechanism, it has become clear that lncRNAs are key factors in plant transcriptional modulation with significant impacts in both development and stress. This presents many opportunities to exploit these modes of regulation for fine-tuning plant transcription in agronomic contexts. Future work is needed in this area to help overcome the challenges associated in translating all this fundamental research on lncRNAs into agronomical applications.

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References

1. Anderson, D. M., Anderson, K. M., Chang, C.-L., Makarewich, C. A., Nelson, B. R., et al. (2015) A Micropeptide Encoded by a Putative Long Noncoding RNA Regulates Muscle Performance. Cell 160, 595–606. DOI: 10.1016/j.cell.2015.01.009
2. Bardou, F., Merchan, F., Ariel, F., and Crespi, M. (2011) Dual RNAs in plants. Biochimie 93, 1950–1954. DOI: 10.1016/j.biochi.2011.07.028
3. Huang, J.-Z., Chen, M., Chen, D., Gao, X.-C., Zhu, S., et al. (2017) A Peptide Encoded by a Putative lncRNA HOXB-AS3 Suppresses Colon Cancer Growth. Molecular Cell 68, 171-184.e6. DOI: 10.1016/j.molcel.2017.09.015
4. Hubé, F., Velasco, G., Rollin, J., Furling, D., and Francastel, C. (2010) Steroid receptor RNA activator protein binds to and counteracts SRA RNA-mediated activation of MyoD and muscle differentiation. Nucleic Acids Research 39, 513–525. DOI: 10.1093/nar/gkq833
5. Verheggen, K., Volders, P.-J., Mestdagh, P., Menschaert, G., Van Damme, P., et al. (2017) Noncoding after All: Biases in Proteomics Data Do Not Explain Observed Absence of lncRNA Translation Products. Journal of Proteome Research 16, 2508–2515. DOI: 10.1021/acs.jproteome.7b00085
6. Marguerat, S., Schmidt, A., Codlin, S., Chen, W., Aebersold, R., et al. (2012) Quantitative Analysis of Fission Yeast Transcriptomes and Proteomes in

Proliferating and Quiescent Cells. Cell 151, 671–683. DOI: 10.1016/j.cell.2012.09.019
7. Frankish, A., Diekhans, M., Jungreis, I., Lagarde, J., Loveland, J. E., et al. (2020) GENCODE 2021. Nucleic Acids Research 49, D916–D923. DOI: 10.1093/nar/gkaa1087
8. Di Marsico, M., Paytuvi Gallart, A., Sanseverino, W., and Aiese Cigliano, R. (2021) GreeNC 2.0: a comprehensive database of plant long non-coding RNAs. Nucleic Acids Research 50, D1442–D1447. DOI: 10.1093/nar/gkab1014
9. Szcześniak, M. W., Bryzghalov, O., Ciomborowska-Basheer, J., and Makałowska, I. (2019) CANTATAdb 2.0: Expanding the Collection of Plant Long Noncoding RNAs. Methods in Molecular Biology, 415–429. DOI: 10.1007/978-1-4939-9045-0_26
10. Statello, L., Guo, C.-J., Chen, L.-L., and Huarte, M. (2020) Gene regulation by long non-coding RNAs and its biological functions. Nature Reviews Molecular Cell Biology 22, 96–118. DOI: 10.1038/s41580-020-00315-9
11. Kindgren, P., Ard, R., Ivanov, M., and Marquardt, S. (2018) Transcriptional read-through of the long non-coding RNA SVALKA governs plant cold acclimation. Nature Communications 9. DOI: 10.1038/s41467-018-07010-6
12. Kong, X., Wang, H., Zhang, M., Chen, X., Fang, R., et al. (2022) A SA-regulated lincRNA promotes Arabidopsis disease resistance by modulating pre-rRNA processing. Plant Science 316, 111178. DOI: 10.1016/j.plantsci.2022.111178
13. Qin, T., Zhao, H., Cui, P., Albesher, N., and Xiong, L. (2017) A Nucleus-Localized Long Non-Coding RNA Enhances Drought and Salt Stress Tolerance. Plant Physiology 175, 1321–1336. DOI: 10.1104/pp.17.00574
14. Seo, J. S., Diloknawarit, P., Park, B. S., and Chua, N. (2018) ELF18-INDUCED LONG NONCODING RNA 1 evicts fibrillarin from mediator subunit to enhance PATHOGENESIS-RELATED GENE 1 (PR1) expression. New Phytologist 221, 2067–2079. DOI: 10.1111/nph.15530
15. Wunderlich, M., Groß-Hardt, R., and Schöffl, F. (2014) Heat shock factor HSFB2a involved in gametophyte development of Arabidopsis thaliana and its expression is controlled by a heat-inducible long non-coding antisense RNA. Plant Molecular Biology 85, 541–550. DOI: 10.1007/s11103-014-0202-0
16. Franco-Zorrilla, J. M., Valli, A., Todesco, M., Mateos, I., Puga, M. I., et al. (2007) Target mimicry provides a new mechanism for regulation of microRNA activity. Nature Genetics 39, 1033–1037. DOI: 10.1038/ng2079
17. Liu, C., Muchhal, U. S., and Raghothama*, K. G. (1997). Plant Molecular Biology 33, 867–874. DOI: 10.3389/fpls.2022.906603
18. Liu, F., Xu, Y., Chang, K., Li, S., Liu, Z., et al. (2019) The long noncoding RNAT5120 regulates nitrate response and assimilation in Arabidopsis. New Phytologist 224, 117–131. DOI: 10.1111/nph.16038
19. Wang, T., Xing, J., Liu, Z., Zheng, M., Yao, Y., et al. (2019) Histone acetyltransferase GCN5-mediated regulation of long non-coding RNA At4 contributes to phosphate starvation response in Arabidopsis. Journal of Experimental Botany 70, 6337–6348. DOI: 10.1093/jxb/erz359
20. Ariel, F., Jegu, T., Latrasse, D., Romero-Barrios, N., Christ, A., et al. (2014) Noncoding Transcription by Alternative RNA Polymerases Dynamically Regulates

