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Current Opinion in Plant Biology

Linking transcriptional silencing with chromatin remodeling, folding, and positioning in the nucleus

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Abstract

Chromatin organization is important for many DNA-templated processes in eukaryotic cells such as replication and transcription. Recent studies have uncovered the capacity of epigenetic modifications, phase separation, and nuclear architecture and spatial positioning to regulate chromatin organization in both plants and animals. Here, we provide an overview of the recent progress made in understanding how chromatin is organized within the nucleus at both the local and global levels with respect to the regulation of transcriptional silencing in plants. To be concise while covering important mechanisms across a range of scales, we focus on how epigenetic modifications and chromatin remodelers alter local chromatin structure, how liquid-liquid phase separation physically separates broader chromatin domains into distinct droplets, and how nuclear positioning affects global chromatin organization.

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Current Opinion in Plant Biology 2022, **69:**102261

This review comes from a themed issue on **Epigenetics and gene regulation**

Edited by **Bob Schmitz** and **Ortrun Mittelsten Scheid**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.pbi.2022.102261

Keywords

Epigenetic modifications, Chromatin organization, Transcriptional regulation.

Introduction

In eukaryotes, the nucleosome is the fundamental unit of chromatin composed of DNA wrapped around a histone octamer [1]. Nucleosome position and density are highly correlated with the accessibility of the underlying DNA because they can impact transcriptional activity by preventing other factors, such as transcription factors and DNA methyltransferases, from accessing their binding sites [2,3]. Genomic regions with low nucleosome density are generally accessible, correlate with the transcription start or termination sites of actively transcribed genes [4,5], and are enriched with activating epigenetic marks such as H3K27ac [5]. Conversely, regions with high nucleosome density are generally inaccessible, contain inactive genes or repressed transposons, and are enriched with silencing epigenetic marks such as DNA methylation and H3K27me3 [6**]. In *Arabidopsis*, the strongest interchromosomal interactions are observed within pericentromeric heterochromatin and telomeric regions [7,8]. Heterochromatic regions with silencing epigenetic marks (e.g., DNA methylation and H3K9me2) are generally associated with higher-order chromatin structures. The formation of both local chromatin interactions and higher-order chromatin structures is largely dependent on chromatin remodelers. On the one hand, the proper positioning of nucleosomes requires these chromatin remodelers to establish specific epigenetic modifications. On the other hand, chromatin remodelers and other chromatin reader proteins can recognize epigenetic marks to modify chromatin structure.

Chromatin liquid-liquid phase separation (LLPS) physically separates broader chromatin domains into distinct droplets and impacts global chromatin organization and transcription [9]. The formation of membrane-less nuclear bodies via LLPS has also been found to widely participate in the processes of chromatin modification and transcriptional regulation [10]. At the global scale of chromatin positioning within the nucleus, recent studies have started to uncover mechanisms by which plants can spatially organize chromatin within specific compartments and have identified molecular factors that may be functional equivalents of established organizational mechanisms in animals.

To provide a brief overview of transcriptional regulation across multiple scales of chromatin organization in plants, this review focuses on the relationship between silencing epigenetic marks (e.g., DNA methylation and H3K27me3) and local chromatin structure in both constitutive and facultative heterochromatin, the physical separation of broader chromatin domains into distinct droplets by LLPS, and the function of nuclear positioning in global chromatin organization.

