Non-coding and epigenetic mechanisms in the regulation of seed germination in *Arabidopsis thaliana*

Benjamin J.M. Tremblay and Julia I. Qüesta

Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra 08193, Barcelona, Spain

Highlight

By examining the well-studied seed-to-seedling transition in *Arabidopsis thaliana*, we can learn fundamental insights into how non-coding and epigenetic mechanisms regulate transcription during developmental transitions in plants.

Abstract

Seed germination as a developmental process has been extensively studied using the model plant Arabidopsis thaliana. Its seed biology is generally well understood, from the regulation of seed maturation and dormancy to germination and the post-germinative transition. These events require, and are the result of, extensive transcriptional reprogramming which importantly are mediated by essential epigenetic mechanisms such as DNA methylation, different histone variants and modifications, as well as by non-coding regulatory RNAs. Studying these mechanisms therefore is essential for understanding the regulation of gene expression during germination. In this review we summarize our current knowledge of these mechanisms in the context of *Arabidopsis thaliana* seed biology and discuss aspects requiring further study.

Keywords

Arabidopsis thaliana, epigenetics, gene regulation, seed germination, seedling development, seed dormancy, chromatin regulation, DNA methylation, histone modifications, non-coding RNAs

Introduction

Eukaryotes, including plants, have evolved a set of highly complex non-coding and epigenetic mechanisms which work in concert to regulate gene expression. These mechanisms begin at the genome with the methylation of cytosines in DNA. This mark can have a strong repressive effect on transcription and plays a role in silencing transposable elements. The histones, composing the nucleosomes around which DNA is wrapped, are themselves involved in the regulation of gene expression by the presence of various modifications of the histone tails which can both activate or repress transcription. Transcription itself produces a host of various products, many of which are non-coding. These non-coding RNAs, which include long non-coding RNAs (lncRNAs), can influence gene expression through various mechanisms including interacting with both chromatin and proteins which regulate transcription. Regulation of gene expression continues even after transcription with mRNAs targeted for cleavage and degradation by microRNAs (miRNAs).

Developmental transitions in plants require switches in active gene regulatory networks, which occur by the action of non-coding and epigenetic mechanisms. The control of flowering time by vernalization was known to involve the antisense lncRNA COOLAIR to initiate transcriptional shutdown of the flowering repressor FLOWERING LOCUS C (FLC) by coordinating the removal of the active histone mark H3K36me3 (Swiezewski et al., 2009; Csorba et al., 2014). Later, this is followed by deposition of the histone repressive mark H3K27me3 over FLC, which provides stable epigenetic repression of the gene (De Lucia et al., 2008). DNA methylation is also necessary for flowering, as its presence over the FLOWERING WAGENINGEN (FWA) locus prevents ectopic transcription of the gene, which delays flowering (Soppe et al., 2000). The activity of miRNAs can also be seen during all periods of development. For example, miR166 controls the outcomes of adaxial and abaxial cell identities during leaf formation by promoting cleavage of the transcripts of the genes PHABULOSA (PHB), REVOLUTA (REV), and CORONA (CNA) on the abaxial side (Liu et al., 2011). Despite the strong links between the many examples of the individual importance of these mechanisms in development, it is important to consider that they are all necessary in combination.

In *Arabidopsis thaliana* and other flowering plants alike during the seed-to-seedling transition, development of the seed is initiated after the onset of embryogenesis. The seed maturation program is carefully regulated by transcription factors, among which are LEAFY COTYLEDON1 (LEC1), LEC2, ABA INSENSITIVE3 (ABI3), and FUSCA3 (FUS3), collectively known as the LAFL network (Jia *et al.*, 2014). These factors up-regulate pathways responsible for the accumulation of seed storage proteins and lipids, the process of seed desiccation, and biosynthesis of the phytohormone abscisic acid (ABA), the latter of which maintains seed dormancy and prevents germination. In *Arabidopsis thaliana*, this period of primary dormancy can be broken either by after-ripening or cold stratification, which reduce ABA levels and represses expression of

the *DELAY OF GERMINATION1* (*DOG1*) gene, a major factor maintaining dormancy via an ABA-independent pathway (Iwasaki *et al.*, 2022). However if germination takes place in unfavourable conditions such as elevated temperatures, imbibed seeds can instead induce an additional phase of dormancy known as secondary dormancy, which is poorly understood but likely involves the restarting of ABA biosynthesis (Buijs, 2020). Under normal conditions the lack of ABA signaling allows for the biosynthesis of the phytohormone gibberellin (GA), which itself promotes germination (Seo *et al.*, 2006). The initial stages of germination are characterized by rehydration and cellular expansion, which drives the emergence of the radicle from the seed. Finally, cell division is initiated after a period of transcriptional reprogramming, followed by the post-germinative transition to seedling growth (Carrera-Castaño *et al.*, 2020; Tremblay *et al.*, 2024). In this review we focus on non-coding and epigenetic mechanisms regulating the various stages of the seed-to-seedling transition, providing a comprehensive view of how these can work in concert to coordinate complex changes in gene expression necessary for the massive rise in cellular differentiation (Figure 1).

Transcriptional regulation by long non-coding RNAs

LncRNAs are a class of RNAs, longer than 200 nt, which generally lack coding potential but can share many other similarities with mRNAs, including being transcribed by RNAPII, a 5′ cap, spliced introns, and a polyA tail. This class of RNA is a common feature of eukaryotes, including plants, and estimates for their abundance in *Arabidopsis* generally range from several thousands to tens of thousands depending on the sensitivity of the methods of identification (Liu *et al.*, 2012; Wang *et al.*, 2014*a*; Zhao *et al.*, 2018; Sun *et al.*, 2020; Corona-Gomez *et al.*, 2022; Palos *et al.*, 2022; Kornienko *et al.*, 2024). Despite this, only a fraction of lncRNAs have thus far been experimentally characterized in depth and found to play essential roles in development and stress response in *Arabidopsis* (Tremblay and Qüesta, 2023). This includes several which have been shown to play both positive and negative roles in promoting germination.

