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SPECIAL ISSUE REVIEW

Conserved functions of chromatin regulators in basal Archaeplastida

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SUMMARY

Chromatin is a dynamic network that regulates genome organization and gene expression. Different types of chromatin regulators are highly conserved among Archaeplastida, including unicellular algae, while some chromatin genes are only present in land plant genomes. Here, we review recent advances in understanding the function of conserved chromatin factors in basal land plants and algae. We focus on the role of Polycomb-group genes which mediate H3K27me3-based silencing and play a role in balancing gene dosage and regulating haploid-to-diploid transitions by tissue-specific repression of the transcription factors KNOX and BELL in many representatives of the green lineage. Moreover, H3K27me3 predominantly occupies repetitive elements which can lead to their silencing in a unicellular alga and basal land plants, while it covers mostly protein-coding genes in higher land plants. In addition, we discuss the role of nuclear matrix constituent proteins as putative functional lamin analogs that are highly conserved among land plants and might have an ancestral function in stress response regulation. In summary, our review highlights the importance of studying chromatin regulation in a wide range of organisms in the Archaeplastida.

Keywords: chromatin regulation, H3K27me3, evolutionary conservation, haploid-to-diploid transition, nuclear matrix constituent protein, transposable element, KNOX/BELL, genome integrity, stress response, H3K9me.

INTRODUCTION

DNA forms highly condensed three-dimensional structures to fit into the nucleus. 146 bp DNA wrap around one histone octamer containing two copies of the core histones H2A, H2B, H3, and H4 to form a nucleosome. Nucleosome chains interact with proteins to form condensed chromatin structures. Chromatin can be distributed into highly compacted heterochromatin, a region of gene silencing, and less densely packaged euchromatin, where genes are accessible to the transcriptional machinery and gene expression can occur. The distribution into eu- and heterochromatin is not static. In fact, chromatin compaction and accessibility are highly dynamic and influenced by a multitude of factors. A whole palette of proteins that can maintain or modify the chromatin structure and regulate the expression of genes has been identified. Particularly the histone tails are highly posttranslationally modified, including acetylation, methylation, phosphorylation, and monoubiquitination. Different modifications can have different effects on the expression of the occupied genes. A major silencing mark is trimethylated histone 3 lysine 27 (H3K27me3) that can be found at transcriptionally silenced loci. H3K27 is methylated by Polycomb Repressive Complex 2 (PRC2), one of the Polycomb Group (PcG) protein complexes, which was initially discovered as a regulator of cell identity and tissue development in the fruit fly *Drosophila melanogaster* (Lewis, 1978). Drosophila PRC2 consists of four subunits: the nucleosome remodeling factor p55, Suppressor of zeste (Su(z)12) which enables nucleosome-PRC2-binding, Enhancer of zeste (E(z)) which catalyzes histone methylation, and Extra Sex Combs (ESC), which maintains complex integrity (Schwartz & Pirrotta, 2013). In 1997, the presence of PRC2 proteins in plants was first demonstrated in *Arabidopsis thaliana* (Goodrich et al., 1997).

Subsequent studies revealed a high conservation of PcG proteins across Archaeplastida species (Huang et al., 2017), a major evolutionary lineage of photosynthetic organisms, which is comprised of Glaucophyta (unicellular

algae), Rhodophyta (uni- and multicellular red algae), and Viridiplantae (Burki et al., 2020). The latter group contains aquatic organisms such as the green algae Chlorophyta and Charophyta, as well as terrestrial plants, the Embryophyta (Burki et al., 2020; Ruhfel et al., 2014; Wang, Liang, et al., 2021). The *A. thaliana* genome contains three *E(z)* homologs: *CURLY LEAF*, *MEDEA*, and *SWINGER*. They were shown to be involved in the regulation of flowering timing, seed development, and stress memory (Bouyer et al., 2011; Jiang et al., 2008; Müller-Xing et al., 2014; Sani et al., 2013; Tang et al., 2012). Homologs of *E(z)* are also present in the unicellular model green alga *Chlamydomonas reinhardtii* and the unicellular model red alga *Cyanidioschyzon merolae*, indicating a function for PRC2 and H3K27me3 also in unicellular organisms that do not produce seeds or develop flowers (Huang et al., 2017; Mikulski et al., 2017; Schubert, 2019; Shaver et al., 2010). A second PcG complex, PRC1, that mediates H2A monoubiquitination is also highly conserved, though only a subset of complex members is present in (some) algae.