an Auxin-Driven Chromatin Loop. Molecular Cell 55, 383–396. DOI: 10.1016/j.molcel.2014.06.011
21. Bardou, F., Ariel, F., Simpson, C. G., Romero-Barrios, N., Laporte, P., et al. (2014) Long Noncoding RNA Modulates Alternative Splicing Regulators in Arabidopsis. Developmental Cell 30, 166–176. DOI: 10.1016/j.devcel.2014.06.017
22. Csorba, T., Questa, J. I., Sun, Q., and Dean, C. (2014) Antisense COOLAIR mediates the coordinated switching of chromatin states at FLC during vernalization. Proceedings of the National Academy of Sciences 111, 16160–16165. DOI: 10.1073/pnas.1419030111
23. Ding, J., Lu, Q., Ouyang, Y., Mao, H., Zhang, P., et al. (2012) A long noncoding RNA regulates photoperiod-sensitive male sterility, an essential component of hybrid rice. Proceedings of the National Academy of Sciences 109, 2654–2659. DOI: 10.1073/pnas.1121374109
24. Fajkus, P., Peška, V., Závodník, M., Fojtová, M., Fulnečková, J., et al. (2019) Telomerase RNAs in land plants. Nucleic Acids Research 47, 9842–9856. DOI: 10.1093/nar/gkz695
25. Fang, X., Wu, Z., Raitskin, O., Webb, K., Voigt, P., et al. (2020) The 3′ processing of antisense RNAs physically links to chromatin-based transcriptional control. Proceedings of the National Academy of Sciences 117, 15316–15321. DOI: 10.1073/pnas.2007268117
26. Fedak, H., Palusinska, M., Krzyczmonik, K., Brzezniak, L., Yatusevich, R., et al. (2016) Control of seed dormancy in Arabidopsis by a cis -acting noncoding antisense transcript. Proceedings of the National Academy of Sciences 113. DOI: 10.1073/pnas.1608827113
27. Henriques, R., Wang, H., Liu, J., Boix, M., Huang, L., et al. (2017) The antiphasic regulatory module comprising CDF5 and its antisense RNA FLORE links the circadian clock to photoperiodic flowering. New Phytologist 216, 854–867. DOI: 10.1111/nph.14703
28. Heo, J. B., and Sung, S. (2011) Vernalization-Mediated Epigenetic Silencing by a Long Intronic Noncoding RNA. Science 331, 76–79. DOI: 10.1126/science.1197349
29. Hisanaga, T., Okahashi, K., Yamaoka, S., Kajiwara, T., Nishihama, R., et al. (2019) A cis -acting bidirectional transcription switch controls sexual dimorphism in the liverwort. The EMBO Journal 38. DOI: 10.15252/embj.2018100240
30. Kim, D.-H., and Sung, S. (2017) Vernalization-Triggered Intragenic Chromatin Loop Formation by Long Noncoding RNAs. Developmental Cell 40, 302-312.e4. DOI: 10.1016/j.devcel.2016.12.021
31. Kramer, M. C., Kim, H. J., Palos, K. R., Garcia, B. A., Lyons, E., et al. (2022) A Conserved Long Intergenic Non-coding RNA Containing snoRNA Sequences, lncCOBRA1, Affects Arabidopsis Germination and Development. Frontiers in Plant Science 13. DOI: 10.3389/fpls.2022.906603
32. Liu, F., Marquardt, S., Lister, C., Swiezewski, S., and Dean, C. (2010) Targeted 3′ Processing of Antisense Transcripts Triggers Arabidopsis FLC Chromatin Silencing. Science 327, 94–97. DOI: 10.1126/science.1180278