One such lncRNA was identified from a screen of nuclear RNAs in young seedlings, which had uncovered 14 lncRNAs containing RNA-binding protein (RBP) interaction sites with significant levels of conservation with *Brassica rapa* named *CONSERVED IN BRASSICA RAPA 1-14* (*lncCOBRA1-14*) (Gosai *et al.*, 2015; Kramer *et al.*, 2022). Of these, three, lncCOBRA1, lncCOBRA3, and lncCOBRA5, were found to have high levels of germination-specific expression patterns with nuclear subcellular localization, each differing in tissue specificity. A more in-depth molecular characterization of *lncCOBRA1* revealed that it encodes two small nucleolar RNAs (snoRNAs), lacks a polyA tail, and undergoes post-transcriptional modifications to trim it from a length of over 1000 nt to a final RNA between 500 and 600 nt. Mutants lacking *lncCOBRA1* had significantly reduced rates of germination and reduced vegetative size, indicating its role in promoting germination and seedling growth. Attempts to functionally

characterize the lncRNA indicated it does not interact with rRNAs, despite its two snoRNAs, though 113 interacting proteins could be identified with high confidence. These interacting proteins were associated with various germination-related pathways, though a major subset of these were ribosome-associated proteins. A network analysis uncovered the highly conserved scaffolding protein RECEPTOR FOR ACTIVATED C KINASE 1A (RACK1A) and many of its previously known interactors, leading the authors to hypothesize *lncCOBRA1* may serve as a scaffold to bring together a network of proteins promoting germination (Kramer *et al.*, 2022).

Another evolutionarily conserved lncRNA, *HIDDEN TREASURE 1* (*HID1*), was first identified in a large-scale reverse-genetic screen of intermediate size lncRNAs (50-300 nt) and was initially shown to regulate light-directed seedling growth via the red light-mediated seedling photomorphogenesis pathway (Figure 2a) (Wang *et al.*, 2014*c*,*b*). This lncRNA is 236 nt long and constitutively expressed in seedlings. Functional characterization revealed it to be chromatin-bound and interacting with the promoter of *PHYTOCHROME-INTERACTING FACTOR 3* (*PIF3*) in a manner which negatively regulates its transcription (Wang *et al.*, 2014*b*). Subsequent research revealed HID1's role in promoting light-dependent germination (Wang *et al.*, 2022). Using a phytochrome B (phyB)-dependent germination protocol, in which seeds are briefly exposed to red and far-red light before being germinated in the dark, mutants lacking *HID1* showed significantly reduced rates of germination, suggesting *HID1* is a downstream positive regulator of phyB-dependent germination. In these conditions, phyB promotes increased expression of *HID1* in the radicle. This accumulation results in *HID1* binding to the chromatin of the ABA biosynthesis gene *9-CIS-EPOXYCAROTENOID DIOXYGENASE* (*NCED9*), disrupting binding of the methyltransferase ARABIDOPSIS TRITHORAX-RELATED7 (ATXR7), inhibiting enrichment of the active histone mark H3K4me3 and ultimately leading to a transcriptional down-regulation of *NCED9*. The reduction of ABA biosynthesis then releases repression of GA biosynthesis, the primary signaling molecule promoting germination.

Finally, transcription of the gene *DOG1*, which maintains dormancy in mature dry seeds (Bentsink *et al.*, 2006), has been shown to be regulated by both positive and negative-acting lncRNAs (Figure 2b) (Fedak *et al.*, 2016; Yatusevich *et al.*, 2017; Montez *et al.*, 2023). One of these, known as *asDOG1*, is an antisense lncRNA originating from the final exon that is 5′ capped, polyadenylated, and between 1200 to 1300 nt long (Fedak *et al.*, 2016). A T-DNA mutant disrupting asDOG1's promoter increases *DOG1* transcription and reduces germination, suggesting *asDOG1* acts in opposition to the dormancy maintenance effect of *DOG1* in order to promote germination. The lncRNA was shown to act only in *cis* despite the relative stability of the RNA, as heterozygous *asDOG1*-deficient mutants still had displayed increased *DOG1* transcript levels and dormancy, though the exact mechanism remains unknown. In addition to *asDOG1*, several lncRNA isoforms were also detected being initiated 1.5 kb upstream of the *DOG1* transcriptional start sites, collectively named *PUPPIES* (Montez *et al.*, 2023).

These lncRNAs were shown to be upregulated in seeds imbibed in the presence of salt and induce increased transcription of *DOG1*, thus delaying germination under salt stress conditions. Similar to *asDOG1*, *PUPPIES* are believed to act in *cis* by interacting with the chromatin of the *DOG1* gene and increasing the level of RNA Polymerase II (RNAPII) loading at the gene as well increasing splicing efficiency of the *DOG1* transcript. In sum, the control of *DOG1* expression by this collection of lncRNAs perfectly exemplifies the possible high levels of complexity involved in non-coding regulation of gene expression.

Beyond known cases of lncRNAs regulating genes determining the timing or rate of germination, we have recently detected active transcription initiation at thousands of sites of non-coding transcription during all stages of the seed-to-seedling transition (Tremblay *et al.*, 2024). A substantial portion of these were found to be present in the antisense orientation overlapping genes with known functions during germination, such as a novel isoform of *asDOG1* expressed specifically during cold stratification of seeds. In total approximately 20% of all protein coding genes detectable during this transition also had detectable antisense transcription, with the majority of sense-antisense pairs correlating throughout the various stages. Such pairs also tended to be less expressed than those which were anti-correlating, and those without any detectable antisense transcription, suggesting that antisense lncRNAs may regulate many genes activated during germination (Figure 2c). The study also identified numerous lncRNAs from enhancer regions known as enhancer RNAs (eRNAs), which in other species have been shown to interact with transcriptional regulators of target genes however in plants their functional role remains unclear (Sartorelli and Lauberth, 2020).

Post-transcriptional regulation by microRNAs

MicroRNAs (miRNAs) are a class of small RNAs between 20 and 24 nt, which post-transcriptionally regulate gene expression via sequence complementarity with other mRNAs, leading to cleavage by ARGONAUTE (AGO) proteins or translation inhibition (Yu *et al.*, 2017). These small RNAs originate from miRNA (*MIR*) genes, from which RNAPII transcribes a type of lncRNA known as a primary miRNA (pri-miRNA), afterwhich it must undergo several processing steps before becoming a mature miRNA. Currently the miRNA database *miRBase* lists 428 miRNA sequences for *Arabidopsis thaliana* (Kozomara *et al.*, 2019). miRNAs are known to play essential roles in the development of *Arabidopsis thaliana*, including during germination (Zhan and Meyers, 2023), where many miRNAs are dynamically expressed (Narsai *et al.*, 2017).