Another major silencing modification is methylated H3K9 (H3K9me1/2/3) that represses transcription and can be found within heterochromatic areas and at transposable elements (TEs), while acetylated H3K9 (H3K9Ac) promotes gene expression and can thus be found at loci of actively transcribed genes (Nakayama et al., 2001; Rea et al., 2000; Zhou et al., 2010). H3K9 methylation is mediated by SU (VAR) histone methyltransferases (HMTs) that have three homologs in the Arabidopsis genome, namely SU (VAR) HOMOLOG (SUVH) 4, SUVH5, and SUVH6 (Jackson et al., 2002, 2004; Li et al., 2018). Histone acetylation is mediated by histone acetyl transferases (HATs, reviewed by Boycheva et al., 2014; Roth et al., 2001). The addition of negatively charged acetyl groups reduces the positive charge of histones and thereby decreases their affinity for the negatively charged DNA and can increase the accessibility of transcription factors to the chromatin (Görisch et al., 2005). HMTs and HATs are antagonized by histone demethylases (HDMTs) and histone deacetylases (HDACs), which can remove the methyl and acetyl groups, respectively. H3K9 methylation and acetylation are both conserved in land plants (Li et al., 2018; Montgomery et al., 2020; Widiez et al., 2014; Zhang et al., 2023; Zhao et al., 2020). Chromatin regulation can also occur by direct modification of the DNA: DNA methyltransferases methylate cytosine residues to form 5-methylcytosine (5mC) and DNA glycolases such as DEMETER (DME) remove methyl groups from cytosines (reviewed by Zhang et al., 2018). Methylated DNA is a target of H3K9 HMTs in a pattern-specific manner: AtSUVH4 preferably binds to methylated CWG (W can be A or T), AtSUVH5 interacts with CCG and AtSUVH6 can bind to both sequence patterns (Li et al., 2018). In turn, H3K9me2 was shown to recruit the DNA methyltransferases CHROMOMETHYLASE (CMT) 2 and CMT3 (Bernatavichute et al., 2008; Du et al., 2012; Johnson et al., 2007; Zemach et al., 2013). Thereby, DNA and H3K9 methylation maintain heterochromatin regulation and compaction in a positive feedback loop manner (reviewed by Du et al., 2015). Moreover, 5mC has a function in TE silencing and in regulating plant development and stress response (Hu et al., 2020; Nguyen et al., 2022; Ramakrishnan et al., 2021). DNA methylation is highly conserved in eukaryotes (Aguilar-Cruz et al., 2019; Huff & Zilberman, 2014). However, 5mC is not detectable in the genomes of several organisms such as *Cy. merolae*, raising the question how these organisms epigenetically maintain usually 5mC-regulated processes.

The combination of different chromatin marks shapes the chromatin state at a specific locus and determines whether it is active or silent (Sequeira-Mendes et al., 2014). Chromatin states can be bivalent, possessing activating and silencing marks as recently shown for biosynthesis genes of camalexin, which is produced during plant–pathogen interactions (Zhao, Kong, et al., 2021). Canonical histones within nucleosomes can be replaced by histone variants that exhibit different properties or organize the arrangement of chromatin within the nucleus and thereby control the accessibility of genes (Dechat et al., 2009; Herrmann et al., 2009). Histone variants are inserted by chromatin remodelers that utilize energy from ATP hydrolysis to restructure nucleosome arrangements and enable or impede accessibility for other regulators and the transcriptional machinery. Also, these factors are highly conserved among eukaryotes (Becker & Hörz, 2002). Lastly, chromatin regulation strongly depends on nuclear organization, with the nuclear lamina and envelope having a key role in regulating gene expression.

The fine-tuning of chromatin regulation results in specific gene expression patterns that are fundamental for genome integrity and heterochromatin regulation, cell proliferation, determination and maintenance of cell fate, adaptation to changes of environmental conditions, and various other processes. However, how conserved chromatin factors contribute to genome regulation in unicellular and basal land plants is less clear and has been addressed only recently, as more and more genome sequence are available and genomics tools have been developed. Here, we therefore review recent findings on chromatin regulators in basal land plants and algae (Box 1). We compare their roles in Archaeplastida species that emerged at different time points during plant evolution, discuss their potential functional conservation and regulatory roles, and point out open questions (Box 2).

CONTROL OF CELL IDENTITY AND HAPLOID-TO-DIPLOID TRANSITION

Plant life cycles are characterized by an alternation of two distinct life forms: haploid gametes fuse to form diploid

Box 1. Summary

- While the PRC2 targets *KNOX* and *BELL* regulate sporophyte cell identity in flowering plants such as *A. thaliana*, they regulate haploid-to-diploid transitions in lower land plants and aquatic phototrophs, indicating that this is their ancestral function.
- H3K27me3 controls gene dosage in *A. thaliana* and in the bryophyte *Marchantia polymorpha*.
- Silenced TEs in land plants predominantly carry H3K9me1/2, while in unicellular, aquatic organisms such as *Cy. merolae* or *Phaeodactylum tricornutum*, H3K27me3 is the main mark that can be found at silenced TEs.
- NMCPs regulate chromatin organization and accessibility by tethering chromosomes to the NE and function in stress responses in *A. thaliana* and *M. polymorpha*.

Box 2. Open questions

- What is the function of *KNOX* and *BELL* and their regulation by PRC2 in *Cy. merolae*, a unicellular organism that was never shown to undergo haploid-to-diploid transitions?
- What is the predominant mechanism by which green algae control TE repression?
- Do genomes of organisms that lack NMCP homologs encode proteins that function as structural components in the nuclear periphery and regulate higher order genome organization and gene expression?

