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Regulation of biological accuracy, precision, and memory by plant chromatin organization

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Accumulating evidence points toward diverse functions for plant chromatin. Remarkable progress has been made over the last few years in elucidating the mechanisms for a number of these functions. Activity of the histone demethylase IBM1 accurately targets DNA methylation to silent repeats and transposable elements, not to genes. A genetic screen uncovered the surprising role of H2A.Z-containing nucleosomes in sensing precise differences in ambient temperature and consequent gene regulation. Precise maintenance of chromosome number is assured by a histone modification that suppresses inappropriate DNA replication and by centromeric histone H3 regulation of chromosome segregation. Histones and noncoding RNAs regulate *FLOWERING LOCUS C*, the expression of which quantitatively measures the duration of cold exposure, functioning as memory of winter. These findings are a testament to the power of using plants to research chromatin organization, and demonstrate examples of how chromatin functions to achieve biological accuracy, precision, and memory.

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Introduction

Chromatin is an amalgam of DNA and associated modifications, histones and other proteins with post-translational modifications, and RNA in the nuclei of eukaryotic cells. Chromatin has numerous known functions in plant biology, many of which are likely conserved between eukaryotes. These include DNA transcription [1,2], replication [3,4] and repair [5,6], chromosome segregation through centromere function [7••], silencing and preventing proliferation of transposable elements (TEs) [8,9], genomic imprinting [10], vernalization [11,12], and paramutation [13].

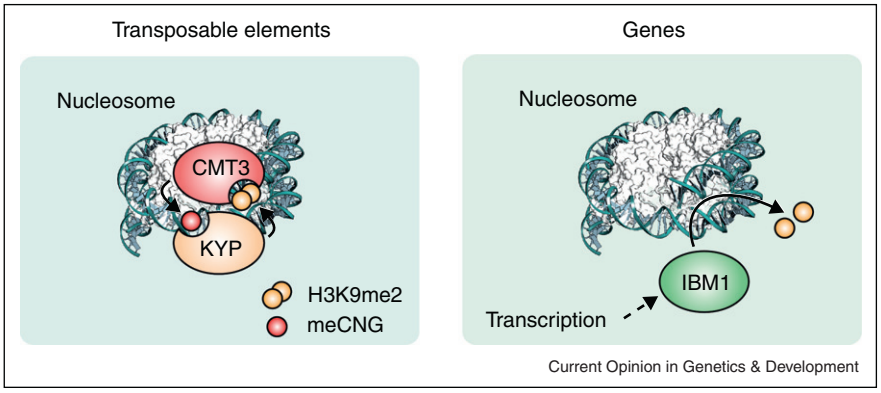
Detailed maps of chromatin features across the genomes of *Arabidopsis thaliana* [14•,15–17], maize [18], and rice [19,20], as well as basally diverged plants and distantly related green algae [21•,22•], reveal rich tapestries. However, knowledge of these genomic patterns leads unavoidably to mechanistic questions about how these patterns are established and maintained and of their relationships to function. Here, we highlight chromatin systems for which studying *A. thaliana* has led to mechanistic insights about the interplay of chromatin molecules in the genome and their functions.

Accuracy: DNA methylation at the right places

Cytosine DNA methylation is well characterized in *A. thaliana* [8]. Methylation of TEs causes transcriptional silencing and prevents proliferation of these elements and associated genome rearrangements [23,24]. Methylation of CG dinucleotides is common in both genes (where its function remains unknown) and TEs, whereas methylation of CNG sequences by CHROMOMETHYLASE 3 (CMT3) is present in TEs and excluded from genes [25]. CNG DNA methylation is maintained by a self-reinforcing biochemical loop involving histone methylation (Figure 1). At its core is the reciprocal binding of two enzymes to each other's product. CMT3 bears a chromodomain that binds dimethylated lysine 9 of histone H3 (H3K9me2) [26], primarily catalyzed by the histone methyltransferase KRYPTONITE (KYP) [27,28]. Likewise, KYP has an SRA domain that binds directly to methylcytosine in DNA, preferring CNG sequences [29]. This ensures that DNA and histone methylation of TEs will be perpetuated indefinitely, but does not address how this process is excluded from genes.