ABA is the major phytohormone that represses germination, making its biosynthesis, signaling, and turnover critical for this process. MiRNAs have been long predicted to have an important role in this process, as mutants lacking a key miRNA processing subunit, HYPONASTIC LEAVES1 (HYL1), are hypersensitive to ABA and nearly completely fail to germinate when grown in the presence of exogenous ABA (Lu and

Fedoroff, 2000). Expression of *HYL1* itself is also down-regulated by ABA, suggesting processing of miRNAs may be involved in a negative feedback loop with ABA signaling. Specific cases of miRNAs participating in ABA regulation have also been found, such as miR159 and miR160 (Liu *et al.*, 2007*b*; Reyes and Chua, 2007). In the former case, ABA leads to an induction of the transcription factor *ABSCISIC ACID-INSENSITIVE3* (*ABI3*) which in turn up-regulates miR159 expression. Accumulation of this miRNA leads to cleavage of the mRNA of two target transcription factors, *MYB33* and *MYB101*, both positive regulators of ABA signaling. This negative feedback loop was proposed to serve as a way to recover from temporary stress conditions which have led to elevated ABA levels by quickly degrading the pool of mRNAs involved in positive ABA signaling (Reyes and Chua, 2007). Similarly, miR160 has been shown to repress ABA signaling by targeting the mRNA of the auxin signaling transcription factor *AUXIN RESPONSE FACTOR10* (*ARF10*), which also leads to a down-regulation of ABA-responsive genes. Plants with a transgenic *ARF10* gene resistant to miR160 display ABA hypersensitivity, suggesting crosstalk between ABA and auxin signaling networks during germination (Liu *et al.*, 2007*b*). Both these results highlight the crucial roles of miRNAs in promoting germination in conditions which drive increased ABA accumulation.

Beyond direct ABA signaling, other processes related to germination have been implicated in regulation by miRNAs. For example, miR163 has instead been shown to play a role in germination via a light-activated pathway (Chung *et al.*, 2016). Under light conditions during germination, an up-regulation of this miRNA leads to increased cleavage of its target *PARAXANTHINE METHYLTRANSFERASE1* (*PXMT1*), a methyltransferase methylating 1,7-paraxanthine. In the absence of miR163, *PXMT1* displays increased accumulation in the radicle, slowing the rate of germination and reducing radicle growth, and later leading to increased lateral root growth in the seedling stage. Additionally, in the absence of functional phyA, PIF1 binds the promoter of the miR408 pri-miRNA, repressing its transcription and leading to an increase in the miR408 target *PLANTACYANIN* (*PCY*), a phytocyanin and negative regulator of germination (Jiang *et al.*, 2021). Under phyA-dependent conditions, PIF1 is inactivated and instead allows for increased miR408 via up-regulation by *ELONGATED HYPOCOTYL5* (*HY5*) to promote germination via cleavage of *PCY* (Zhang *et al.*, 2014). These results suggest miRNAs such as miR163 and miR408 play key roles in promoting light-regulated germination.

Additional pathways involved in regulating germination are likely regulated by miRNAs as well. Expression of miR156 is sustained in seeds through a DOG1-dependent mechanism and targets the mRNAs of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE genes for degradation, which are necessary for upregulating expression of the germination-promoting miR172 (Huo *et al.*, 2016). Both miR395c and miR395e have been proposed as having opposing roles in regulating germination via control of the sulfur assimilation pathway, which has been shown to aid plant growth in stressful conditions (Kim *et al.*, 2010*b*). Under salt stress conditions, plants

overexpressing miR395c show increased cleavage of *ATP SULFURYLASE1* (*APS1*), *APS4*, and *SULFATE TRANSPORTER 2;1* (*SULTR2;1*), resulting in reduced germination rates likely due to reduced sulfur assimilation and transport. In contrast, in such conditions plants overexpressing miR395e show an up-regulation of the same target genes and a down-regulation of *APS3*, resulting in increased germination. Finally, miR402, a miRNA up-regulated by salt, dehydration, and cold stresses (Sunkar and Zhu, 2004), has been shown to down-regulate *DEMETER-LIKE PROTEIN3* (*DML3*), a DNA demethylase (Kim *et al.*, 2010*a*). This regulation was shown to promote germination under such conditions, suggesting maintaining higher levels of DNA methylation may help repress negative regulators of germination during stress.

DNA methylation and gene silencing by small RNAs

DNA methylation involves the covalent addition of a methyl group onto cytosines to form 5-methylcytosine within DNA, which can be present in CG, CHG (H = A/G/T), or CHH contexts. In plants, CG methylation is found within gene bodies, though the impact this has on gene expression is not well understood. CG methylation can also co-occur with methylation at CHG and CHH, typically within transposable elements (TEs) or other repeat regions, with strong silencing effects. DNA methylation is primarily established by the RNA-directed DNA methylation (RdDM) pathway, in which small RNAs (sRNAs), the products of RNAs transcribed by RNAPIV, direct the *de novo* methyltransferase machinery to the genome. This methylation is then maintained through the action of METHYLTRANSFERASE1 (MET1) in the case of CG methylation, and CHROMOMETHYLASE2 (CMT2) and CMT3 in the case of methylation at CHH and CHG, respectively. The genome of *Arabidopsis thaliana* undergoes many changes in DNA methylation patterns during its lifecycle which are essential for normal developmental progression (Hemenway and Gehring, 2023).

That DNA methylation plays a role in embryogenesis and seed formation is well established (Markulin *et al.*, 2021). Mutants lacking *MET1* and *CMT3* undergo abnormal embryo development and suffer reduced seed viability (Finnegan *et al.*, 1996; Xiao *et al.*, 2006*b*), and maternal plants lacking *MET1* or *DECREASE IN DNA METHYLATION1* (*DDM1*), a chromatin remodeler needed to maintain DNA methylation, produce enlarged seeds (Xiao *et al.*, 2006*a*). This effect may be related to its inability to undergo the reduction in nuclear size which occurs during seed maturation, as loss of these proteins leads to unstable genome architecture and loss of heterochromatin (Soppe *et al.*, 2002; Mathieu *et al.*, 2003, 2007; van Zanten *et al.*, 2011). In contrast, plants lacking any methylation at CHG and CHH show no defects in seed size or nuclear shrinkage, suggesting these processes may be specifically dependent on CG methylation (Lin *et al.*, 2017). Despite these links, a strong association between global DNA methylation levels and germination rates under normal conditions for non-dormant seeds has not been shown, with no or minor effects on germination seen in various DNA methylation mutants (Lin *et al.*, 2017; Kim *et al.*, 2021; Malabarba *et al.*, 2021; Sato *et al.*, 2021; He *et al.*, 2022). Germinating seeds in the presence of the DNA methylation inhibitor 5-