zygotes that grow into sporophytes. Sporophyte cells can undergo meiosis to generate haploid spores that eventually grow into gamete-producing gametophytes to close the cycle. Remarkably, both life forms are expressed by one single genome. This requires the suppression of gametophyte genes in the sporophyte and *vice versa*. A highly conserved mechanism that controls haploid-to-diploid transition is based on the epigenetic silencing of genes encoding KNOX (KNOTTTED-like) and BELL (BEL-Like) homeoproteins by H3K27me3 (Lafos et al., 2011; Lodha et al., 2013; Tan et al., 2022). KNOX and BELL belong to the family of TALE HD (three amino acid loop extension homeodomain) transcription factors and heterodimerize to regulate gene expression (Bellaoui et al., 2001). In gametophytes of the moss *Physcomitrium patens, PpBELL1* is epigenetically silenced by H3K27me3, and its ectopic overexpression induces sporophyte development (Horst et al., 2016). Land plant KNOX proteins can be subdivided into two classes KNOX1 and KNOX2 with distinct functions (Mukherjee et al., 2009). *KNOX1* expression in *P. patens* sporophytes ensures cell proliferation while *KNOX2* expression suppresses the gametophyte program (Sakakibara et al., 2008, 2013). Similarly, in the liverwort *M. polymorpha*, MpBELL3/4 and MpKNOX1 interact to initiate the zygotic program, while MpBELL1 and MpKNOX2 heterodimerize to enable sporophyte development (Dierschke et al., 2021; Hisanaga et al., 2021). Interestingly, *MpKNOX1* expression is egg-specific (Dierschke et al., 2021; Hisanaga et al., 2021). The mechanism by which it is repressed in sperm remains elusive. Since *KNOX/ BELL* are conserved targets of PRC2, and it was shown that knockout of *MpE(z)1* results in lethality at the haploid stage, while knockout of *MpE(z)2/3* impedes sporophyte development, it is possible that *MpKNOX* is silenced by H3K27me3 in egg cells (Flores-Sandoval et al., 2016; Montgomery et al., 2022). This hypothesis is supported by the finding that *KNOX* and *BELL* loci are covered by H3K27me3 in vegetative tissues of the Marchantia gametophytes (Hisanaga et al., 2022). These findings indicate that PRC2 regulates haploid-to-diploid transitions in land plants with gametophyte-dominant life forms (Figure 1). However, most higher land plants are sporophyte dominant and only their gametes are haploid. Zygote formation occurs by egg and sperm fusion and thereby produced diploid seeds grow into sporophytes with differentiated tissues. Here, KNOX and BELL rather function in the control of cell and tissue identity than in the induction of haploid-to-diploid transition. For instance, in the model plant *A. thaliana*, interaction of KNOX homologs KNAT2 and KNAT6 (KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 2/6) with the BELL protein REPLUMLESS (RPL) in the SAM was shown to maintain the prevailing undifferentiated cell state (Semiarti et al., 2001; Smith & Hake, 2003). In leaves, *KNAT2* and *KNAT6* are transcriptionally silenced to enable leaf cell differentiation (Lafos et al., 2011; Semiarti et al., 2001). Analysis of leaf cell epigenomes revealed that *KNAT2/6* loci were covered with the repressive H3K27me3 mark, indicating that PRC2 has a function in regulating the identity of sporophyte cells in higher land plants that exhibits sporophyte dominant life forms (Lafos et al., 2011).

Interestingly, *KNOX*, *BELL*, and PRC2 are also present in genomes of unicellular species of the green lineage, which may not be in need of regulators of tissue differentiation (Vigneau & Borg, 2021). Investigating the role of the KNOX/BELL program in these organisms allows to draw conclusions on the function of PRC2 in unicellular ancestors of land plants. In the unicellular green alga *C. reinhardtii*, the *minus* gamete expresses the *KNOX* gene *GSM1* (*GAMETE-SPECIFIC MINUS1*) and the *plus* gamete expresses the *BELL* gene *GSP1* (*GAMETE-SPECIFIC PLUS1*; Kurvari et al., 1998; Lee et al., 2008). Upon gamete fusion,