33. Rigo, R., Bazin, J., Romero-Barrios, N., Moison, M., Lucero, L., et al. (2020) The Arabidopsis lncRNA ASCO modulates the transcriptome through interaction with splicing factors. EMBO reports 21. DOI: 10.15252/embr.201948977
34. Rosa, S., Duncan, S., and Dean, C. (2016) Mutually exclusive sense–antisense transcription at FLC facilitates environmentally induced gene repression. Nature Communications 7. DOI: 10.1038/ncomms13031
35. Roulé, T., Christ, A., Hussain, N., Huang, Y., Hartmann, C., et al. (2022) The lncRNA MARS modulates the epigenetic reprogramming of the marneral cluster in response to ABA. Molecular Plant 15, 840–856. DOI: 10.1016/j.molp.2022.02.007
36. Sousa, C., Johansson, C., Charon, C., Manyani, H., Sautter, C., et al. (2001) Translational and Structural Requirements of the Early Nodulin Gene enod40 , a Short-Open Reading Frame-Containing RNA, for Elicitation of a Cell-Specific Growth Response in the Alfalfa Root Cortex. Molecular and Cellular Biology 21, 354–366. DOI: 10.1128/MCB.21.1.354-366.2001
37. Sun, Q., Csorba, T., Skourti-Stathaki, K., Proudfoot, N. J., and Dean, C. (2013) R-Loop Stabilization Represses Antisense Transcription at the Arabidopsis FLC Locus. Science 340, 619–621. DOI: 10.1126/science.1234848
38. Swiezewski, S., Liu, F., Magusin, A., and Dean, C. (2009) Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. Nature 462, 799–802. DOI: 10.1038/nature08618
39. Wang, Y., Fan, X., Lin, F., He, G., Terzaghi, W., et al. (2014) Arabidopsis noncoding RNA mediates control of photomorphogenesis by red light. Proceedings of the National Academy of Sciences 111, 10359–10364. DOI: 10.1073/pnas.1409457111
40. Wang, Y., Luo, X., Sun, F., Hu, J., Zha, X., et al. (2018) Overexpressing lncRNA LAIR increases grain yield and regulates neighbouring gene cluster expression in rice. Nature Communications 9. DOI: 10.1038/s41467-018-05829-7
41. Wu, J., Liu, C., Liu, Z., Li, S., Li, D., et al. (2018) Pol III-Dependent Cabbage BoNR8 Long ncRNA Affects Seed Germination and Growth in Arabidopsis. Plant and Cell Physiology 60, 421–435. DOI: 10.1093/pcp/pcy220
42. Wu, Z., Fang, X., Zhu, D., and Dean, C. (2019) Autonomous Pathway: FLOWERING LOCUS C Repression through an Antisense-Mediated Chromatin-Silencing Mechanism. Plant Physiology 182, 27–37. DOI: 10.1104/pp.19.01009
43. Yatusevich, R., Fedak, H., Ciesielski, A., Krzyczmonik, K., Kulik, A., et al. (2017) Antisense transcription represses Arabidopsis seed dormancy QTL DOG1 to regulate drought tolerance. EMBO reports 18, 2186–2196. DOI: 10.15252/embr.201744862
44. Zhao, X., Li, J., Lian, B., Gu, H., Li, Y., et al. (2018) Global identification of Arabidopsis lncRNAs reveals the regulation of MAF4 by a natural antisense RNA. Nature Communications 9. DOI: 10.1038/s41467-018-07500-7
45. Zhao, Y., Zhu, P., Hepworth, J., Bloomer, R., Antoniou-Kourounioti, R. L., et al. (2021) Natural temperature fluctuations promote COOLAIR regulation of FLC. Genes & Development 35, 888–898. DOI: 10.1101/gad.348362.121
46. Zhou, H., Liu, Q., Li, J., Jiang, D., Zhou, L., et al. (2012) Photoperiod- and thermo-sensitive genic male sterility in rice are caused by a point mutation in a novel

noncoding RNA that produces a small RNA. Cell Research 22, 649–660.DOI: 10.1038/cr.2012.28
47. Cabili, M. N., Trapnell, C., Goff, L., Koziol, M., Tazon-Vega, B., et al. (2011) Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes & Development 25, 1915–1927. DOI: 10.1101/gad.17446611
48. Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Djebali, S., et al. (2012) The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Research 22, 1775–1789. DOI: 10.1101/gr.132159.111
49. Lubelsky, Y., and Ulitsky, I. (2018) Sequences enriched in Alu repeats drive nuclear localization of long RNAs in human cells. Nature 555, 107–111. DOI: 10.1038/nature25757
50. Aillaud, M., and Schulte, L. N. (2020) Emerging Roles of Long Noncoding RNAs in the Cytoplasmic Milieu. Non-Coding RNA 6, 44. DOI: 10.3390/ncrna6040044
51. Hsu, P. Y., Calviello, L., Wu, H.-Y. L., Li, F.-W., Rothfels, C. J., et al. (2016) Super-resolution ribosome profiling reveals unannotated translation events in Arabidopsis. Proceedings of the National Academy of Sciences 113. DOI: 10.1073/pnas.1614788113
52. Camilleri-Robles, C., Amador, R., Klein, C. C., Guigó, R., Corominas, M., et al. (2022) Genomic and functional conservation of lncRNAs: lessons from flies. Mammalian Genome 33, 328–342. DOI: 10.1007/s00335-021-09939-4
53. Hawkes, E. J., Hennelly, S. P., Novikova, I. V., Irwin, J. A., Dean, C., et al. (2016) COOLAIR Antisense RNAs Form Evolutionarily Conserved Elaborate Secondary Structures. Cell Reports 16, 3087–3096. DOI: 10.1016/j.celrep.2016.08.045
54. Banani, S. F., Lee, H. O., Hyman, A. A., and Rosen, M. K. (2017) Biomolecular condensates: organizers of cellular biochemistry. Nature Reviews Molecular Cell Biology 18, 285–298. DOI: 10.1038/nrm.2017.7
55. Roden, C., and Gladfelter, A. S. (2020) RNA contributions to the form and function of biomolecular condensates. Nature Reviews Molecular Cell Biology 22, 183–195. DOI: 10.1038/s41580-020-0264-6
56. Unfried, J. P., and Ulitsky, I. (2022) Substoichiometric action of long noncoding RNAs. Nature Cell Biology 24, 608–615. DOI: 10.1038/s41556-022-00911-1
57. Wu, M., Yang, L.-Z., and Chen, L.-L. (2021) Long noncoding RNA and protein abundance in lncRNPs. RNA 27, 1427–1440. DOI: 10.1261/rna.078971.121
58. Klemm, S. L., Shipony, Z., and Greenleaf, W. J. (2019) Chromatin accessibility and the regulatory epigenome. Nature Reviews Genetics 20, 207–220. DOI: 10.1038/s41576-018-0089-8
59. Allis, C. D., and Jenuwein, T. (2016) The molecular hallmarks of epigenetic control. Nature Reviews Genetics 17, 487–500. DOI: 10.1038/nrg.2016.59
60. Kan, R. L., Chen, J., and Sallam, T. (2022) Crosstalk between epitranscriptomic and epigenetic mechanisms in gene regulation. Trends in Genetics 38, 182–193. DOI: 10.1016/j.tig.2021.06.014
61. Brockdorff, N., Bowness, J. S., and Wei, G. (2020) Progress toward understanding chromosome silencing by Xist RNA. Genes & Development 34, 733–744. DOI: 10.1101/gad.337196.120