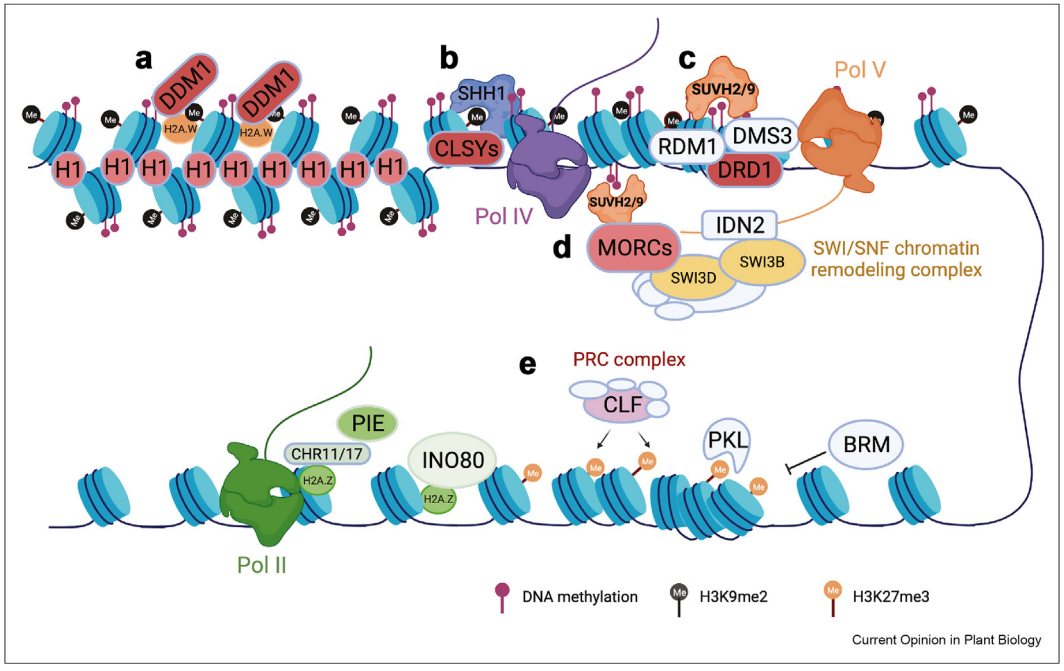
Local chromatin remodeling

The eukaryotic genome is partitioned into transcriptionally active euchromatin and silent heterochromatin. Constitutive heterochromatin, composed of tandem DNA repeats and transposons, is stably condensed and usually enriched with DNA methylation and H3K9 methylation. In contrast, facultative heterochromatin is associated with genomic regions enriched for H3K27 methylation. Emerging evidence indicates that chromatin remodelers are widely involved in the formation of constitutive and facultative heterochromatin by modulating DNA methylation and H3K27me3, respectively. In eukaryotes, there are four families of chromatin remodelers including switch/sucrose non-fermentable (SWI/SNF), imitation switch (ISWI), chromodomain helicase DNA-binding (CHD), and INOsitol requiring 80 (INO80). The ability of these chromatin remodelers to regulate transcriptional state by opening and closing chromatin architecture has been well documented [11]. Here, we review the recent progress outlining the relationship between chromatin remodelers and local chromatin structure in transcriptional silencing.

Chromatin remodeling in DNA methylation-marked heterochromatin

Constitutive heterochromatin is composed of highly condensed chromatin largely enriched with DNA methylation, which contributes to the establishment and maintenance of higher-order chromatin structures. In *Arabidopsis*, the chromatin remodeler DDM1 (Decreased DNA Methylation 1) is required for the maintenance of DNA methylation in constitutive heterochromatin [12], whereas the interspersed DNA methylation within transposon- or repeat-containing euchromatic regions is mainly mediated by the RNA-directed DNA methylation (RdDM) pathway [13]. The RdDM pathway requires small interfering RNAs (siRNAs) and long noncoding scaffold RNAs produced