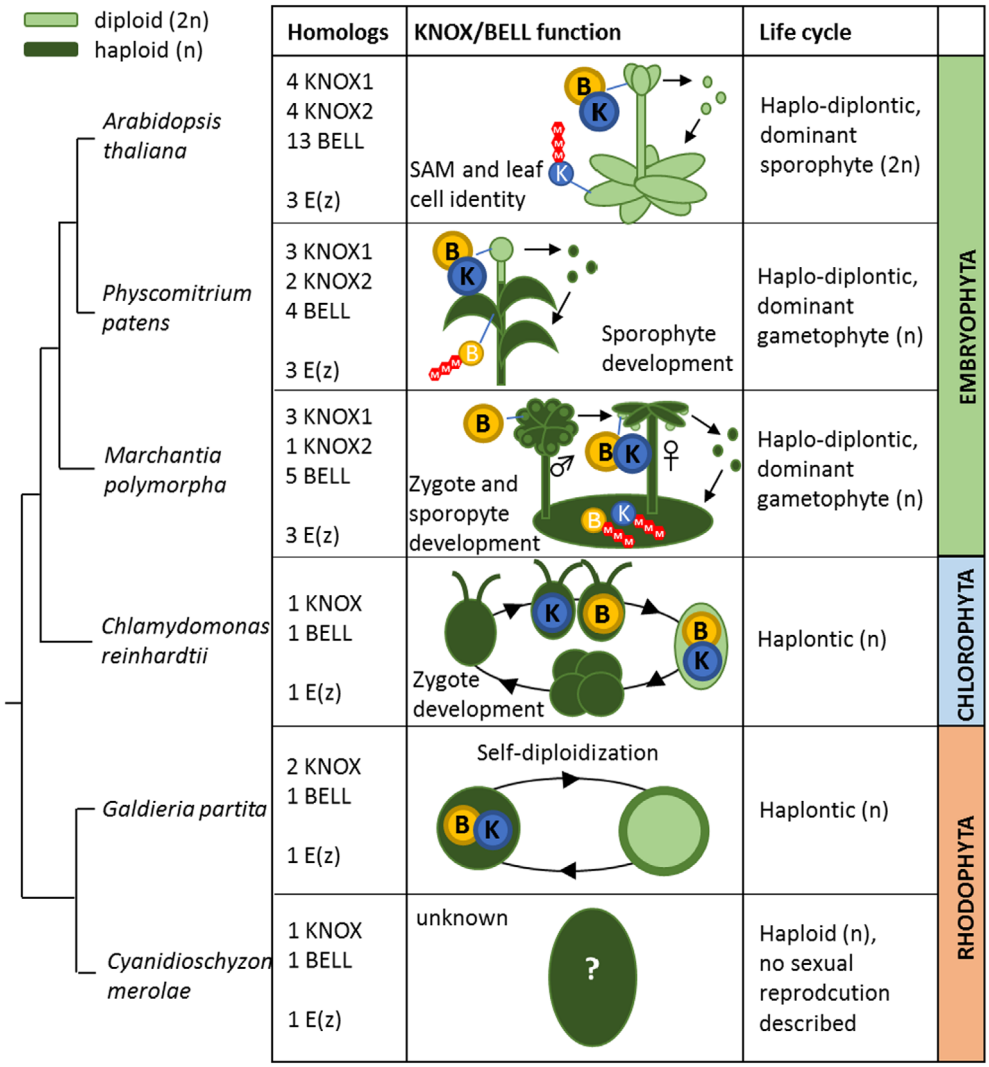


Figure 1. Function of the PRC2 targets *KNOX/BELL* in different Archaeplastida representatives (based on Furumizu et al., 2015).
During the evolution of Archaeplastida, the number of homologs of the TALE homeoproteins KNOTTED-Like (KNOX; K) and BEL-Like (BELL; B) as well as of the PRC2 histone methyltransferase Enhancer of zeste (E(z)) increased. Gene duplication caused the presence of two *KNOX* gene classes, *KNOX1* and *KNOX2* in land plants (Mukherjee et al., 2009). The conserved function of *KNOX/BELL* is the development and maintenance of the diploid program. H3K27me3 is indicated as red hexagons targeting genes (not proteins). How *KNOX/BELL* expression is regulated in *Chlamydomonas reinhardtii* and *Galdieria partita* remains to be elucidated. Moreover, the function of *KNOX/BELL* in *Cyanidioschyzon merolae* is not clear. Phylogenetic tree was created based on Miyagishima et al. (2017), Wang, Karaaslan, et al. (2021), and Hirooka et al. (2022) and does not reflect real genetic distances.

GSM1 and GSP1 proteins heterodimerize and translocate to the pronucleus to initiate the zygotic program (Lee et al., 2008). Aside from the diploid zygotic stage, *C. reinhardtii* lives a haploid life. Loss of *GSM1* or *GSP1* results in the formation of zygotes that fail to undergo nuclei fusion or flagella resorption, suggesting that similarly as in gametophyte-dominant early land plants, in unicellular Archaeplastida the PRC2-controlled KNOX/BELL program regulates haploid-to-diploid-transitions (Kariyawasam et al., 2019). Hirooka et al. (2022) showed that the unicellular red alga *Galdieria partita* can reproduce sexually. Prior to this study, *G. partita* was only known as a diploid cell-walled organism that reproduces asexually by mitotic cell division. Hirooka et al. (2022) uncovered that cultivation in a low pH medium triggers the cells to undergo meiosis. The emerging haploid gametes are cell wall-free and can switch between a motile tadpole shape or a nonmotile spheric shape. Haploid-to-diploid-transition occurs either if one gamete undergoes self-diploidization or if two gametes fuse (Hirooka et al., 2022). Two complementary isogamous mating types were identified. In contrast to *C. reinhardtii*, *G. partita* gametes of both mating types express *KNOX* and *BELL* (Hirooka et al., 2022). Knockout of either of the TALE HD TFs causes the loss of the

ability to endoreduplicate, indicating that they are necessary for self-diploidization. However, it was not shown whether *KNOX/BELL* loss affected mating. The Galdieria genome encodes one protein with homology to a PRC2 H3K27 methyltransferases (Kim et al., 2015). However, it remains to be elucidated whether it is involved in regulation of *KNOX* and *BELL* and phase transitions in this unicellular alga.