62. Budak, H., Kaya, S. B., and Cagirici, H. B. (2020) Long Non-coding RNA in Plants in the Era of Reference Sequences. Frontiers in Plant Science 11. DOI: 10.3389/fpls.2020.00276
63. Chen, L., Zhu, Q.-H., and Kaufmann, K. (2020) Long non-coding RNAs in plants: emerging modulators of gene activity in development and stress responses. Planta 252. DOI: 10.1007/s00425-020-03480-5
64. Jampala, P., Garhewal, A., and Lodha, M. (2021) Functions of long non-coding RNA in Arabidopsis thaliana. Plant Signaling & Behavior 16, 1925440. DOI: 10.1080/15592324.2021.1925440
65. Wang, H.-L. V., and Chekanova, J. A. (2017) Long Noncoding RNAs in Plants. Advances in Experimental Medicine and Biology, 133–154. DOI: 10.1007/978-981-10-5203-3_5
66. Waseem, M., Liu, Y., and Xia, R. (2020) Long Non-Coding RNAs, the Dark Matter: An Emerging Regulatory Component in Plants. International Journal of Molecular Sciences 22, 86. DOI: 10.3390/ijms22010086
67. Wierzbicki, A. T., Blevins, T., and Swiezewski, S. (2021) Long Noncoding RNAs in Plants. Annual Review of Plant Biology 72, 245–271. DOI: 10.1146/annurev-arplant-093020-035446
68. Brockdorff, N. (2013) Noncoding RNA and Polycomb recruitment. RNA 19, 429–442. DOI: 10.1261/rna.037598.112
69. Veluchamy, A., Jégu, T., Ariel, F., Latrasse, D., Mariappan, K. G., et al. (2016) LHP1 Regulates H3K27me3 Spreading and Shapes the Three-Dimensional Conformation of the Arabidopsis Genome. PLOS ONE 11, e0158936. DOI: 10.1371/journal.pone.0158936
70. Blackledge, N. P., and Klose, R. J. (2021) The molecular principles of gene regulation by Polycomb repressive complexes. Nature Reviews Molecular Cell Biology 22, 815–833. DOI: 10.1038/s41580-021-00398-y
71. Davidovich, C., and Cech, T. R. (2015) The recruitment of chromatin modifiers by long noncoding RNAs: lessons from PRC2. RNA 21, 2007–2022. DOI: 10.1261/rna.053918.115
72. Almeida, M., Bowness, J. S., and Brockdorff, N. (2020) The many faces of Polycomb regulation by RNA. Current Opinion in Genetics & Development 61, 53–61. DOI: 10.1016/j.gde.2020.02.023
73. Wu, H.-W., Deng, S., Xu, H., Mao, H.-Z., Liu, J., et al. (2018) A noncoding RNA transcribed from the AGAMOUS (AG) second intron binds to CURLY LEAF and represses AG expression in leaves. New Phytologist 219, 1480–1491. DOI: 10.1111/nph.15231
74. Zhao, J., Sun, B. K., Erwin, J. A., Song, J.-J., and Lee, J. T. (2008) Polycomb Proteins Targeted by a Short Repeat RNA to the Mouse X Chromosome. Science 322, 750–756. DOI: 10.1126/science.1163045
75. Costa, S., and Dean, C. (2019) Storing memories: the distinct phases of Polycomb-mediated silencing of Arabidopsis FLC. Biochemical Society Transactions 47, 1187–1196. DOI: 10.1042/BST20190255
76. Zhu, P., Lister, C., and Dean, C. (2021) Cold-induced Arabidopsis FRIGIDA nuclear condensates for FLC repression. Nature 599, 657–661. DOI: 10.1038/s41586-021-04062-5