Figure 1



Chromatin remodelers function in chromatin organization and transcriptional regulation. (**a**) The SWI/SNF2 chromatin remodeling protein DDM1 binds H2A.W and changes the properties of chromatin to allow DNA methyltransferases to access histone H1 containing regions. (**b**) The CLSY family of chromatin remodelers associates with the H3K9me2 reader protein SHH1 and enables Pol IV recruitment. (**c**) DRD1 interacts with DMS3 and RDM1 to change nucleosome positioning and facilitate the association of Pol V with chromatin. (**d**) SWI3B, a subunit of the SWI/SNF chromatin remodeling complex, functions with the Pol V transcript-binding protein IDN2 to establish nucleosome positioning and mediate *de novo* DNA methylation and transcriptional silencing. MORC family proteins interact with SWI3D and the DNA methylation binding proteins SUVH2/9 to mediate heterochromatin condensation and gene silencing. (**e**) Chromatin remodelers function in PRC-enriched facultative heterochromatin regulation. Important abbreviations: DDM1, Deceased in DNA Methylation 1; CLSY, SNF2 domain-containing protein CLASSY; SHH1, SAWADEE Homeodomain Homolog 1; Pol IV, DNA-dependent RNA polymerase IV; DRD1, Defective in RNA-Directed DNA methylation 1; DMS3, Defective in Meristem Silencing 3; RDM1, RNA-Directed DNA Methylation 1; Pol V, DNA-dependent RNA polymerase V; IDN2, Involved in De Novo 2; MORC, Microrchidia; SUVH2/9, SU(VAR)3-9 homolog protein 2 and SU(VAR)3-9 homolog protein 9; Pol II, DNA-dependent RNA polymerase II. CHR11/17, imitation of switch (ISWI)-like chromatin-remodeling protein 11 and 17; PIE, Photoperiod-Independent Early flowering; INO80, INOsitol requiring 80; PRC complex, Polycomb Repressive Complex; CLF, Curly LeaF; PKL, PicKLe, CHD3-type chromatin remodeling protein; BRM, BRahMa, SWI/SNF chromatin remodeling protein.

by the DNA-dependent RNA polymerases Pol IV and Pol V to mediate DNA methylation in all sequence contexts (CG, CHG, and CHH, where H = A, C or T) [14].

DDM1 encodes a SWI/SNF2 chromatin remodeler that can shift nucleosomes in an ATP-dependent manner *in vitro* [15]. Loss of DDM1 causes a profound reduction of DNA methylation and H3K9me2, leading to transcriptional activation in heterochromatin [16,17]. Given that nucleosomes are barriers to DNA methyltransferases, DDM1 enables cytosine methylation of nucleosome-wrapped DNA by remodeling heterochromatin to allow DNA methyltransferases to access histone H1-containing regions [12,18,19]. DDM1 was also reported to directly bind H2A.W and mediate its deposition, resulting in the alteration of chromatin properties and transposon silencing (Figure 1a) [20*]. In *Arabidopsis*, H2A.W uniquely marks silent genes and transposons in constitutive heterochromatin [21]. While the loss of H2A.W has no impact on the repression of genes and transposons, it would be interesting to further investigate how the incorporation of H2A.W by DDM1 in heterochromatin contributes to transposon silencing. Additionally, a recent study showed that the combined loss of H1 and H2A.W greatly increases chromatin accessibility and DNA methylation in heterochromatin, suggesting that the maintenance of heterochromatin requires both H2A.W and H1 [22*].

The CLASSY (CLSY) family of putative SWI2/SNF2 chromatin remodeler proteins facilitates Pol IV occupancy and the *de novo* establishment of DNA methylation [23]. CLSY1 and CLSY2 associate with SAWADEE HOMEODOMAIN HOMOLOG1 (SHH1) and are required for H3K9me2 recognition and Pol IV recruitment for chromatin remodeling in leaf tissue, while CLSY3 and CLSY4 recognize CG methylation and are specifically expressed in the ovules (Figure 1b) [23,24*]. The tissue-specific expression of different CLSYs indicates that chromatin remodelers may be important for DNA methylation patterning and gene regulation during plant cell differentiation.

DRD1 (Defective in RNA-directed DNA methylation 1) is another helicase-like SWI2/SNF2 chromatin remodeler, which physically interacts with DEFECTIVE IN MERISTEM SILENCING 3 (DMS3) and RNA-DIRECTED DNA METHYLATION 1 (RDM1) to form the DDR complex [25]. DRD1 acts both downstream and in conjunction with DMS3 and RDM1, serving as a bridge between the DDR complex and Pol V [26]. A recent cryo-EM structure of the DDR complex revealed that the binding of DRD1 can stabilize the coiled-coil domain of DMS3, which converts the DMS3 dimer within the complex from an open to a closed state [26]. This indicates that DRD1 triggers the formation of the DDR complex, which subsequently recruits Pol V to specific genomic loci (Figure 1c).