Overall, the land plant evolutionary trend goes toward diploid sporophytes being the dominant life form with a function for the KNOX/BELL program in controlling the differentiation of sporophyte cells (Figure 1). However, in early land plants as well as in aquatic, unicellular phototrophs, PRC2-controlled expression of KNOX and BELL function in the transition from the haploid to the diploid phase, indicating that this is an evolutionary conserved function of PRC2 in the green lineage (Vigneau & Borg, 2021). Interestingly, the unicellular cell wall-free red alga *Cy. merolae* represents an exception. It reproduces asexually and so far only a haploid life form has been described. Still, its genome contains one *KNOX* and one *BELL* homolog of yet uncharacterized functions (Hirooka et al., 2022). A genome-wide analysis of H3K27me3 distribution in *Cy. merolae* revealed that the *KNOX/BELL* homologs are targets of this epigenetic repressor (Mikulski et al., 2017). Hirooka et al. (2022) hypothesize that *Cy. merolae* might exist as diploid in nature, however, the laboratory strain may contain only one mating type. In addition, the conditions under which a transition to a diploid stage may occur need to be established.

MAINTENANCE OF GENOME DOSAGE AND INTEGRITY

Changes in gene dosage, as they occur for instance in the event of fertilization or with unequal numbers of sex chromosomes, can have dramatic effects on the phenotype and heavily affect the viability of an organism if not properly compensated. Dosage compensation is achieved by the epigenetic silencing of either the maternal or the paternal allele (or genome), a phenomenon termed genomic imprinting, or by complete silencing of sex chromosomes (reviewed by Bai & Settles, 2014; Ferguson-Smith & Bourc'his, 2018). Genomic imprinting requires DNA and histone modifications. In the maternal gametophyte of Arabidopsis, the DNA glycosylase DME is required for demethylation of the PcG gene *MEA* and other paternally imprinted genes (Choi et al., 2002; Schoft et al., 2011). Furthermore, PRC2-mediated H3K27me3 was shown to repress paternal alleles of maternally expressed genes and maternal alleles of paternally expressed genes (Baroux et al., 2006; Fitz Gerald et al., 2009; Köhler et al., 2003, 2005; Makarevich et al., 2006; Weinhofer et al., 2010). Recent studies showed that histone methylation also plays a role in genomic imprinting in basal land plants: Most of the genes expressed in embryos of *M. polymorpha* are transcribed from the maternal genome and all eight paternal autosomes as well as the one sex chromosome are enriched with H3K27me3 (Montgomery et al., 2022). This suggests a role for PRC2 in gene dosage control by paternal chromosome repression in bryophytes. In fact, loss of maternal *E(z)2* and E*(z)3*, which unlike *E(z)1* are only expressed in embryos, restores paternal gene expression in this tissue (Montgomery et al., 2022). Moreover, mutant embryos that express the biparental transcriptome are severely affected in growth and mature sporophytes produce either non-viable spores or no spores at all (Montgomery et al., 2022). This emphasizes the importance of gene dosage control during ploidy changes and highlights the indispensable role of H3K27me3-mediated imprinting during paternal chromosome repression. It remains unclear how unicellular Archaeplastida such as *C. reinhardtii* or *G. partita* regulate gene dosage during ploidy transitions. Thus, further investigations are needed to draw conclusions on a potential evolutionary conservation of this function of H3K27me3. Moreover, it might be interesting to analyze the role of DNA methylation in genomic imprinting as it was shown that egg cells of *M. polymorpha* exhibit higher DNA methylation levels than sperm cells (Schmid et al., 2018), but proof of a direct link to targeted silencing of maternal alleles are missing.

To study how flowering plants compensate gene dosage imbalances derived from changes in gene copy numbers, Lopez et al. (2021) generated transgenic *A. thaliana* lines that had strongly reduced 45S rDNA gene copy numbers (down to 10%). Despite this tremendous change in gene dosage, the mutant plants exhibited similar rRNA levels as the wild type (Lopez et al., 2021). Wildtype rDNA loci harbored significantly more of the heterochromatic silencing mark H3K9me2 than the mutant lines. Moreover, rDNA loci of transgenic lines were enriched with the activating H3K9Ac mark (Lopez et al., 2021) suggesting that rDNA dosage compensation is achieved by modifications of H3K9. Whether this is a conserved function remains elusive, as studies in lower land plants and unicellular phototrophs are missing.

Another potential threat for a genome's integrity is the presence of TEs. TEs were first discovered in maize, where they make up around 85% of the genome (McClintock, 1950; Schnable et al., 2009). Plant TEs can be subdivided into two classes (Pedro et al., 2021): Retrotransposons are propagated by a "copy-and-paste" mechanism via RNA intermediates that are reverse transcribed into a cDNA copy which integrates into the genome, while DNA transposons are excised and reintegrate elsewhere in a "cut-and-paste" manner. Since uncontrolled gene insertion can disrupt essential genes and negatively affect fitness, precise TE regulation is essential for an organism's viability. In flowering plants, like Arabidopsis, the predominant chromatin marks that silence transposons are H3K9

