Propagation of patterns of gene expression through the cell cycle requires prompt restoration of epigenetic marks after the twofold dilution caused by DNA replication. Here we show that the transcripational repressive mark H3K27me3 (histone H3 lysine 27 trimethylation) is restored in replicating plant cells through DNA replication–coupled modification of histone variant H3.1. Plants evolved a mechanism for efficient K27 trimethylation on H3.1, which is essential for inheritance of the silencing memory from mother to daughter cells. We illustrate how this mechanism establishes H3K27me3-mediated silencing during the developmental transition to flowering. Our study reveals a mechanism responsible for transmission of H3K27me3 in plant cells through cell divisions, enabling H3K27me3 to function as an epigenetic mark.

Epigenetic marks participate in maintaining expression patterns from mother to daughter cells. During DNA replication, the passage of the replication fork disrupts chromatin structure (1). Maintenance of a stable pattern of transcription after DNA replication requires restoration of epigenetic marks in the S phase soon after DNA replication. At replication sites, nucleosomes are assembled from recycled histones and newly synthesized, largely unmodified histones. In contrast to the histone H3 variant H3.3, the variant H3.1 is deposited at the replication fork by CAF1 (chromatin assembly factor 1) (2, 3). Maintenance of transcriptional repressive states by histone H3 lysine 27 trimethylation (H3K27me3) deposited by Polycomb repressive complex 2 (PRC2) is a conserved mechanism in animals and plants (4, 5), which involves positive-feedback loops (6–9). After DNA replication in mammalian cells, full levels of H3K27me3 are only restored at the next G1 phase (10–12), likely as a result of the step-wise methylation of K27 by PRC2, from H3K27me1 by this histone H3 lysine 27 trimethylation (H3K27me3) deposited by Polycomb repressive complex 2 (PRC2) is a conserved mechanism in animals and plants (4, 5), which involves positive-feedback loops (6–9). After DNA replication in mammalian cells, full levels of H3K27me3 are only restored at the next G1 phase (10–12), likely as a result of the step-wise methylation of K27 by PRC2, from H3K27me1 to H3K27me3 during S phase to achieve H3K27me3 during the next G2 phase (10–14).

In contrast to animals, plants evolved the PRC2-independent methyltransferases ATXR5 and ATXR6 (Arabidopsis TRITHORAX-RELATED PROTEIN 5 and 6, hereafter referred to as ATXR5/6), which deposit H5K27me1 on H3.1 (15, 16). Hence, the kinetics of H3K27me3 restoration might be different between plants and animals. To address this hypothesis, we synchronized the cell cycle of tobacco BY-2 cells (fig. S1A and B) and measured total levels of H3K27me1 and H3K27me3. Levels of H3K27me1 were not reduced during S phase (Fig. 1A and fig. S1C), consistent with the association of ATXR5/6 at replication foci (15). In contrast, levels of H3K27me3 dropped during S phase, likely reflecting the incorporation of newly synthesized, unmodified histones. Subsequently, H3K27me3 levels were restored early in G2 phase and remained stable until the next G1 phase (Fig. 1A and fig. S1C). These observations contrast with the slow restoration of H3K27me3 in animal cells, suggesting that plants have a specific mechanism dedicated to rapid restoration of H3K27me3 after DNA replication.
This up-regulation correlated with a decreased that are involved in the activation of stem cells in the overall patterns of H3K27me3 were preserved (fig. S7, A and B; and table S1) (17, 18), consistent with the growth of ectopic leaflets (fig. S5C). Thus, knockdown of H3.1 compromises deposition of H3K27me3. Loss of H3K27me3 in h3.1kd was not due to misexpression of PcG genes or H3K27 demethylases, nor did it originate from a perturbed cell cycle (fig. S8). To test whether the lack of H3.1 as a substrate for trimethylation at K27 caused impaired H3K27me3 maintenance, we created point-mutated H3.1K4A, H3.1K9A, and H3.1K27A (A, alanine). Only H3.1K27A failed to rescue the developmental defects, reduction of H3K27me3 levels, and derepression of class I KNOX genes in h3.1kd-1 (fig. 2F and fig. S9, A to C), suggesting the importance of H3.1 as a substrate for inheritance of H3K27me3. PRC2 does not prefer H3.1 over H3.3 as a substrate (15, 20), and H3.1 and H3.3 carry comparable levels of H3K27me3 in Arabidopsis (fig. S9D). Four residues at positions 31, 41, 57, and 90 distinguish H3.1 from H3.3, but A31 alone confers the specificity of H3.1 monomethylation by ATXR5/6 (fig. S9E) (15). We mutated each of the four amino acids in H3.1 to the corresponding H3.3 amino acid. Only the mutation A31T (Ala31→Thr15) failed to rescue the developmental defects and the reduction of H3K27me3 levels in h3.1kd-1 (fig. 2F and fig. S9, F and G), suggesting that monomethylation of K27 on H3.1 is responsible for maintenance of H3K27me3. The degree of reduction of H3K27me3 levels in h3.1kd lines was correlated with the enrichment of H3K27me3 in the wild type (fig. S10A). Moreover, a moderate global loss of H3K27me1 in a hypomorphic amiRNA-resistant mutant reduced H3K27me3 enrichment at genes most deprived of H3K27me3 in h3.1kd lines (fig. S10, B to D) (16). Together, these results suggest that in the context of the replication fork, ATXR5/6 selectively monomethylates K27 of H3.1 and provides H3K27me3 as a substrate for rapid methylation by PRC2 to generate H3K27me3.