azacytidine does not appear to have a significant impact on germination rate, though likely due to lack of cell divisions during early germination required for the incorporation of the compound into the genome (Morgan and Donohue, 2022). However, loss of the demethylase *REPRESSORS OF SILENCING1* (*ROS1*) has been reported to increase seed dormancy and reduce germination rate of freshly harvested seed via down-regulation of *DOG1-LIKE4* (*DOGL4*), a negative regulator of seed dormancy, from increased promoter methylation of the paternal allele in endosperm cells as a result of maternal imprinting (Zhu *et al.*, 2018). H3K9me2 alongside CHG methylation marking the binding site of the histone demethylase RELATIVE OF EARLY FLOWERING6 (REF6) also blocks its binding and removal of the repressive histone mark H3K27me3 over maternally imprinted dormancy-promoting genes in the endosperm, such as ABI3 (Figure 3a) (Sato *et al.*, 2021). Additionally, non-canonical RdDM of CHH in the promoter of the paternal allele of the dormancy repressor *ALLANTOINASE* (*ALN*) in cold-matured seeds reduces its expression and increases dormancy (Iwasaki *et al.*, 2019). These findings suggest that while proper DNA methylation is necessary for seed development, dormancy and seedling establishment, it may not be essential for germination itself.

Indeed, several methylome studies profiling global DNA methylation dynamics during germination indicate that DNA methylation plays a minor role in germination (Bouyer *et al.*, 2017; Kawakatsu *et al.*, 2017; Lin *et al.*, 2017; Narsai *et al.*, 2017). These studies reported the only major change in global DNA methylation was from an approximate doubling of CHH methylation in mature seeds compared to vegetative tissues, usually over TEs, and likely due to excess sRNAs exported from hypomethylated endosperm nuclei. It was further reported that increased CHH methylation is gradually lost over the course of germination as cellular divisions dilute methylated CHH, though after nuclear decondensation has occurred, again supporting that this type of methylation is unrelated to nuclear size (van Zanten *et al.*, 2011). Increased transcription from TE genes could be detected in mutants lacking CHG and CHH methylation for a small number of hypomethylated TEs, suggesting the CHH hypermethylation patterns specific to seeds could serve as an additional layer of silencing or protection from DNA damage, but otherwise this deficiency had no noticeable impact on germination under normal conditions (Lin *et al.*, 2017; Underwood *et al.*, 2018). While a small fraction of genes expressed during germination had nearby germination-specific changes in DNA methylation, these were rarely associated with the actual expression patterns of the genes. In fact, most mature seed and germination-specific genes reside in regions generally devoid of DNA methylation (Lin *et al.*, 2017). However some exceptions have been reported for genes associated with tuning the timing of germination under stress conditions. Loss of RdDM-dependent methylation of CHH in the promoter of the transcription factor *MYB74* leads to reduced germination rates in salt stress conditions (Xu *et al.*, 2015), and a later study of genome-wide methylation changes in RdDM-deficient mutants identified changes for many other genes associated with the altered germination rates of these mutants in salt stress conditions (Palomar *et al.*, 2021). Furthermore, loss of *DML3* leads to faster germination rates during salt, osmotic, and

cold stresses, possibly due to silencing of genes repressing germination in unfavourable conditions (Kim *et al.*, 2010*a*). Overall, DNA methylation appears to help optimize germination success in suboptimal environmental conditions.

This still leaves in question the purpose of the seed-specific CG methylation-dependent nuclear shrinkage. One possibility is a requirement for the specific silencing of genes required to maintain dormancy, which may be supported by increased dormancy seen in mutants lacking *ROS1* (Zhu *et al.*, 2018). However, this may yet be independent of genome-wide condensation and it does not explain why under specific conditions seeds can enter a secondary dormancy if genome decondensation occurs during imbibition, though the effects of these conditions on decondensation dynamics have not been reported (Toh *et al.*, 2008; van Zanten *et al.*, 2011; Auge *et al.*, 2015). A second possibility involves a general shutdown of transcription during germination as a result of tightly packed chromatin restricting RNAPII activity (Kawakatsu *et al.*, 2017), though no study has reported measuring transcription rates in dry seeds of wild-type or CG hypomethylated mutants. Finally, a third possibility involves protection of DNA from damage (Kawakatsu *et al.*, 2017) such as from insertions of active transposable elements or random mutations, which can occur more frequently in accessible chromatin regions (Monroe *et al.*, 2022). Mature seeds also gradually accumulate DNA double-strand breaks which are not repaired until early imbibition, though the ability for the seed to repair these declines with age (Sano *et al.*, 2016). While CG hypomethylation does not have a negative effect on the DNA damage response pathway (Shaked *et al.*, 2006; Choi *et al.*, 2019), the lack of genome condensation could nevertheless render it more vulnerable to accumulating increased damage over time, as the level of genome-wide chromatin accessibility has been shown to be correlated with DNA damage (Kolářová *et al.*, 2021; Layat *et al.*, 2021). Ultimately, an increased accumulation of DNA damage reduces the longevity of seeds and makes them less able to withstand challenging environments.

The essential roles of histones in seed biology

The four core histone proteins H2A, H2B, H3, and H4 make up the nucleosome, the most basic unit of chromatin. Their positioning along the DNA has a direct effect on its availability to complexes such as RNAPII, as well as the various possible posttranslational modifications of the histone tails. Various histone variants and modifications are broadly associated with greater chromatin states, such as the highly compacted heterochromatin, which has a silencing effect on transcription, and the more relaxed euchromatin, which is associated with active genes (Foroozani *et al.*, 2022; Jamge *et al.*, 2023). Commonly studied marks such as H3ac and H3K4me3 are associated with transcriptional activation, whereas others such as H3K9me2 and H3K27me3 instead with transcriptional repression. Whereas some regions of the genome can be constitutively marked by the same modifications or variants, other regions, such as where many developmental genes are located, are dynamically

regulated during the plant life cycle to carefully control switches between transcription programs (Yu *et al.*, 2023).

One of the most studied marks which plays a major role in seed formation and germination (but also in all other developmental transitions) is H3K27me3. The deposition of this mark is mediated by Polycomb Repressive Complex 2 (PRC2), which exists in three core combinations in *Arabidopsis thaliana* (Yu *et al.*, 2023). The FIS-PRC2 complex includes FERTILIZATION-INDEPENDENT SEED2 (FIS2), MULTICOPY SUPPRESSOR OF IRA1 (MSI1), FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) and MEDEA (MEA), essential for all reproductive processes, including gametophyte formation and embryogenesis (Grossniklaus and Paro, 2014; Simonini *et al.*, 2021) The EMF-PRC2 complex, composed of EMBRYONIC FLOWER2 (EMF2), MSI1, FIE, CURLY LEAF (CLF), and SWINGER (SWN), has a unique role in repressing embryonic genes during seedling development (Mozgova and Hennig, 2015). Finally, the VRN-PRC2 complex is composed of VERNALIZATION2 (VRN2), MSI1, FIE, CLF and SWN, which is specifically needed for regulation of vernalization (Mozgova and Hennig, 2015).(Yu *et al.*, 2023). In combination these three complexes are active in all parts of the plant life cycle but only have partially overlapping functions.