and DNA methylation (Miura et al., 2001; Veiseth et al., 2011) though H3K27me3-mediated silencing of some discrete TEs has been reported as well (Zervudacki et al., 2018). Interestingly, 5mC is absent from the *Cy. merolae* epigenome and H3K9me1 abundance is low, raising the question how this unicellular alga silences mobile genes to maintain genome integrity (Hisanaga et al., 2022; Huff & Zilberman, 2014). It was shown that around half of all TEs are targets of H3K27me3, while in Arabidopsis only 4% of TEs carry H3K27me3 (Johnson et al., 2004; Lafos et al., 2011; Mikulski et al., 2017; Oh et al., 2008). Moreover, knock-out of the one *E(z)* homolog that is present in the alga's genome resulted in complete loss of H3K27me3 which correlated with an induction of a large fraction of normally repressed TEs, suggesting that PRC2-mediated H3K27me3 deposition is an important TE silencing mechanism in this unicellular organism (Hisanaga et al., 2022). It is worth mentioning that while 25% of the Arabidopsis genome is comprised of TEs, only 0.7% of the *Cy. merolae* genome consists of transposons. To address the question whether TE silencing is potentially an ancestral function of H3K27me3, it is necessary to look at more representatives of the green lineage, that derived at different evolutionary stages. Suppression of the *E(z)* homolog in *C. reinhardtii* results in a decrease of transposon silencing, supporting the idea that H3K27me3 has a key role in TE regulation in unicellular organisms (Shaver et al., 2010). While a genome-wide analysis of chromatin marks associated with TEs is still missing for *C. reinhardtii* and for other green algae, it was recently shown in non-phototrophic eukaryotes such as the ciliates *Paramecium tetraurelia* and *Tetrahymena thermophila*, and the marine diatom *Phaeodactylum tricornutum* that PcG-catalyzed H3K27me3 plays a major role in transposon silencing (Frapporti et al., 2019; Zhao et al., 2019; Zhao, Rastogi, et al., 2021). In the bryophyte *M. polymorpha* repetitive elements make up around 27% of the genome (Montgomery et al., 2020). Around 43% of TEs are silenced by H3K9me1, H3K27me1, and DNA methylation, while 20% are enriched with H3K27me3 (Montgomery et al., 2020). A total of 57% of the genome of the moss *P. patens* are TEs and the predominant silencing mark is likely H3K9me2 (Widiez et al., 2014). H3K27me3 targets mostly genic regions as it was also shown for higher land plants (Lafos et al., 2011; Oh et al., 2008). This suggests that TE silencing is a key function of H3K27me3 in basal Archaeplastida, and that mechanisms which maintain genome integrity became more and more complex during land plant evolution with a shift toward DNA and H3K9 methylation being the predominant marks (Deleris et al., 2021). Genome-wide mapping showed that TE chromosomal distribution varied between different Archaeplastida species (Figure 2). In the red alga *Cy. merolae*, repetitive elements cluster predominantly in telomeric and subtelomeric regions, in *P. patens* and *M. polymorpha*, TEs alternate with protein coding genes and are interspersed over the whole chromosome, and in the flowering plant *A. thaliana*, TEs are additionally enriched around the centromers forming distinct regions of pericentric heterochromatin (Lang et al., 2018; Mikulski et al., 2017; Montgomery et al., 2020). It remains to be elucidated whether this tendency to distribute toward the chromocenters is an evolutionary phenomenon, coincidental or a consequence of different genome organization. Interestingly, several independent studies showed that protein coding genes that are located next to repetitive elements can exhibit the same chromatin state and thus be regulated in

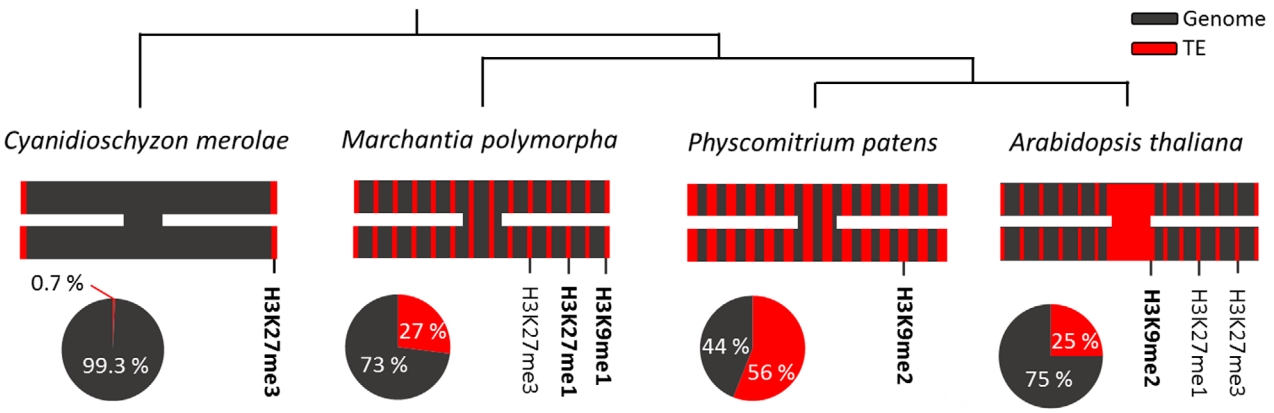
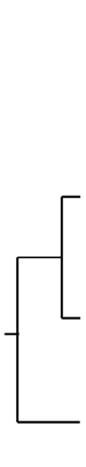