77. Wu, Z., Ietswaart, R., Liu, F., Yang, H., Howard, M., et al. (2015) Quantitative regulation of FLC via coordinated transcriptional initiation and elongation. Proceedings of the National Academy of Sciences 113, 218–223. DOI: 10.1073/pnas.1518369112
78. Liu, F., Quesada, V., Crevillén, P., Bäurle, I., Swiezewski, S., et al. (2007) The Arabidopsis RNA-Binding Protein FCA Requires a Lysine-Specific Demethylase 1 Homolog to Downregulate FLC. Molecular Cell 28, 398–407. DOI: 10.1016/j.molcel.2007.10.018
79. Fang, X., Wang, L., Ishikawa, R., Li, Y., Fiedler, M., et al. (2019) Arabidopsis FLL2 promotes liquid–liquid phase separation of polyadenylation complexes. Nature 569, 265–269. DOI: 10.1038/s41586-019-1165-8
80. Yu, N., Nützmann, H.-W., MacDonald, J. T., Moore, B., Field, B., et al. (2016) Delineation of metabolic gene clusters in plant genomes by chromatin signatures. Nucleic Acids Research 44, 2255–2265. DOI: 10.1093/nar/gkw100
81. Nützmann, H., Huang, A., and Osbourn, A. (2016) Plant metabolic clusters – from genetics to genomics. New Phytologist 211, 771–789. DOI: 10.1111/nph.13981
82. Turck, F., Roudier, F., Farrona, S., Martin-Magniette, M.-L., Guillaume, E., et al. (2007) Arabidopsis TFL2/LHP1 Specifically Associates with Genes Marked by Trimethylation of Histone H3 Lysine 27. PLoS Genetics 3, e86. DOI: 10.1371/journal.pgen.0030086
83. Berry, S., Rosa, S., Howard, M., Bühler, M., and Dean, C. (2017) Disruption of an RNA-binding hinge region abolishes LHP1-mediated epigenetic repression. Genes & Development 31, 2115–2120. DOI: 10.1101/gad.305227.117
84. Gomez, J. A., Wapinski, O. L., Yang, Y. W., Bureau, J.-F., Gopinath, S., et al. (2013) The NeST Long ncRNA Controls Microbial Susceptibility and Epigenetic Activation of the Interferon-γ Locus. Cell 152, 743–754. DOI: 10.1016/j.cell.2013.01.015
85. Luo, S., Lu, J. Y., Liu, L., Yin, Y., Chen, C., et al. (2016) Divergent lncRNAs Regulate Gene Expression and Lineage Differentiation in Pluripotent Cells. Cell Stem Cell 18, 637–652. DOI: 10.1016/j.stem.2016.01.024
86. Yang, Y. W., Flynn, R. A., Chen, Y., Qu, K., Wan, B., et al. (2014) Essential role of lncRNA binding for WDR5 maintenance of active chromatin and embryonic stem cell pluripotency. eLife 3. DOI: 10.7554/eLife.02046
87. Chen, F. X., Smith, E. R., and Shilatifard, A. (2018) Born to run: control of transcription elongation by RNA polymerase II. Nature Reviews Molecular Cell Biology 19, 464–478. DOI: 10.1038/s41580-018-0010-5
88. Kuehner, J. N., Pearson, E. L., and Moore, C. (2011) Unravelling the means to an end: RNA polymerase II transcription termination. Nature Reviews Molecular Cell Biology 12, 283–294. DOI: https://doi.org/10.1038/nrm3098
89. Schier, A. C., and Taatjes, D. J. (2020) Structure and mechanism of the RNA polymerase II transcription machinery. Genes & Development 34, 465–488. DOI: 10.1101/gad.335679.119
90. Latos, P. A., Pauler, F. M., Koerner, M. V., Şenergin, H. B., Hudson, Q. J., et al. (2012) Airn Transcriptional Overlap, But Not Its lncRNA Products, Induces Imprinted Igf2r Silencing. Science 338, 1469–1472. DOI: 10.1126/science.1228110