To further understand the connection between H3.1-dependent H3K27me3 propagation and the inheritance of the memory of silencing during DNA replication, we engineered an inducible knockdown of H3.1 by triggering the expression of amiRNA-HTR13 in the htr1 knockout mutant upon dexamethasone (DEX) treatment (fig. 3, A and B). To monitor the impact of the treatment, we examined transcript levels of AT5G60250 and AT5G60610 because these genes showed a strong loss of H3K27me3 and concomitant up-regulation in h3.1kd lines (fig. 3, D and E, and fig. S7, D to F). HTR13 transcript levels were reduced after DEX treatment for 48 hours, but reduction of H3K27me3 and up-regulation of AT5G60250 and AT5G60610 only took place at 96 hours (fig. 3, C and D). The cell cycle duration in Arabidopsis ranges from 17 to 48 hours (21, 22), suggesting that, upon the loss of H3.1, dilution of H3K27me3 levels by at least one or two cycles of DNA replication causes transcriptional activation. Inhibition of DNA replication by olomoucine or roscovitine (23) (fig. 3B and fig. S11) blocked DEX-induced loss of

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**Fig. 2.** H3.1 incorporation facilitates maintenance of H3K27me3. (A) Quantification of H3K4me3, H3K27me1, and H3K27me3 Western blot signals from fig. S6A. Values are fold changes over Col. Unpaired t test: *P* < 0.05; **P** < 0.01; ***P*** < 0.001; n.s., not significant. (B) Normalized average distribution of H3K27me3 at all H3K27me3-marked genes in reference-adjusted reads per million (RPPM). TSS, transcription start site; TES, transcription end site. (C) Venn diagrams of up-regulated genes in h3.1kd lines determined by RNA-seq and genes marked by H3K27me3. P values are based on the hypergeometric test. (D) Gene expression determined by quantitative real-time polymerase chain reaction (qPCR). Transcriptional up-regulation in h3.1kd lines of all genes examined is statistically significant. Unpaired t test: *P* < 0.001. (E) ChIP-qPCR analysis of H3K27me3 enrichment at the indicated regions (fig. S7F). The loss of H3K27me3 in h3.1kd lines at all regions examined is statistically significant. Unpaired t test: *P* < 0.001. (F) Number of T1 transgenic plants that showed no rescue, partial rescue, and complete rescue of the h3.1kd-1 phenotype, as defined in fig. S5E. The number (n) of T1 plants analyzed for each construct is indicated. For all experiments, error bars represent SD from three biological replicates.
**Fig. 3.** H3.1-dependent propagation of H3K27me3 maintains the memory of silencing during DNA replication. 
(A) GR-LhG4 expressed under the control of the HTR13 promoter (solid arrow) can be activated by DEX and subsequently binds to the pOp3 promoter to activate amiHTR13-1 transcription (dashed arrow). 
(B) Experimental design of treatment with DEX and the cell cycle inhibitors olo- moucine (OLO) and roscovitine (ROS). 
(C) Gene expression upon DEX and cell cycle inhibitor treatment. 
(D) Relative H3K27me3 levels at AT5G60250 and AT5G60610 chromatin in seedlings treated with DEX and cell cycle inhibitors. For all experiments, error bars represent SD from three biological replicates. Unpaired t test: *P < 0.05; **P < 0.01; ***P < 0.001; n.s., not significant.