Figure 2. Predominant chromatin marks and localization of transposable elements (TEs) on chromosomes in different members of the green lineage.
In the unicellular alga *Cyanidioschyzon merolae*, TEs (indicated in red) can be found predominantly at the telomeres and subtelomeres, while in the bryophytes *Marchantia polymorpha* and *Physcomitrium patens* and in the flowering plant *Arabidopsis thaliana*, they are distributed over the whole chromosome, alternating with protein coding genes (Lang et al., 2018; Mikulski et al., 2017; Montgomery et al., 2020). Additionally, in flowering plants, TEs are enriched around the centromers, forming pericentric heterochromatin. The predominant histone marks associated with TEs are highlighted in bold. Pie charts indicate the proportions of repetitive elements in relation to the genome size, while chromosomes indicate the localization of TEs and are not reflecting the proportion of TEs. Phylogenetic tree was created based on Miyagishima et al. (2017), Wang, Karaaslan, et al. (2021) and Hirooka et al. (2022) and does not reflect real genetic distances.

a similar way. In *M. polymorpha*, for instance, around 60% of H3K27me3-silenced genes were found to be in close proximity to H3K27me3-silenced TEs (Hisanaga et al., 2022; Montgomery et al., 2020). In line with this, a colocalization of PRC2-regulated genes and TEs was found in Arabidopsis (Dong et al., 2012; Engelhorn et al., 2017; Hisanaga et al., 2022). Several studies showed that during evolution TEs were domesticated as *cis*-regulatory elements in mammals and plants (Batista et al., 2019; Chuong et al., 2017; Pehrsson et al., 2019; Schmitz et al., 2022; Xie et al., 2013; Zhao et al., 2018). In this context, it might be possible that TE silencing is an ancestral function of H3K27me3, while the function of H3K27me3 shifted toward gene regulation in higher land plants, possibly by the co-option of TEs as *cis*-regulatory elements. Alternatively, PRC2 might be specifically targeted to certain TE sequences, resulting in a sequence-dependent bias in species with high H3K27me3 targeting of TEs.

FUNCTION OF CHROMATIN REGULATORS IN BIOTIC AND ABIOTIC STRESS RESPONSES

Several studies have demonstrated a role for histone and DNA-modifying enzymes in stress responses in the past (reviewed by Avramova, 2015; Halder et al., 2022; Lämke & Bäurle, 2017; Probst & Mittelsten Scheid, 2015). A series of recent studies reported the involvement of a structural protein that is associated with the nuclear envelope (NE) in plant stress responses (Choi et al., 2019; Guo et al., 2017; Jarad et al., 2019; Sakamoto et al., 2020). NEs consist of an outer and an inner nuclear membrane (ONM and INM), nuclear pore complexes, and the nuclear lamina (NL). The NL is located below the INM and provides mechanical support for the nucleus. Animal NL are made up of nuclear lamins, which are protein filaments that function as structural components and chromatin regulators (reviewed by Dechat et al., 2009; Herrmann et al., 2009). Homologs of lamins are lacking in plant genomes. However, proteins with similar function and localization have been identified and were termed nuclear matrix constituent proteins (NMCPs). They contain coiled-coil domains that provide their function as structural proteins and are highly conserved among land plants (Ciska et al., 2019). Phylogenetic analyses revealed that a gene duplication event caused the emergence of two *NMCP* classes in higher land plants (Figure 3, Ciska et al., 2013, 2019; Tamura et al., 2015). Functional differences and redundancies between the two classes are not fully understood yet. While dicots possess two to three *NMCP1* homologs, monocots possess one *NMCP1* and both possess a single *NMCP2* (Ciska et al., 2013, 2019). The Arabidopsis genome encodes four NMCPs, *CROWDED NUCLEUS 1–4* (*CRWN1-4*), of which *CRWN1-3* are classified as *NMCP1* and *CRWN4* belongs to the *NMCP2* class (Mikulski et al., 2019; Wang et al., 2013). CRWN proteins were shown to regulate the nuclear morphology, as nuclei of knockout mutants exhibit decreased sizes and abnormal shapes (Sakamoto & Takagi, 2013; Wang et al., 2013). Additionally, CRWN1 contributes to the higher order genome organization by tethering the chromatin to the nuclear periphery (Hu et al., 2019). In line with this, it was shown that loss of *CRWN1* causes disturbance of chromocenter formation and heterochromatin condensation, further supporting a role for this protein in chromatin regulation in plants (Poulet, Duc, et al., 2017; Wang et al., 2013). Interestingly, while methylated H3K9 is crucial for chromatin anchoring to the NL in animals, knockout of H3K9 methylases in Arabidopsis did not affect



	Organism	NMCP homologs	Function in: Stress response	Function in: Chromatin organization	Function in: Nuclear size and morphology
EMBRYOPHYTA	*Arabidopsis thaliana*	3 NMCP1 1 NMCP2	**yes**	**yes**	**yes**
EMBRYOPHYTA	*Physcomitrium patens*	2	not tested	not tested	not tested
EMBRYOPHYTA	*Marchantia polymorpha*	1	**yes**	no	no
CHARO-PHYTA	*Spirogyra pratensis*	1	not tested	not tested	not tested
CHARO-PHYTA	*Coleochaete orbicularis*	1	not tested	not tested	not tested
CHLORO-PHYTA	*Volvox carteri*	0	--	--	--
CHLORO-PHYTA	*Chlamydomonas reinhardtii*	0	--	--	--