The SU(VAR)3-9 homolog proteins SUVH2/9 interact with the DDR complex and bind methylated DNA to recruit Pol V to specific regions [27,28]. Pol V then produces long noncoding RNAs that interact with the RNA-binding protein INVOLVED IN DE NOVO 2 (IDN2), which associates with the SWI/SNF complex protein SWI3B to establish nucleosome positioning and mediate transcriptional silencing [29]. These results suggest that ATP-dependent chromatin remodelers are involved in positioning nucleosomes, facilitating DNA methylation, and transcriptional silencing.

The *Arabidopsis* MICRORCHIDIA (AtMORC1-7) family of proteins is required for heterochromatin condensation and transcriptional silencing [30–32]. Loss of AtMORC6 induces large-scale nuclear reorganization and chromatin interaction changes coupled with the de-repression of silenced genes and transposons [7]. In *C. elegans*, ceMORC1 displays no DNA sequence preference but can trap and compact loops of DNA to form topologically entrapped foci that enforce chromatin compaction [33]. AtMORC6 interacts with the DNA methylation binding protein SUVH9 and the SWI/SNF chromatin remodeler SWI3D to mediate transcriptional silencing and chromatin condensation (Figure 1d) [30]. A recent study showed that MORC7, which associates with Pol V and RdDM components *in vivo*, is localized to specific chromatin regions and facilitates the establishment of RdDM [32*]. These results indicate that MORCs are recruited to RdDM sites via interactions with RdDM components, and then function as molecular intermediaries or a memory component between DNA methylation, higher-order chromatin structures, and transcriptional silencing [32*].

Chromatin remodeling in PRC-directed facultative heterochromatin

As a key silencing mark in facultative heterochromatin, H3K27me3 is mainly enriched at transcription start sites or within gene bodies and is involved in chromatin organization for both local and long-distance interactions across the *Arabidopsis* genome [7,34*]. Deposition of H3K27me3 is catalyzed by the polycomb repressive complex (PRC), which establishes and maintains the transcriptionally repressed heterochromatin. Emerging evidence shows that chromatin remodelers play an important role in regulating facultative heterochromatin silencing and three-dimensional (3D) genome architecture [35–39**].

In *N. crassa*, the chromatin remodeler ISW (imitation switch) is required for stable PRC-chromatin interaction

in the repression of PRC target genes, proper nucleosome organization, and the assembly of facultative heterochromatin [35,36]. ISW and its accessory proteins interact with each other to form an ATP-utilizing chromatin assembly and remodeling factor complex, which interacts with chromatin targets and remodels the chromatin landscape at H3K27-methylated regions [35,36]. In *Arabidopsis*, the chromodomain helicase DNA binding family remodeler PICKLE (PKL), is thought to promote H3K27me3-harbored nucleosome retention by associating with the SWR1-family remodeler PHOTOPERIOD INDEPENDENT EARLY FLOWERING1 (PIE1) and the PRC complex [37]. PIE incorporates histone variant H2A.Z, which in turn promotes H3K27me3 deposition followed by H3K27me3 retention by PKL and the formation of mature nucleosomes from prenucleosomes [37]. These results indicate that ATP-dependent chromatin remodelers contribute to both the construction and maintenance of H3K27me3-enriched facultative heterochromatin. Additionally, to prevent an inappropriate association and activity of the PRC complex at active genes, the SWI/SNF chromatin remodeler BRAHMA (BRM) directly binds to these regions to restrict the function of the PRC complex [38]. This observation suggests the existence of a high precision mechanism of chromatin remodelers in the regulation of chromatin homeostasis during plant growth and development.