91. Thebault, P., Boutin, G., Bhat, W., Rufiange, A., Martens, J., et al. (2011) Transcription Regulation by the Noncoding RNA SRG1 Requires Spt2-Dependent Chromatin Deposition in the Wake of RNA Polymerase II. Molecular and Cellular Biology 31, 1288–1300. DOI: 10.1128/MCB.01083-10
92. Yang, Z., Zhu, Q., Luo, K., and Zhou, Q. (2001) The 7SK small nuclear RNA inhibits the CDK9/cyclin T1 kinase to control transcription. Nature 414, 317–322. DOI: 10.1038/35104575
93. Xue, Z., Ye, Q., Anson, S. R., Yang, J., Xiao, G., et al. (2014) Transcriptional interference by antisense RNA is required for circadian clock function. Nature 514, 650–653. DOI: 10.1038/nature13671
94. Proudfoot, N. J. (1986) Transcriptional interference and termination between duplicated α-globin gene constructs suggests a novel mechanism for gene regulation. Nature 322, 562–565. DOI: 10.1038/322562a0
95. Martens, J. A., Laprade, L., and Winston, F. (2004) Intergenic transcription is required to repress the Saccharomyces cerevisiae SER3 gene. Nature 429, 571–574. DOI: 10.1038/nature02538
96. Hedtke, B., and Grimm, B. (2009) Silencing of a plant gene by transcriptional interference. Nucleic Acids Research 37, 3739–3746. DOI: 10.1093/nar/gkp241
97. Nielsen, M., Ard, R., Leng, X., Ivanov, M., Kindgren, P., et al. (2019) Transcription-driven chromatin repression of Intragenic transcription start sites. PLOS Genetics 15, e1007969. DOI: 10.1371/journal.pgen.1007969
98. Li, B., Carey, M., and Workman, J. L. (2007) The Role of Chromatin during Transcription. Cell 128, 707–719. DOI: 10.1016/j.cell.2007.01.015
99. Liu, Y., Dang, P., Liu, L., and He, C. (2019) Cold acclimation by the CBF–COR pathway in a changing climate: Lessons from Arabidopsis thaliana. Plant Cell Reports 38, 511–519. DOI: 10.1007/s00299-019-02376-3
100. Yuan, C., Wang, J., Harrison, A. P., Meng, X., Chen, D., et al. (2015) Genome-wide view of natural antisense transcripts in Arabidopsis thaliana. DNA Research 22, 233–243. DOI: 10.1093/dnares/dsv008
101. Dixon, J. R., Selvaraj, S., Yue, F., Kim, A., Li, Y., et al. (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485, 376–380. DOI: 10.1038/nature11082
102. Phillips-Cremins, J. E., Sauria, M. E. G., Sanyal, A., Gerasimova, T. I., Lajoie, B. R., et al. (2013) Architectural Protein Subclasses Shape 3D Organization of Genomes during Lineage Commitment. Cell 153, 1281–1295. DOI: 10.1016/j.cell.2013.04.053
103. Rao, S. S. P., Huntley, M. H., Durand, N. C., Stamenova, E. K., Bochkov, I. D., et al. (2014) A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. Cell 159, 1665–1680. DOI: 10.1016/j.cell.2014.11.021
104. Lai, F., Orom, U. A., Cesaroni, M., Beringer, M., Taatjes, D. J., et al. (2013) Activating RNAs associate with Mediator to enhance chromatin architecture and transcription. Nature 494, 497–501. DOI: 10.1038/nature11884
105. Yang, L., Lin, C., Jin, C., Yang, J. C., Tanasa, B., et al. (2013) lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. Nature 500, 598–602. DOI: 10.1038/nature12451

106. Concia, L., Veluchamy, A., Ramirez-Prado, J. S., Martin-Ramirez, A., Huang, Y., et al. (2020) Wheat chromatin architecture is organized in genome territories and transcription factories. Genome Biology 21. DOI: 10.1186/s13059-020-01998-1
107. Dong, P., Tu, X., Chu, P.-Y., Lü, P., Zhu, N., et al. (2017) 3D Chromatin Architecture of Large Plant Genomes Determined by Local A/B Compartments. Molecular Plant 10, 1497–1509. DOI: 10.1016/j.molp.2017.11.005
108. Dong, Q., Li, N., Li, X., Yuan, Z., Xie, D., et al. (2018) Genome-wide Hi-C analysis reveals extensive hierarchical chromatin interactions in rice. The Plant Journal 94, 1141–1156. DOI:
109. Zhou, S., Jiang, W., Zhao, Y., and Zhou, D.-X. (2019) Single-cell three-dimensional genome structures of rice gametes and unicellular zygotes. Nature Plants 5, 795–800. DOI: 10.1038/s41477-019-0471-3
110. Feng, S., Cokus, S. J., Schubert, V., Zhai, J., Pellegrini, M., et al. (2014) Genome-wide Hi-C Analyses in Wild-Type and Mutants Reveal High-Resolution Chromatin Interactions in Arabidopsis. Molecular Cell 55, 694–707. DOI: 10.1016/j.molcel.2014.07.008
111. Grob, S., Schmid, M. W., Luedtke, N. W., Wicker, T., and Grossniklaus, U. (2013) Characterization of chromosomal architecture in Arabidopsis by chromosome conformation capture. Genome Biology 14, R129. DOI: 10.1186/gb-2013-14-11-r129
112. Grob, S., Schmid, M. W., and Grossniklaus, U. (2014) Hi-C Analysis in Arabidopsis Identifies the KNOT, a Structure with Similarities to the flamenco Locus of Drosophila. Molecular Cell 55, 678–693. DOI: 10.1016/j.molcel.2014.07.009
113. Liu, C., Wang, C., Wang, G., Becker, C., Zaidem, M., et al. (2016) Genome-wide analysis of chromatin packing in Arabidopsis thaliana at single-gene resolution. Genome Research 26, 1057–1068. DOI: 10.1101/gr.204032.116
114. Yang, T., Wang, D., Tian, G., Sun, L., Yang, M., et al. (2022) Chromatin remodeling complexes regulate genome architecture in Arabidopsis. The Plant Cell 34, 2638–2651. DOI: 10.1093/plcell/koac117
115. Wang, C., Liu, C., Roqueiro, D., Grimm, D., Schwab, R., et al. (2014) Genome-wide analysis of local chromatin packing in Arabidopsis thaliana. Genome Research 25, 246–256. DOI: 10.1101/gr.170332.113
116. Huang, Y., Sicar, S., Ramirez-Prado, J. S., Manza-Mianza, D., Antunez-Sanchez, J., et al. (2021) Polycomb-dependent differential chromatin compartmentalization determines gene coregulation in Arabidopsis. Genome Research 31, 1230–1244. DOI: 10.1101/gr.273771.120
117. Ricci, W. A., Lu, Z., Ji, L., Marand, A. P., Ethridge, C. L., et al. (2019) Widespread long-range cis-regulatory elements in the maize genome. Nature Plants 5, 1237–1249. DOI: 10.1038/s41477-019-0547-0
118. Ariel, F., Lucero, L., Christ, A., Mammarella, M. F., Jegu, T., et al. (2020) R-Loop Mediated trans Action of the APOLO Long Noncoding RNA. Molecular Cell 77, 1055-1065.e4. DOI: 10.1016/j.molcel.2019.12.015
119. Moison, M., Pacheco, J. M., Lucero, L., Fonouni-Farde, C., Rodríguez-Melo, J., et al. (2021) The lncRNA APOLO interacts with the transcription factor WRKY42 to trigger root hair cell expansion in response to cold. Molecular Plant 14, 937–948. DOI: 10.1016/j.molp.2021.03.008