In the course of seed development, a global rearrangement of H3K27me3 occurs to repress seed maturation genes and prepare for germination. This is partly mediated by the activity of the transcription factors VIVIPAROUS1/ABI3-LIKE1 (VAL1) and VAL2, which help recruit PRC2 at target genes (Figure 3b) (Yuan *et al.*, 2020; Liang *et al.*, 2022). Loss of VAL1 and VAL2 leads to increased protein content in seeds, reduced H3K27me3 at the *DOG1* locus, and elevated *DOG1* expression, leading to increased dormancy (Schneider *et al.*, 2016; Chen *et al.*, 2020*b*). Similarly, mutants lacking *FIE* also show increased dormancy (Bouyer *et al.*, 2011). This process is highly complex and involves the removal of many other marks to coordinate the switch to transcriptional repression. Deacetylases are recruited by VALs to reduce H3ac levels over *DOG1* as part of its repression, including HISTONE DEACETYLASE 2A (HD2A) and HD2B (Han *et al.*, 2023). Further deacetylases may also be needed for this, such as HISTONE DEACETYLASE9 (HDA9) (van Zanten *et al.*, 2014). The action of deacetylases is not always in coordination with gain of H3K27me3 however; for example, the histone deacetylase complex members SWI-INDEPENDENT3 (SIN3)-LIKE1 (SNL1) and SNL2 reduce activation of the transcription factor *AUXIN RESISTANT1* (*AUX1*) in seeds, which later up-regulates genes that will accelerate radicle growth and germination during imbibition (Wang *et al.*, 2016). Additionally, H3K4me3 present at *DOG1* and a number of other loci is recognized by ALFIN1-LIKE6 (AL6) and AL7, which in turn recruit the PRC1 components ARABIDOPSIS THALIANA RING 1A (AtRING1a) and ARABIDOPSIS THALIANA B LYMPHOMA MOLONEY MURINE LEUKEMIA VIRUS INSERTION REGION 1 HOMOLOG 1B (AtBMI1b) to deposit H2Aub, though this could be in coordination with VALs to aid recruitment of PRC2 (Yang *et al.*, 2013; Molitor *et al.*, 2014). Additional factors, such RNA-binding proteins RZ-1A and RZ-1B, coordinate deacetylation and removal of H3K4me3 by *ABI3* and ABA

biosynthesis genes including *NCED6*, also facilitating later H3K27me3 deposition (Yang *et al.*, 2022). Proteins LYSINE SPECIFIC DEMETHYLASE LIKE1 (LDL1) and LDL2, which demethylate H3K4me3, also contribute to transcriptional repression (Zhao *et al.*, 2015). Removal of some level of H2Bub is likely necessary as well, as mutants lacking the H2B ubiquitination genes *HISTONE MONOUBIQUITINATION1* (*HUB1*) and *HUB2* do not accumulate *DOG1* and *NCED9* transcript in seeds, demonstrating its importance for the regulation of dormancy as a positive regulator of transcription (Liu *et al.*, 2007*a*).

Elsewhere in the genome changes in H3K27me3 are also affected by REF6, which is transiently accumulated on chromatin before seed maturation (Pan *et al.*, 2022). The activity of REF6 is necessary to remove H3K27me3 and derepress the ABA catabolism genes *CYP707A1* and *CYP707A3*, without which leads to increased ABA content in seeds and elevated dormancy (Figure 3a) (Chen *et al.*, 2020*a*). Another histone demethylase, EARLY FLOWERING6 (ELF6), also contributes in a partially non-redundant manner to reducing dormancy at this time (Sato *et al.*, 2021). During imbibition, many H3K27me3-marked genes related to hormones and expansins are derepressed to accelerate germination (Sato *et al.*, 2021; Pan *et al.*, 2022). Not all REF6 targets are demethylated however, and a number of dormancy-related genes are additionally marked by H3K9me2 and methylated CHG, blocking REF6 binding (Moreno-Romero *et al.*, 2019; Sato *et al.*, 2021). Loss of H3K9me2 and gain of H3ac leads to increased transcript accumulation of various dormancy-related genes such as *DOG1*, *ABI3*, *NCED6* and *NCED9* in seeds, highlighting the broader importance of this mark in maintaining transcriptional repression (Zheng *et al.*, 2012; Gu *et al.*, 2019; Zhou *et al.*, 2020). By the end of seed maturation, all REF6 activity has ceased and it is depleted from chromatin, where it is not detected until several days after the start of imbibition (likely to prevent increased PRC2 activity later in germination from repressing its targets), though its absence largely will no longer affect H3K27me3 dynamics after this point (Pan *et al.*, 2022). In fact global H3K27me3 levels undergo very little change during early germination, and while germination speed is reduced in mutants lacking FIE or CLF and SWN, its progression is unhindered until the post-germinative transition (Bouyer *et al.*, 2011).

By early germination transcription regulation now occurs independent of changes in H3K27me3, including the transcriptional suppression of a subset of embryonic and seed maturation genes still free of H3K27me3. These include genes related to seed maturation activated by the LAFL network (Jia *et al.*, 2014), themselves up-regulated during embryogenesis by the transcription factor AGAMOUS-LIKE15 (AGL15), which is not targeted for H3K27me3 deposition until later during germination (Figure 3b) (Zheng *et al.*, 2009). This transcriptional suppression may occur at least in part due to the action of deacetylases such as HDA6 and HDA19, without which the expression of *LEC1*, *FUS3*, and *ABI3* are up-regulated during germination (Tanaka *et al.*, 2008). Some genes are also transcriptionally suppressed via a RZ-1A and RZ-1B-dependent mechanism, as these proteins promote the loss of the active marks H3ac and

H3K4me3 during early germination before the deposition of H3K27me3 (Yang *et al.*, 2022). Deacetylation is generally an important regulator of germination, as inhibiting it with trichostatin A strongly reduces germination speed (Tanaka *et al.*, 2008; Temmerman *et al.*, 2023). The protein HISTONE DEACETYLASE COMPLEX1 (HDC1), which coordinates deacetylation at a number of stress-related genes during germination, is also necessary to regulate germination during salt stress (Perrella *et al.*, 2024). Regardless of the mechanisms behind the transcriptional down-regulation of such genes, they cease to be effective during the post-germinative transition as they reactivate in the absence of their targeting by VALs, PRC1, and PRC2 during this time (Figure 3b) (Tsukagoshi *et al.*, 2007; Bouyer *et al.*, 2011; Yang *et al.*, 2013; Mozgová *et al.*, 2017; Chen *et al.*, 2018; Ruta *et al.*, 2019, page 2; Yuan *et al.*, 2020). Thus far, the timing or regulatory mechanism involved in this reactivation remains an open question. Additionally, the beginning of cell divisions during this time requires active PRC2 machinery to maintain the repression of genes marked with H3K27me3 during seed development and prevent dilution of the mark (Pan *et al.*, 2022).