To investigate the mechanisms of diverse chromatin remodelers in 3D chromatin organization, a recent study examined genome-wide chromatin interactions in mutants of all four families of chromatin remodelers by using Hi-C and showed that BRM, INO80, and PKL are involved in nucleosome density, while the ISWI chromatin remodelers CHR11 and CHR17 regulate nucleosome distribution patterns [39**]. In all these chromatin remodeler complex mutants, the genome-wide H3K27me3 reduction was accompanied by chromatin-interaction compartment switch [39**]. This revealed the different regulatory mechanisms of chromatin remodelers in both linear nucleosome distribution pattern and density, which promote PRC-dependent H3K27me3 deposition and 3D chromatin structure formation (Figure 1e).

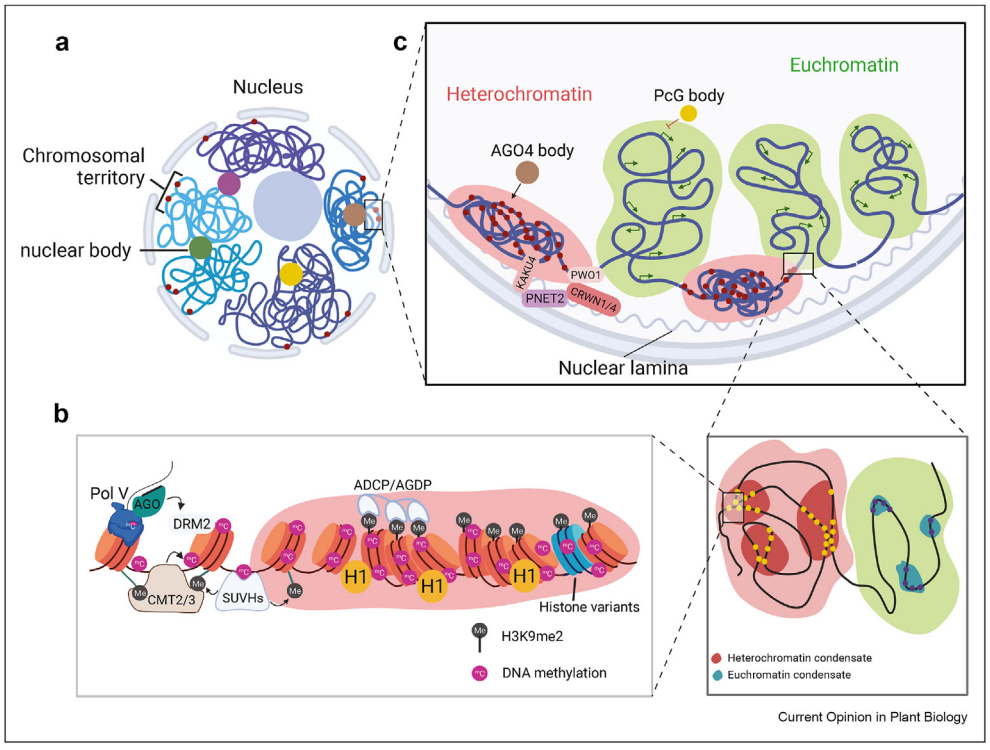
Phase separation and chromatin condensation