**Fig. 4.** Replication-dependent H3.1 deposition facilitates H3K27me3 enrichment at FLC by vernalization. 
(A) Flowering time after vernalization. The number (n) of plants counted for each line is indicated. 
(B) FLC transcript levels in FRI-Col and h3.1kd lines. NV, no vernalization; V40, 40-day cold treatment; T10, 40-day cold treatment followed by 10-day growth at warm temperatures. Error bars represent SD from three biological replicates. 
(C) Schematic view of the FLC gene structure, indicating regions examined by ChIP-qPCR. Black boxes represent exons, and lines represent intergenic regions and introns. The arrow indicates the TSS, bp, base pairs. 
(D) Relative H3K27me3 levels at FLC during vernalization. Error bars represent SD from three biological replicates. 
(E) Flowering time of cell cycle inhibitor–treated plants. The number of plants counted for each line is indicated. 
(F) FLC transcript levels in cell cycle inhibitor–treated seedlings. Error bars represent SD from three biological replicates. 
(G) Relative H3K27me3 enrichment at FLC in cell cycle inhibitor–treated seedlings. Error bars represent SD from three biological replicates. For all experiments, unpaired t test: **P < 0.01; ***P < 0.001; n.s., not significant.
H3K27me3 and gene activation (Fig. 3, C and D). Together, these results indicate that replication-coupled propagation of H3K27me3 on H3.1 is required to maintain the memory of silencing during DNA replication (Fig. S12).

We further investigated the establishment of H3K27me3-mediated silencing in the context of flowering, the transition from vegetative growth to reproductive growth. Vernalization, a prolonged cold treatment, induces H3K27me3 enrichment that stably silences the flowering repressor FLC (FLOWERING LOCUS C) (24). H3K27me3 enrichment is induced at the nucleation region around the transcription start site (TSS) of FLC at low temperatures, from where it spreads to the FLC gene body during subsequent growth in warm temperatures and is retained as the epigenetic memory of vernalization (24). We introduced a functional FRI (FRIGIDA) allele, which confers a requirement for vernalization to accelerate flowering, into h3.1kd lines. After vernalization, flowering was delayed in h3.1kd lines (Fig. 4A and fig. S13A), and FLC expression increased (Fig. 4B). Cold-induced nucleation of H3K27me3 was observed at the TSS of FLC in h3.1kd, but H3K27me3 maintenance and spreading over FLC was impaired (Fig. 4, C and D, and fig. S13B). During vernalization, H3.3 enrichment at FLC decreased, whereas H3.1 enrichment increased and balanced total H3 levels (Fig. S13, B and C). In contrast to seedlings, mature Arabidopsis leaves that are devoid of replicating cells had impaired H3K27me3 enrichment after vernalization (25), and H3.1 did not increase in response to vernalization (Fig. S13, D and E). To address the link between H3.1, DNA replication, and spreading of H3K27me3, we impeded cell cycle progression after cold exposure (Fig. S14A). Plants treated with cell cycle inhibitors flowered later than untreated plants (Fig. 4E and fig. S14B), showed higher FLC expression levels (Fig. 4F), and reduced spreading of H3K27me3 at FLC (Fig. 4G and fig. S14C). Together, these data demonstrate that H3.1 deposition by DNA replication facilitates establishment of H3K27me3 at FLC chromatin during vernalization, although spreading of H3K27me3 at FLC might be independent from maintenance of H3K27me3 at the initial nucleation site (fig. S14D).

We demonstrate that plant-specific mono-methylation of H3.1 and tethering of PcG proteins to the replication fork restores H3K27me3 to maintain the memory of silencing. Similarly, DNA replication has been implicated in the silencing of heterochromatin by facilitating the propagation of H3K9 methylation in fission yeast (26, 27). In the context of postembryonic development, plants also evolved a mechanism that evicts PcG proteins from the chromatin and dilutes H3K27me3 in a cell cycle–dependent manner during plant cell fate determination (28). In contrast with that of most animals, plant development is largely postembryonic and plastic. The mechanism reported in this study enables plant cells to face a high potential of regeneration and loss of cell fate. Hence, engineering of the epigenetic memory of silencing in a replication-coupled manner might allow manipulation of developmental plasticity and regeneration in eukaryotes.
DNA replication–coupled histone modification maintains Polycomb gene silencing in plants
Danhua Jiang and Frédéric Berger

**Managing gene silencing through replication**
Vernalization is the process in plants by which wintertime chill stimulates springtime flowering. Yang *et al.* and Jiang and Berger show how chill is recorded in *Arabidopsis* epigenetically by methylation of histones. Specialized components of the Polycomb group of proteins remodel DNA to establish the methylation marks and are linked to DNA replication. Long-term stable epigenetic status follows rapid establishment of metastable epigenetic marks. This epigenetic strategy may be key to the developmental requirement of both secure and nimble fate decisions, allowing plant cells to change fates.

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