Beyond the extensive work into the various modifications of H3, several studies have also reported the role of histone variants in shaping seed genome regulation. The variant H3.3, abundant in dry seeds through the histone chaperone HISTONE REGULATOR A (HIRA), helps repress dormancy and enhance longevity during genotoxic stress (Layat *et al.*, 2021). While not essential for seed formation itself, its loss severely impacts germination and the post-germinative transition (Zhao *et al.*, 2022). It is present at both 5′ and 3′ ends of genes in seed euchromatin, as opposed to only the 3′ end in seedlings. This facilitates 5′ chromatin opening during germination and suppresses transcription from the 3′ end, and is necessary to facilitate increased GA biosynthesis gene expression. This action is likely in combination with another variant, H2A.Z, which likely helps facilitate 5′ chromatin opening (Zhao *et al.*, 2022). Chromatin accessibility more generally may play an important role in germination. In a recent study we observed a tight correlation between the loss of ABA hypersensitivity during the post-germinative transition and the loss of promoter accessibility for genes related to ABA signaling, which could explain why these genes are no longer upregulated upon exposure to exogenous ABA (Figure 3c-d) (Lopez-Molina *et al.*, 2001; Tremblay *et al.*, 2024). However, how promoter accessibility is dynamically controlled during germination remains to be investigated. H2A.Z has also been implicated in regulating other aspects of germination, including transcriptional down-regulation of *SOM*, a negative regulator of germination during heat stress. Under normal conditions the protein POWERDRESS (PWR) directs deacetylation of H3ac at *SOM* and deposition of H2A.Z, blocking its up-regulation by *ABI3*, whereas under high temperature conditions *PWR* transcription is down-regulated (Yang *et al.*, 2019).

Conclusions

In conclusion, the regulation of the genome in the formation of seeds and during germination is highly complex and involves a myriad of non-coding and epigenetic

mechanisms, including lncRNAs, miRNAs, DNA methylation, and histone variants and their modifications. Many aspects of these mechanisms remain understudied, including the large numbers of uncharacterized lncRNAs expressed during germination and the many other possible not-yet characterized histone modifications and variants. More specific aspects of these mechanisms are poorly understood as well, including the dynamics of transcriptional shutdown in seeds and genome compaction. Furthermore, non-coding regulation of gene expression by the 3D genome is increasingly being recognized as an essential aspect of transcriptional regulation (Schmitz *et al.*, 2022; Marand *et al.*, 2023). Despite its small genome, the study of chromatin looping in Arabidopsis thaliana has revealed its role in complex transcriptional coordination (Deng *et al.*, 2023). Studying the 3D genome can be achieved by the use of powerful techniques such as chromosome conformation capture coupled with sequencing (Hi-C) as well as variants which profile chromosomal interactions specific to certain histone modifications such HiChIP which couples Hi-C with chromatin immunoprecipitation (ChIP), both which have been used successfully with *Arabidopsis thaliana* (Belton *et al.*, 2012; Grob *et al.*, 2014; Mumbach *et al.*, 2016; Huang *et al.*, 2021).

Non-coding and epigenetic mechanisms act in concert during all stages of the seed-to-seedling transition, making studying how these mechanisms interact to regulate the same pathways critical. Future research will need to investigate how these elements co-regulate the seed-to-seedling transition to make sense of poorly understood aspects such as the condensation and subsequent decondensation of the genome in maturing and germinating seeds, respectively. Furthermore, it is now obvious that the seed-to-seedling transition is dependent on the presence of many distinct cell type-specific transcriptional programs (Liew *et al.*, 2024)Together, these advances will pave the way for applying our understanding of seed biology to solutions for agriculture.

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Author Contributions

B.J.M.T. performed the review of the literature and wrote the manuscript. B.J.M.T. and J.I.Q. prepared the figures and edited the manuscript. J.I.Q. supervised the work.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

This manuscript does not present new data.

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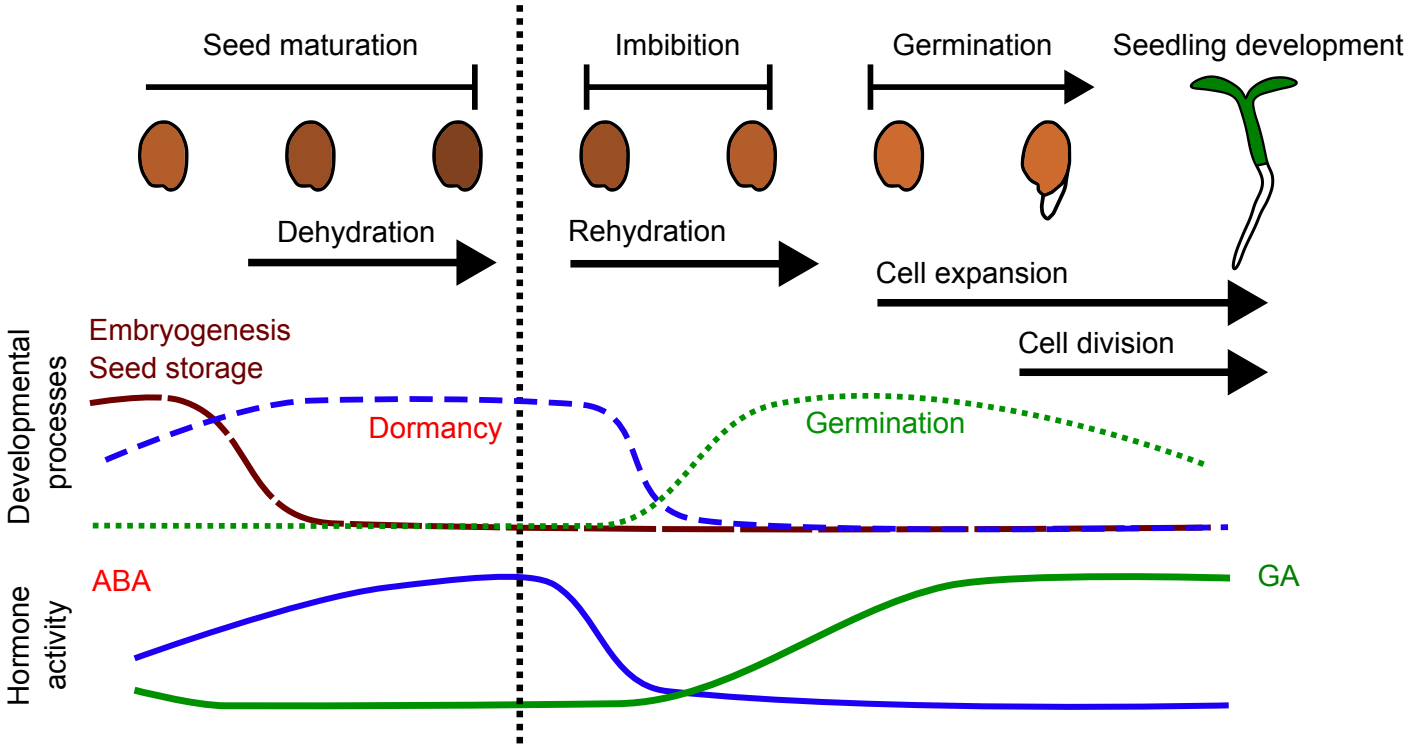
Figure Legends