Beyond local chromatin structure, broader chromatin domains can organize by forming liquid- or gel-like droplets through either the intrinsic phase separation properties of chromatin itself or through interactions with other associated factors in the nucleus [9]. The LLPS property of chromatin is correlated with chromatin organization and gene transcription [34]. Histone tail-driven LLPS of chromatin is highly dependent on nucleosome spacing and the associated linker histone H1 [40]. Together with DNA methylation, H1 increases nucleosome spacing, condenses heterochromatin, and globally controls nucleosome positioning to silence transposons and suppress intragenic antisense transcripts [41,42]. H1 also enforces the separation of euchromatic and heterochromatic regions by impeding the RdDM pathway in GC-rich heterochromatin [43*]. The highly disordered C-terminal tail of H1 and DNA have been shown to act as scaffolds for phase-separated heterochromatin both *in vitro* and in HeLa cells [44,45]. It is possible that *Arabidopsis* H1.1 and H1.2 proteins, predicted to also contain C-terminal intrinsically disordered regions (IDR, http://www.pondr.com), may have similar function in the formation of heterochromatin foci. Before reaching this conclusion, more experiments are needed to exclude the possibility that H1 increases nucleosome spacing via its mere binding to linker DNA to condense heterochromatin independent of phase separation. Besides, it has been shown that histone variant H2A.W is able to promote chromatin condensation though long-range, fiber-to-fiber interaction by its C-terminal SPKK motif *in vitro* and organize heterochromatin into chromocenters in the central cell nucleus [46]. Furthermore, histone variant H2B.8 has also been shown to aggregate transcriptionally inactive chromatin into phase-separated condensates in sperm cells [47*]. Together, these studies suggest that histone H1 and histone variants may directly regulate nucleosome stability and chromatin environment by phase separation (Figure 2b).

Apart from the intrinsic phase separation properties of chromatin, LLPS-driven chromatin condensation also occurs through interactions with chromatin reader proteins. Heterochromatin protein 1 (HP1) couples its self-oligomerization and H3K9me3 binding capacity to drive the formation of phase-separated condensates and chromatin compaction [48,49]. In plants, the H3K9me2 reader protein ADCP1, a functional analog to HP1, also mediates heterochromatin phase separation and chromocenter formation [50,51].

Additionally, many membrane-less subnuclear organelles and nuclear bodies that are formed by LLPS have been found to participate in chromatin modifications and gene regulation in plants (Figure 2c) [10]. In the nucleus, these membrane-less condensates are involved in the formation of the nucleolus, which functions in rRNA biosynthesis and ribosome biogenesis [52]; nuclear speckles, which are involved in gene regulation and the association of transcription factors and chromatin remodeling factors [53]; and Cajal bodies, which are involved in RNA silencing and snRNP biogenesis [54]. For example, ARGONAUTE4 (AGO4) forms a specific nuclear body that colocalizes with the Pol V subunit NRPD1b and Cajal bodies [55]. This suggests that AGO4 and its related siRNA complex both function in

Figure 2



Phase separation and chromatin condensation at the nuclear periphery. (**a**) In the nucleus, chromatin is highly organized into different chromosome territories. Heterochromatin within gene-poor regions is typically found at the nuclear periphery, whereas gene-rich euchromatin is in the nuclear interior. (**b**) ADCP/AGDP binds H3K9me2, which is established by a self-reinforcing loop between DNA methyltransferases (DRM2 and CMT2/3) and H3K9 methyltransferases (SUVHs), and mediates heterochromatin phase separation and chromocenter formation. Histone linker H1 is proposed to condense heterochromatin and regulate nucleosome position. Histone variants (e.g., H2A.W and H2B.8) are also involved in promoting the formation of nuclear condensates. (**c**) Plant lamin-like proteins CRWN1/4 and PWO1 function together to link inaccessible chromatin domains and the nuclear matrix. PNET2 is a nuclear membrane protein associated with CRWN1 and KAKU4, and drives heterochromatin to the nuclear periphery. Phase-separated assemblies of proteins and nucleic acids are termed nuclear bodies (e.g., AGO4 body and PcG body). AGO4 bodies colocalize with Pol V subunits and Cajal bodies to establish DNA methylation. PcG bodies are condensates of Polycomb-group (PcG) proteins, which control H3K27me3. Important abbreviations: Pol V, DNA-dependent RNA polymerase V; AGO4, Argonaut 4; CRWN1/4, CROWDED NUCLEI 1 and CROWDED NUCLEI 4; PWO1, PROLINE-TRYPTOPHAN-TRYPTOPHAN-PROLINE INTERACTOR OF POLYCOMBS1; PNET2, Plant Nuclear Envelope Transmembrane 2; DRM2, Domains Rearranged Methyltransferase 2; CMT2/3, CHROMOMETHYLASE 2 and CHROMOMETHYLASE 3; SUVHs, SU(VAR)3-9 HOMOLOG proteins; ADCP/AGDP, Agenet Domain Containing Protein 1/Agenet Domain (AGD)-Containing P1.