Increase in bonsai methylation 1 (*ibm1*) was identified in a screen for increased DNA methylation in the *BNS* gene [30]. IBM1 is a JmjC domain lysine demethylase that removes H3K9me2 (Figure 1). The activity of IBM1 is tied to transcription, resulting in the absence of H3K9me2 within genes [30,31,32••]. Without constant surveillance to remove H3K9me2 in genes, the self-reinforcing loop of KYP and CMT3 causes CNG methylation to accumulate in some genes in addition to its normal localization in TEs. Thus, IBM1 ensures accurate genomic CNG methylation by blocking aberrant H3K9me2 in transcribed genes. Intriguingly, loss of IBM1 causes inappropriate CNG methylation of those genes that already contain CG methylation [31,32••]. H3K9me2 deposition might be accelerated by CG methylation, some of which is in a CGG context (also a type of

Figure 1



Accurate genomic CNG methylation. A single nucleosome core particle on each side was rendered from Protein Data Bank Accession 1kx5 with DNA in green and histones in white. At left is the self-reinforcing loop at transposable elements of CHROMOMETHYLASE 3 (CMT3, red) methylating CNG sequence in DNA (meCNG) and binding histone H3 lysine 9 dimethylation (H3K9me2) through a chromodomain. Likewise, KRYPTONITE (KYP, orange) catalyzes H3K9me2 and binds meCNG through an SRA domain. At right is the removal of H3K9me2 by INCREASE IN BONSAI METHYLATION 1 (IBM1, green), which is targeted to genes by transcription.

CNG site), which would bind the SRA domain of KYP and its paralogs, initiating the self-reinforcing loop.

Precision: temperature sensing by nucleosomes

Ambient temperature is an important input signal for determining flowering time (see 'Memory' section below). Remarkably, an average increase of only 1 °C in the month preceding flowering correlates to an advance in flowering time of four to six days for winter annuals [33]. However, despite its importance, the molecular nature of plant thermometers remains enigmatic [34].

To elucidate the thermometer in *A. thaliana*, Kumar and Wigge [35••] identified *HSP70* expression as a linear output of ambient temperature between 12 and 27 °C and ARP6 as a regulator of this expression. Furthermore, ARP6 is necessary for the regulation between 12 and 27 °C of many, if not most, regulated genes. ARP6 is a subunit of the conserved SWR1 complex, which exchanges soluble H2A.Z/H2B dimers with nucleosomal H2A/H2B dimers, resulting in chromatin localization of H2A.Z [36]. The yeast homolog of ARP6 is important for allowing SWR1 to bind nucleosomes in this reaction [37]. The steady-state localization of H2A.Z is preferentially at the 5′ ends of genes in eukaryotes, including *A. thaliana* [1]. Kumar and Wigge [35••] found that H2A.Z is normally located at temperature-responsive genes, but is lost as the temperature rises, regardless of whether the gene is upregulated or downregulated. Thus, SWR1 and H2A.Z appear necessary for sensing precise changes in ambient temperature by locally regulating the temperature-dependent transcriptome.

Kumar and Wigge [35••] examined data for the yeast *Saccharomyces cerevisiae*, finding evidence that H2A.Z also plays a role in temperature sensing. *S. cerevisiae* H2A.Z is both lost and gained at different genomic loci as the temperature rises, and its localization is influenced by environmental signals unrelated to temperature [38]. By analogy, SWR1 and H2A.Z in *A. thaliana* may have complex localization dynamics and roles outside of temperature sensing. Proper localization of yeast H2A.Z is also aided by removal with an INO80 complex [39], so it may be interesting to investigate the effects of *A. thaliana ino80* mutants [40] on temperature sensing.

Precision: two of each chromosome

A. thaliana centromeres and pericentromeric heterochromatin form 'chromocenters' in interphase nuclei composed of TEs and other repeats marked by extensive DNA methylation and H3K9me2 (see 'Accuracy' section above), and also histone H3 lysine 27 monomethylation (H3K27me1) [26,41]. Jacob and colleagues [42] identified ATXR5 and ATXR6 as histone methyltransferases redundantly required for H3K27me1 near centromeres. Loss of H3K27me1 in the *atxr5 atxr6* mutant results in aberrant re-replication of pericentromeric TEs [43••]. Intriguingly, ATXR5 and ATXR6 each contain a PHD domain that binds the histone H3 N-terminal tail, but is blocked by histone H3 lysine 4 methylation (H3K4me) [43••]. H3K4me is absent from pericentromeric heterochromatin [44], suggesting that this helps target ATXR5/6 and H3K27me1. At the other end of the replication spectrum, normal origins of DNA replication in euchromatin have high levels of H3K4me [17], which is a binding determinant of the PHD domain in ORC1, the large subunit of the Origin Recognition Complex [45]. This suggests that different histone modification states play roles in either blocking over-replication or initiating correct replication, together promoting DNA replication once per strand across chromosomes.