Figure 1: Overview of key developmental and hormonal processes during seed maturation and germination. As embryogenesis nears its end and the seed matures, a combination of processes occur to reach the final desiccated dry seed stage. These include a shutdown in embryogenesis and seed storage-related gene expression, an increase in transcription of dormancy-associated genes, and an accumulation of ABA as well as a general shutdown of transcription. Once the effects of dormancy are released, either by after-ripening or stratification causing a decrease in ABA content and dormancy-related transcripts, imbibition of the dry seed leads to rehydration as transcription resumes during the early stages of germination. During this time germination-promoting genes increase in expression and GA is accumulated. Finally, after a period of cell elongation and the emergence of the radicle, the beginning of the post-germinative transition is accompanied by the resumption of cell division.

Figure 2: LncRNAs participate in both activation and repression of genes preventing germination. (A) Under red and far-red light conditions, phyB promotes accumulation of the *HID1* lncRNA in the radicle of germinating seeds to repress transcription of genes which slow germination. For the repressor *PIF3*, *HID1* RNA directly interacts with the chromatin in its promoter region, repressing transcription. For other genes such as the ABA biosynthesis gene *NCED9*, *HID1* interacts not only interacts with the chromatin in the its promoter region but also with the histone methylase ATXR7 to suppress its ability to deposit the activating gene body mark H3K4me3, thus reducing transcription of *NCED9*. (B) Transcription of the dormancy-promoting gene *DOG1* is induced and repressed by different lncRNAs. During germination, the antisense lncRNA *asDOG1* is expressed and suppresses transcription of *DOG1*. Upon salt stress however, a set of upstream lncRNAs known as *PUPPIES* instead increase RNAPII levels over *DOG1* and increase its expression. (C) We proposed in a recent study that when antisense and sense transcription over the same locus is exclusive, this has the effect of reducing overall transcription and mRNA accumulation (Tremblay *et al.*, 2024). For example, even though genes A (yellow colour) and B (blue colour) may appear transcriptionally active when sampling whole seedlings (shoot and roots), the antisense of gene A (dashed line) is expressed in roots thus preventing accumulation of gene A mRNA (solid line) compared to a gene with a similarly active locus without any antisense transcription. When sampling over time during germination, this has the effect of sense and antisense transcription being correlated but lower total gene mRNA levels.

Figure 3: DNA methylation and active modification of histones are necessary to regulate germination gene expression programs. (A) The histone demethylase REF6 removes the silencing mark H3K27me3 (pink circles) over genes necessary for germination during seed development which encode a REF6 binding site, leaving unmodified H3 (clear circles). However, the activity of REF6 is blocked over certain target genes promoting dormancy by the presence of methylated CHG (green circles)

and H3K9me2 (blue circles), allowing for fine-tuning of REF6 function. (B) As part of the seed maturation process, a host of genes such as the LAFL transcription factor *AGL15* are silenced by the deposition of H3K27me3 (pink circles) via the combined action of VALs, PRC1, and PRC2. These factors are also necessary during the post-germinative transition to maintain the silencing of embryogenesis and seed maturation-related genes. (C) During germination, growth arrest can be incurred by the accumulation of ABA. This can be replicated in laboratory experiments using exogenous ABA provided during the early phases of germination. However, germinating seeds become hyposensitive to the effects of ABA during the post-germinative transition (Lopez-Molina *et al.*, 2001). (D) The loss of sensitivity to growth arrest by exogenous ABA during later germination may be due to the decreased accessibility of promoter accessible chromatin regions (ACRs) we observed for ABA-responsive genes, which become inaccessible during the post-germinative transition (Tremblay *et al.*, 2024). ABA supplementation induces the activity of residual ABI5 protein, which only leads to reactivation of *ABI5*-responsive genes before post-germination, when promoters are still accessible.