the *de novo* DNA methylation process, which then requires distinct nuclear bodies and subnuclear compartments. Furthermore, Polycomb-group (PcG) proteins aggregate to form a PcG body in nuclear speckles and are involved in transcriptional regulation by altering chromatin organization [56]. EMBRYONIC FLOWER1 (EMF1) and LIKE HETEROCHROMATIN PROTEIN1 (LHP1) are required for the formation of PcG bodies and mediate phase-separated condensates to promote H3K27me3 modification [57], and EMBRYO DEFECTIVE 1579 (EMB1579) forms liquid-like condensates and recruits the DNA Damage Binding Protein 1 (DDB1), Cullin 4 (CUL4), and MULTIPLE SUPPRESSOR OF IRA 4 (MSI4) to promote CLF-PRC2 mediated H3K27me3 modification at the FLC locus [58*]. Despite these observations, the phase separation property of the PcG proteins has yet to be demonstrated in plants. It will be interesting to uncover whether *Arabidopsis* PcG proteins are involved in chromatin condensation though LLPS.

Spatial chromatin organization

At a higher scale than phase separation, chromatin positioning within the nucleus also plays a role in chromatin organization. In animals, lamin proteins form the nuclear matrix that interacts with chromatin at the nuclear periphery, thereby acting as the primary scaffold for 3D genome organization [59]. In *S. pombe*, Amo1 associates with the RNA processing complex RIXC and the histone chaperone complex FACT to tether heterochromatin to the nuclear periphery [60]. In mammals, X chromosome inactivation represents an extreme case of nuclear positioning of chromatin whereby the entire inactivated X chromosome is tethered to the nuclear periphery [61]. This suggests that the epigenetic maintenance of heterochromatin may require its

positioning at the nuclear periphery. Of current interest to the plant community is the classification of equivalent factors and mechanisms in plants that could drive the localization of heterochromatin to the nuclear periphery.

In *Arabidopsis*, heterochromatin positioning at the nuclear periphery requires plant-specific, lamin-like CROWDED NUCLEI proteins CRWN1 and CRWN4 in addition to non-CG DNA methylation [62*]. It was shown that CRWN1 binds to inaccessible chromatin domains at the nuclear periphery and that *crwn1* mutants abolish this binding. CRWN1 and CRWN4 localize specifically to the nuclear periphery during interphase [63]. From prometaphase to anaphase, CRWN1 localizes to the condensed chromatin and then localizes back to the nuclear periphery during telophase [64]. Subsequently, during late telophase, CRWN1-4 are all localized to the nuclear periphery [64]. The mechanism by which CRWN1 can localize itself to the nuclear periphery is thought to occur through an interaction with SUN1 and SUN2, which are components of the LINKER OF NUCLEOSKELETON AND CYTOSKELETON (LINC) complex [65]. This suggests that CRWN1 is likely a key factor in chromatin positioning at the nuclear periphery.

In addition, CRWN1 and CRWN4 also physically associate with PROLINE-TRYPTOPHAN-TRYPTOPHAN-PROLINE INTERACTOR OF POLYCOMBS1 (PWO1) [66**]. PWO1 forms foci located partially at the subnuclear periphery and interacts with plant nuclear matrix and nuclear envelope proteins, CRWN1/4 and SUN1. Additionally, *pwo1* and *crwn1crwn2* mutants affect the expression of a similar set of genes, suggesting that PWO1 and CRWN1 act in the same complex to link chromatin repression to the nuclear periphery [66**].