Figure 3. Homologs and functional diversity of nuclear matrix constituent proteins (NMCPs) in different Archaeplastida species.
While NMCP homologs are absent from Chlorophyta, Charophyta were found to possess one NMCP homolog of not yet identified function (Ciska et al., 2019; Poulet, Probst, et al., 2017). A gene duplication event in land plant evolution caused the presence of two classes of NMCPs: NMCP1 and NMCP2, which correlates with an increase of functional diversity (Choi et al., 2019; Ciska et al., 2013, 2019; Guo et al., 2017; Jarad et al., 2019; Sakamoto et al., 2020; Tamura et al., 2015). Phylogenetic tree was created based on Ciska et al. (2019) and does not reflect real genetic distances.

chromosome tethering, suggesting that this process is regulated differently in plants (Hu et al., 2019; Towbin et al., 2012). Recent studies revealed a function for CRWN proteins in the regulation of biotic and abiotic stress responses. Unstressed *crwn1 crwn2* and *crwn1 crwn4* double mutants exhibit increased levels of the defense-related plant hormone salicylic acid (SA; Choi et al., 2019). In line with this, the expression of the SA biosynthesis gene *ISOCHORISMATE SYNTHASE 1* (*ICS1/SID2*) and many genes involved in the response to a bacterial attacks such as *PATHOGENESIS-RELATED 1* (*PR1*) is enhanced in these mutants (Choi et al., 2019; Sakamoto et al., 2020). Consistently, Guo et al. (2017) showed that CRWN1 represses *PR1* by enhancing the binding of the transcription factor NAC WITH TRANSMEMBRANE MOTIF1-LIKE9 (NTL9) to its promoter. Moreover, it was shown that *crwn1 crwn2* double mutants are less susceptible to attacks with *Pseudomonas syringae* pv. *maculicola* (*Psm*) ES4326 and *P. syringae* pv. *tomato* (*Pst*) DC3000, supporting a role for CRWN proteins as negative regulators of immune responses against biotrophic bacteria (Choi et al., 2019; Guo et al., 2017). Jarad et al. (2019) demonstrated that loss of *CRWN1* triggers an enhanced induction of jasmonic acid synthesis genes and knockout mutants exhibited an enhanced resistance against the fungus *Botrytis cinerea*, suggesting a role for *CRWN1* as a positive regulator of defense against necrotrophic pathogens. The authors propose that CRWN1 tethers stress-responsive genes to the NL, which silences them in the absence of a stress stimulus, and releases them during stress exposition allowing for stress gene transcription (Jarad et al., 2019). However, this remains to be demonstrated. Comparative transcriptomics carried out by Sakamoto et al. (2020) identified five copper-associated genes that are tandemly localized on the long arm of Arabidopsis chromosome 5 to be downregulated in *crwn1 crwn4* double mutants. In line with this, they showed that *crwn1 crwn4* double mutants were hypersensitive to excess copper treatments, suggesting an additional function in abiotic stress responses for CRWN proteins (Sakamoto et al., 2020). This functional diversity of NMCPs in flowering plants raises the question about the ancestral function of plant lamina-like proteins. The genome of the moss *P. patens* encodes two NMCP homologs that were shown to localize at the nuclear periphery (Ciska et al., 2013, 2019). However, their function is not yet investigated. While being absent from the genomes of green algae such as *Volvox carteri* and *C. reinhardtii*, NMCP homologs were found to be present in Charophyta (Ciska et al., 2019; Poulet, Probst, et al., 2017). Wang, Karaaslan, et al. (2021) recently studied plant lamin-like proteins in *M. polymorpha*. The liverworts genome encodes one NMCP homolog. As in flowering plants, it is located at the nuclear periphery (Wang, Karaaslan, et al., 2021). Analogous to Arabidopsis double and triple *crwn* mutants, *Mpncmp* thalli exhibit decreased growth (Dittmer et al., 2007; Wang et al., 2013). In contrast to *Atcrwn*, *Mpnmcp* nuclei are not affected in size and shape (Wang, Karaaslan, et al., 2021). Furthermore, expression of *MpNMCP* in *Arabidopsis crwn1 crwn2* does not rescue the small nucleus phenotype (Wang, Karaaslan, et al., 2021). It was shown that *AtCRWN1* is crucial for tethering chromosomes to the nuclear periphery to regulate chromatin organization and gene expression (Choi et al., 2019; Guo et al., 2017; Hu et al., 2019; Jarad et al., 2019; Sakamoto et al., 2020). Similarly, MpNMCP was shown to anchor the male sex chromosome to the NL (Wang, Karaaslan, et al., 2021). However, aside from that, *MpNMCP* does not function in higher order genome organization as its loss does not cause any dramatic changes on chromosome arrangement. Interestingly, the comparison of transcriptomic data obtained from *Mpnmcp* and other stress-related Marchantia gene expression profiles indicated a connection between MpNMCP and biotic stress signaling (Wang, Karaaslan, et al., 2021). This suggests that stress response gene regulation might be the conserved function of NMCPs, while regulation of nuclear size and morphology and chromatin organization might be newly emerged functions (Figure 3). However, further studies are needed to validate this hypothesis and to investigate how exactly MpNMCP is involved in stress reactions. Furthermore, it might be worthwhile to investigate whether organisms that lack NMCP proteins have functional counterparts encoded in their genomes.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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