120. Pacheco, J. M., Mansilla, N., Moison, M., Lucero, L., Gabarain, V. B., et al. (2021) The lncRNA APOLO and the transcription factor WRKY42 target common cell wall EXTENSIN encoding genes to trigger root hair cell elongation. Plant Signaling & Behavior 16. DOI: 10.1080/15592324.2021.1920191
121. Fonouni-Farde, C., Christ, A., Blein, T., Legascue, M. F., Ferrero, L., et al. (2022) The Arabidopsis APOLO and human UPAT sequence-unrelated long noncoding RNAs can modulate DNA and histone methylation machineries in plants. Genome Biology 23. DOI: 10.1186/s13059-022-02750-7
122. Nützmann, H.-W., Doerr, D., Ramírez-Colmenero, A., Sotelo-Fonseca, J. E., Wegel, E., et al. (2020) Active and repressed biosynthetic gene clusters have spatially distinct chromosome states. Proceedings of the National Academy of Sciences 117, 13800–13809. DOI: 10.1073/pnas.1920474117
123. Xu, W., Xu, H., Li, K., Fan, Y., Liu, Y., et al. (2017) The R-loop is a common chromatin feature of the Arabidopsis genome. Nature Plants 3, 704–714. DOI: 10.1038/s41477-017-0004-x
124. Xu, W., Li, K., Li, S., Hou, Q., Zhang, Y., et al. (2020) The R-Loop Atlas of Arabidopsis Development and Responses to Environmental Stimuli. The Plant Cell 32, 888–903. DOI: 10.1105/tpc.19.00802
125. Li, L., Luo, H., Lim, D.-H., Han, L., Li, Y., et al. (2021) Global profiling of RNA–chromatin interactions reveals co-regulatory gene expression networks in Arabidopsis. Nature Plants 7, 1364–1378. DOI: 10.1038/s41477-021-01004-x
126. Davidovich, C., Zheng, L., Goodrich, K. J., and Cech, T. R. (2013) Promiscuous RNA binding by Polycomb repressive complex 2. Nature Structural & Molecular Biology 20, 1250–1257. DOI: 10.1038/nsmb.2679

Figure legends

Figure 1: Overview of example epigenetic mechanisms of actions of plant lncRNAs. A. Prior to vernalization, the flowering gene *MAF4* is only expressed at low basal levels. Upon cold treatment and vernalization, its antisense lncRNA *MAS* increases in expression. This leads to the recruitment of WDR5A and the COMPASS-like complex to the *MAF4* locus and subsequently increases H3K4me3 deposition. Accumulation of H3K4me3 at the *MAF4* TSS leads to increased transcriptional activation of the gene. B. Upon cold exposure, the freezing tolerance gene *CBF1* is activated. After 4 hours, expression of the downstream convergently-oriented lncRNA *SVALKA* is activated. Read-through transcription into the *CBF1* locus leads to transcription of the cryptic antisense RNA *asCBF1*. Transcription on both the sense and antisense strand leads to collision of RNAPII. Stalling of the RNAPII transcribing *CBF1* leads to rapidly-degraded truncated transcripts and a reduction in gene expression. C. The promoter of the auxin transport regulator gene *PID* forms a repressive loop with the locus of the lncRNA *APOLO*. This loop is maintained by recruitment of LHP1 directed to these sites by *APOLO* forming R-loops with target regions, promoting H3K27me3 deposition. Additional RNAPV transcription of *APOLO* also leads to DNA methylation of the *APOLO* locus. Upon auxin treatment, transcription of *APOLO* increases and it decoys LHP1 away from the loop contact regions. This leads to a loss in H3K27me3 and DNA methylation, relaxing the loop and leading to increased expression of *PID*. *APOLO* also acts in *trans*, relaxing repressive loops inhibiting the transcription of other auxin-responsive genes.