Most recently, PNET2 was characterized as a nuclear membrane protein associated with the nuclear matrix of *Arabidopsis* [67**]. Proximity labeling experiments support a close association of PNET2 and KAKU4, another lamin-like protein in *Arabidopsis* [68]. The PNET2 C-terminal domain can also be engaged by KAKU4 at the nuclear periphery, thus strengthening the proposed association between PNET2 and KAKU4. Additionally, both PNET2 and KAKU4 are closely associated with nucleosome core histone H2 proteins. It is believed that PNET2 physically binds CRWN1 to properly localize itself beneath the inner nuclear membrane (Figure 2c).

Despite these discoveries, the exact mechanisms that drive heterochromatin localization to the nuclear periphery remain relatively unknown in plants. Further efforts to understand exactly how the identified nuclear matrix constituents and their associated factors are coordinating the nuclear positioning of chromatin will help to increase our understanding of global chromatin organization in plants.

Furthermore, plants also possess structural maintenance of chromosome (SMC) complexes, which, much like the factors involved in chromatin organization at the nuclear periphery, are less studied compared to their animal homologs. SMC complexes are thought to function in chromosome condensation and chromatin organization through loop extrusion whereby genomic DNA is pulled through the complex to form gradually larger loops of intrachromosomal DNA [69]. In plants, it has been shown that the SMC complexes, condensin and cohesin, are involved in transcriptional silencing [70,71]. SMC4, a core subunit of condensins I and II, contributes to the silencing of pericentromeric transposons without affecting DNA methylation and is also required for chromocenter condensation [71]. It was recently demonstrated that condensin II is also required for the proper positioning of rDNA arrays within the nucleus [72*]. The currently known functions of condensin and cohesin highlight the potential role of SMC complexes in mediating proper spatial chromatin organization in plants.

Perspectives

Genome organization is distinct in different cell types and is also subject to dynamic changes during plant developmental transitions as well as in response to diverse environmental stresses. The past decade has witnessed tremendous progress in our understanding of the function of genome organization on transcriptional regulation and other cellular processes. Epigenetic modifications, including DNA methylation, histone modifications, histone variants, and both short and long-range chromatin interactions have been instrumental in packing and shaping plant genomes (Figure 1). While epigenome profiling and global epigenome perturbation by genetic mutant studies have revealed significant associations between epigenetic features and chromatin organization, these approaches are largely correlative and challenge to dissect the direct and causal roles of epigenetic modifications. Recent structural and biochemical investigations of chromatin remodelers (e.g., DRD1) have contributed significantly to deciphering the regulatory principles of epigenetic modifying machinery in genome organization and transcriptional regulation. However, such studies also offer limited insights into these mechanisms in the context of native chromatin environments under physiological conditions. Recently developed CRISPR/dCas9-mediated epigenome editing technology offers a potentially powerful tool to address these challenges.

Emerging studies have suggested the potential role of liquid–liquid phase separation and spatial chromatin

positioning in genome organization and transcriptional regulation. The phase separation properties (i.e., IDR domains) of histone variants (H2B.8 and linker histone H1), chromatin-binding proteins (ADCP/AGDP and EMB1579), and nuclear bodies (AGO4 bodies and PcG bodies) have been implicated in the regulation of chromatin dynamics and transcription in plants (Figure 2). Despite this progress, direct evidence for the functional significance of phase separation is largely lacking in plants. Delineating the links between the cell signaling pathways upstream of chromatin phase-separation and its biological functions is another challenge for future studies. The development of the mammalian CRISPR genome organization system to reposition specific genomic loci to the nuclear periphery [58] offers an exciting tool to reprogram 3D genome positioning and nuclear organization in plants. Future efforts towards chromatin organization-based epigenome manipulation will further broaden our understanding of transcriptional regulation and lead to the development of innovative epigenetic strategies for agricultural improvements.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Sarah Leichter for the critical reading of this manuscript. This work was supported by NSF (MCB-2043544) and NIH (R35GM124806). Figures were created with Biorender.com. We apologize to colleagues whose work was not included due to space limitations.

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* of special interest
** of outstanding interest

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