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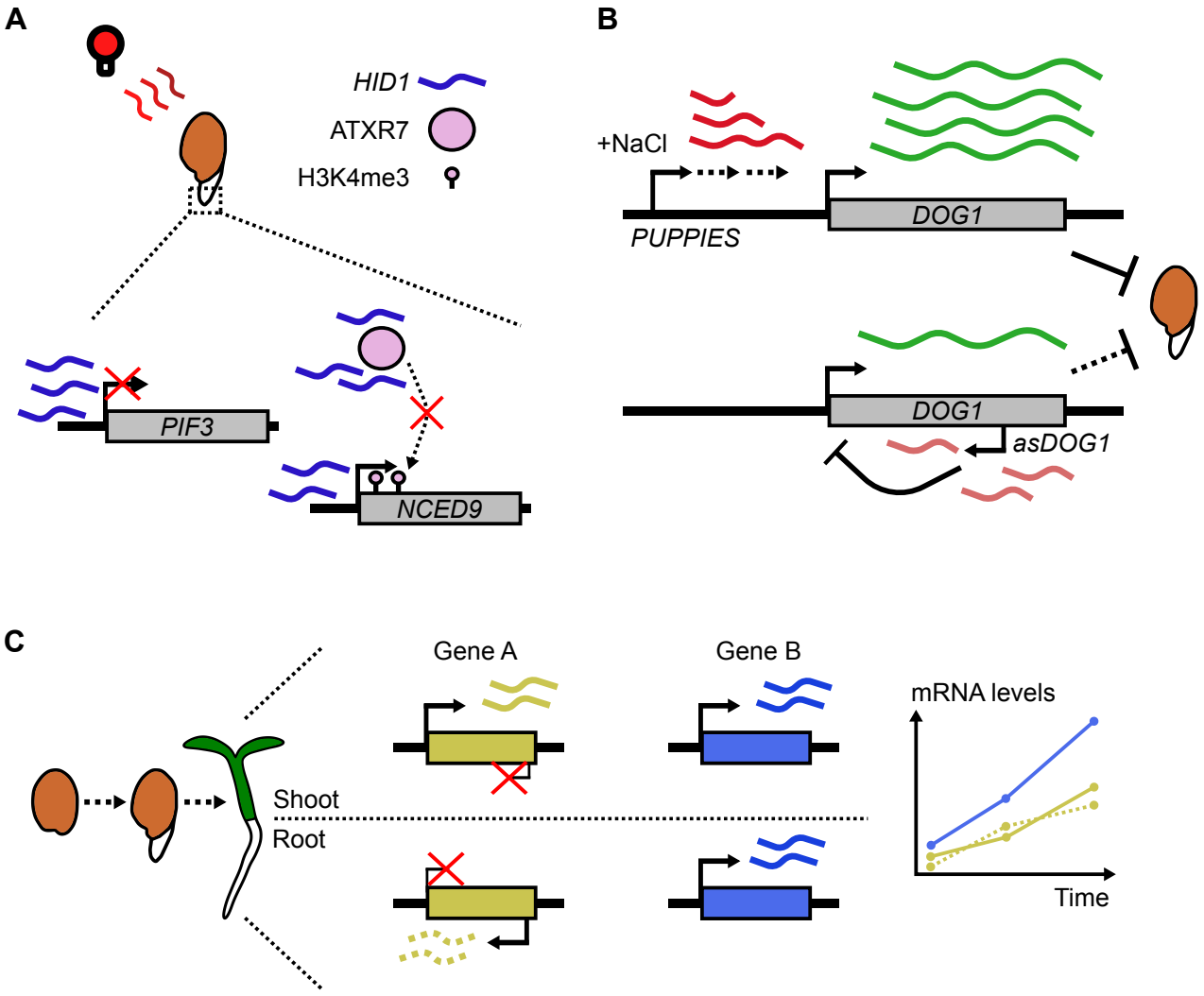

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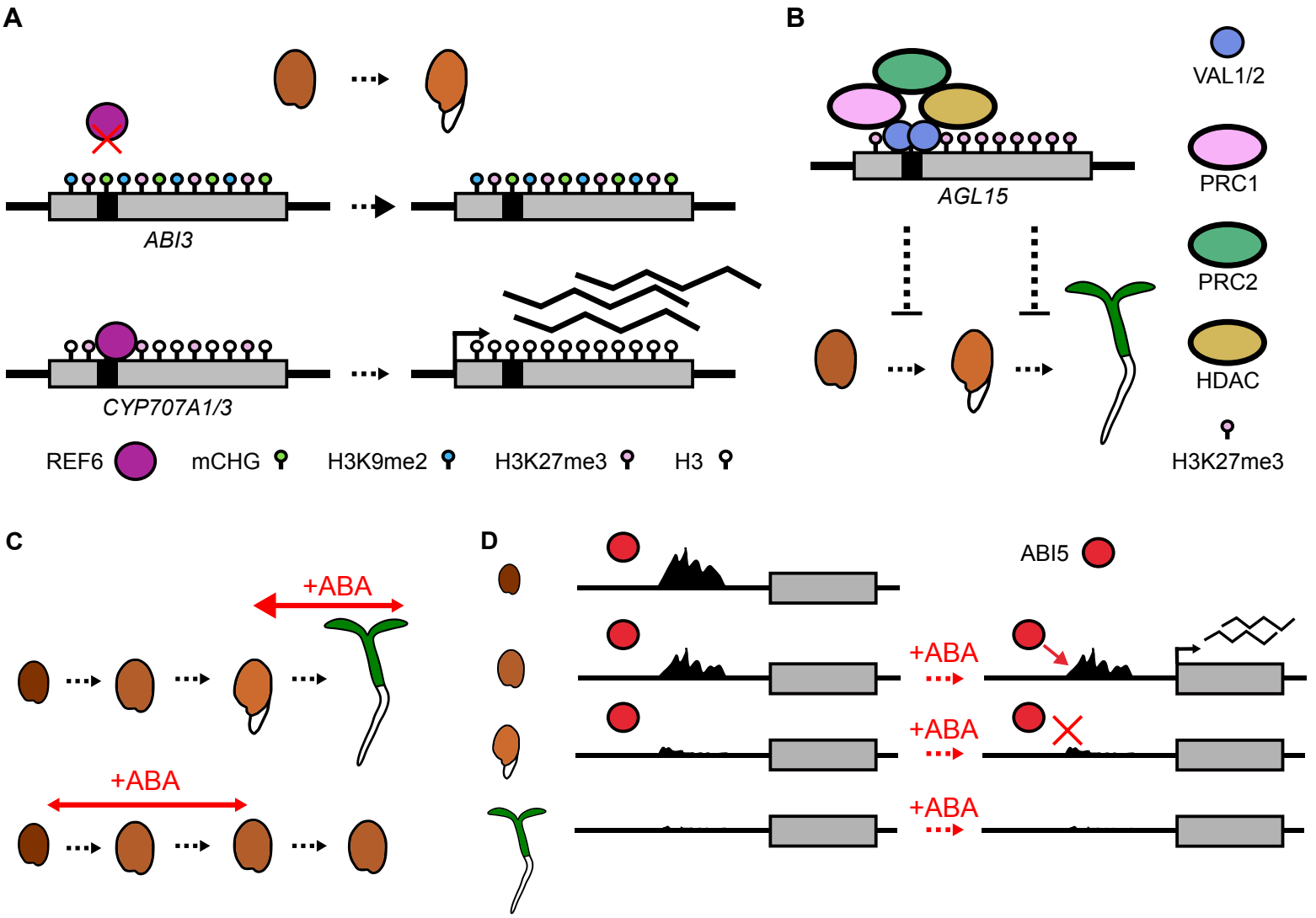

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