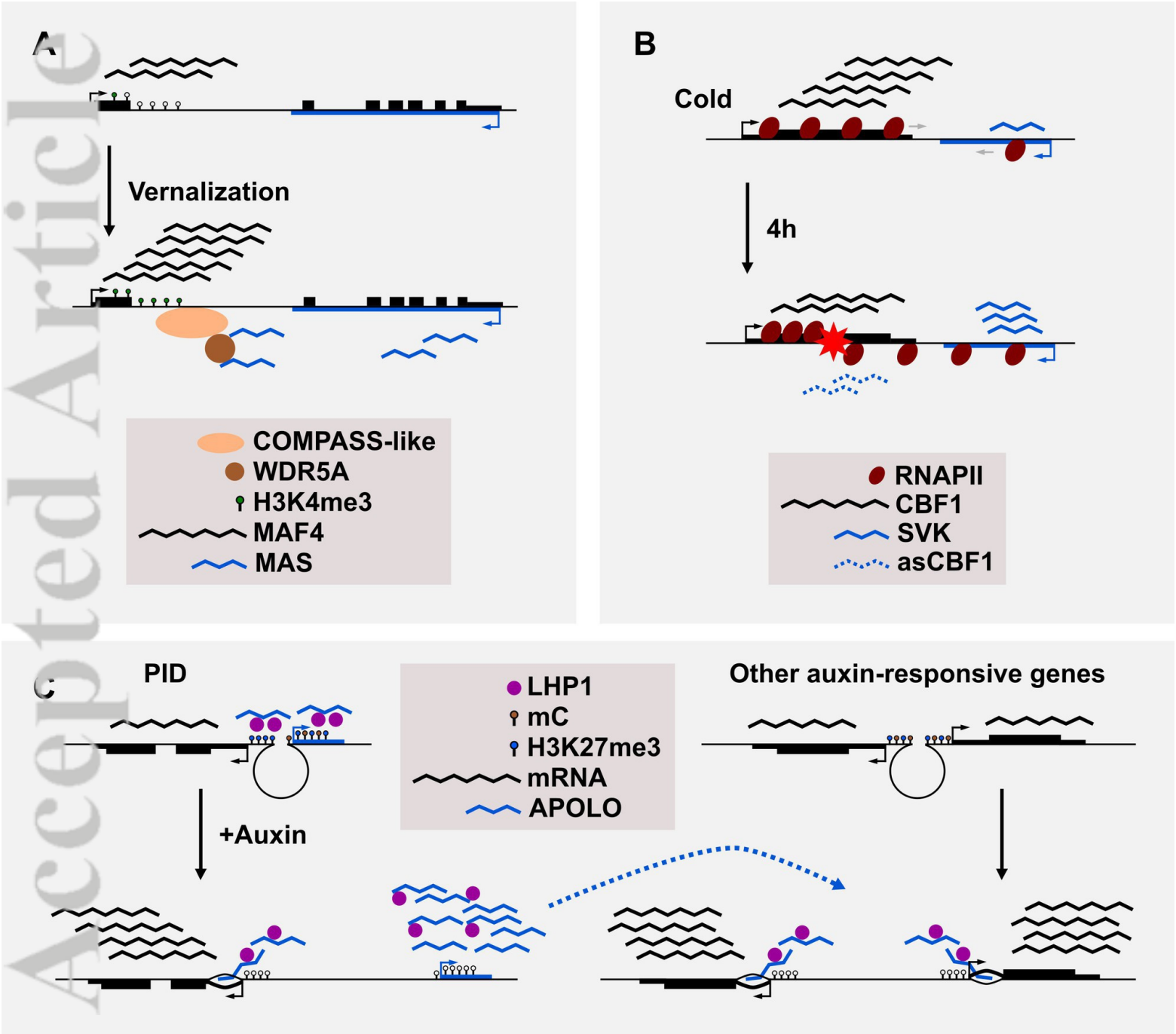

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Table 1: List of plant lncRNAs with characterized epigenetic mechanisms mentioned in this review

lncRNA	Epigenetic mechanism	Target(s)	Reference(s)
AG-incRNA4	Increase H3K27me3 via recruitment of CLF	*AG*	(73)
APOLO	Repressive loop formation and dissociation via recruitment and decoying of LHP1, respectively	*PID, WAG2, AZG2, RHD6, EXT3*	(20, 118-121)
COLDAIR	Increase H3K27me3 via recruitment of CLF	FLC	(28)
COLDWRAP	Repressive loop formation, increase H3K27me3 via recruitment of CLF	*FLC*	(30)
COOLAIR	Switch to H3K27me3 via stabilization of the H3K36me3 demethylation complex	*FLC*	(22, 25, 32, 34, 37, 38, 45)

LAIR	Increase H3K4me3 and H4K16ac via recruitment of OsMOF and OsWDR5	*LRK1, LRK4*	(40)
MARS	Activating loop formation via decoying of LHP1	*MRN1, CYP705A12, MRO*	(35)
MAS	Increase H3K4me3 via recruitment of WDR5A and the COMPASS-like complex	*MAF4*	(44)
T-DNA-QUA1	Transcriptional interference of RNAPII via elongation over *QUA1* TSS	*QUA1*	(97)
T-DNA-RFD1	Transcriptional interference of RNAPII via elongation over *RFD1* TSS	*RFD1*	(96, 97)
SVALKA	Transcriptional interference of RNAPII via read-through transcription of cryptic *asCFB1*	*CBF1*	(11)