Duplication of chromatin following DNA replication requires spatial reorganization of chromatin domains assisted by chromatin assembly factor CAF-1. Here, we tested the genomic consequences of CAF-1 loss and the function of chromatin assembly factor CAF-1 in heterochromatin formation. Genes located in heterochromatic regions are usually silent, and we found that this transcriptional repression persists in the absence of CAF-1 in Arabidopsis. However, using microarrays we observed that genes that are active during late S-phase, when heterochromatin is duplicated, were up-regulated in CAF-1 mutants. Arabidopsis CAF-1 mutants also have reduced cytological heterochromatin content; however, DNA methylation of pericentromeric repeats was normal, demonstrating that CAF-1 is not required for maintenance of DNA methylation. Instead, hypomethylation of the genome, which has only mild effects on the development of wild-type plants, completely arrested development of CAF-1 mutants. These results suggest that CAF-1 functions in heterochromatin formation. CAF-1 and DNA methylation, which is also needed for heterochromatin formation, have partially redundant functions that are essential for cell proliferation. Interestingly, transcriptional repression and heterochromatin compaction can be genetically separated, and CAF-1 is required only for the complete compaction of heterochromatin but not to maintain transcriptional repression of heterochromatic genes.

Chromatin assembly factor CAF-1 has been proposed to function as a histone chaperone during replication-coupled chromatin assembly of newly synthesized DNA during S-phase (1). CAF-1 is a heterotrimeric complex of CAC1/p150/FAS1, CAC2/p60/FAS2, and CAC3/p48/MSI1 in yeast, mammals, and plants, respectively (1), where loss of CAF-1 results in transcription of some silenced genes (2–6). Depletion of mammalian CAF-1 causes S-phase arrest and cell death (7–9). In contrast, loss of CAF-1 is not lethal in Arabidopsis thaliana, which currently is the only known multicellular organism with viable CAF-1 mutants (10).

Mammalian CAF-1 co-localizes with replicating heterochromatin, and the p150 subunit interacts with heterochromatin protein HP1. Therefore, it has been suggested that CAF-1 is involved in heterochromatin formation, but direct functional proof to support this model is still missing (11–13). More recently, a CAF-1-dependent pool of HP1 and a role for the largest subunit of CAF-1 in HP1 deposition during heterochromatin replication in late S-phase were described (14). Here, we tested the hypothesis that CAF-1 is required for normal heterochromatin formation and that transcriptional gene silencing of heterochromatic loci depends on CAF-1. To analyze heterochromatin and gene expression in the absence of CAF-1, the viable Arabidopsis CAF-1 mutants were used. We found that CAF-1 is required for normal heterochromatin compaction but that most silent heterochromatogenic genes remained repressed in CAF-1 mutants.

MATERIALS AND METHODS
Plant Material and Growth Conditions

Plants were grown under long days (16 h of light) in Conviron growth chambers with mixed cold inflorescent and incandescent light (70 μmol m⁻² s⁻¹, 23 °C). Seeds of Columbia (Col), Enkheim (En), and Landsberg erecta (Ler) wild-type accessions and of fas1-1 and fas2-1 (15, 16) mutants were obtained from the Nottingham Arabidopsis Stock Centre, and ddm1 mutant seeds (17) were a courtesy of C. Köhler (Zürich). The previously described CYCB1.1::GUS reporter (18) was introduced into fas1-1 and into fas2-4, a T-DNA insertion FAS2 null allele (data not shown). Because MSI1 participates, in addition to CAF-1, in other essential complexes (for review see Ref. 19), strong reductions of MSI1 cause sterility, and loss of MSI1 leads to embryo lethality (20, 21). We have generated several independent transgenic lines expressing a 35S::MSI1 cDNA antisense construct, and line 1ASb7 was used for all experiments. MSI1 protein levels in these lines are 30–50% of that in WT, but plants do not develop the severe abnormalities observed in MSI1 cosuppression plants and msi1 null mutants (20, 21), and MSI1 knock-down plants were thus more similar to fas1 and fas2 than to the msi1 knock-out mutant.

RNA isolation, Reverse Transcription-PCR, and Southern Blots

RNA was extracted from plants using Trizol (Invitrogen) according to manufacturer’s instructions. For reverse transcription-PCR analysis, 1 μg of total RNA was treated with DNase I and reverse-transcribed using an oligo(dT) primer and Moloney murine leukemia virus reverse transcriptase (Clontech, Palo Alto, CA). Aliquots of the generated cDNA, which equaled 50 ng of total RNA, were used as template for PCR with gene-specific primers (supplemental Table S1). Genomic DNA was prepared with Nucleon Phytopure (Amersham Biosciences) according to the supplier’s recommendations, and Southern blot analysis was performed as described (22).
Array Hybridization and Evaluation

Experimental procedures are described according to MIAME (“minimum information about a microarray experiment”) standards (23).

Experimental Design—A. thaliana (L.) Heynh. seeds (wild types Col, En, and Ler and mutants fas1, fas2, and msi1) were plated on sterile Murashige and Skoog (MS) medium containing 0.8% agar. After stratification for 48 h at 4 °C, plates were incubated in growth chambers (Weiss). Growth conditions consisted of a 16-h photoperiod at 23 °C with white light (70 μmol/m²s). Twelve days after induction of germination, at 4 h after dawn, complete seedlings were harvested into liquid nitrogen. The entire experiment was performed twice, providing independent biological replicates.

Array Design, Samples, and Hybridizations—Affymetrix Arabidopsis ATH1 GeneChips® were used throughout the experiment (Affymetrix, Santa Clara, CA). RNA was prepared, labeled, and hybridized to the arrays as described (24).

Measurements—The arrays were scanned using an Agilent GS 2500 confocal scanner.

Evaluation, Normalization, and Data Analysis—Signal values were derived from Affymetrix *.cel files using GCRMA (25) in the statistic package R (version 1.9.0) (26).

Bioinformatic Analysis

To identify differentially expressed genes, the nonparametric rank product algorithm was used (27). This algorithm was found to be extremely powerful even with very few replicates (27). The algorithm calculates false discovery rates (FDR) that are inherently corrected for multiple testing. Genes were considered significantly changed if the FDR value for the corresponding probe set was smaller than 0.05. To enrich for biologically relevant changes, probe sets were selected that were changed at least 1.5-fold in both replicate experiments. Genes were considered as significantly changed in two or more mutants if at least one of two criteria was fulfilled: (i) the gene was significantly changed in two mutants independently; (ii) the FDR value of the rank product algorithm, which was applied simultaneously to the data for the two mutants, was smaller than 0.05. In addition, probe sets were selected only when they had a fold-change of at least 1.5 for both mutants in both replicate experiments. Genes were grouped into collapsed functional gene ontology categories (obtained from www.arabidopsis.org). The significance of enrichment was estimated based on the hypergeometric test and multiple testing corrections according to Benjamini and Hochberg (28). Pericentromeric genes were defined operationally by examining averaged gene expression around centromeres. Blocks with low transcriptional activity in wild-type samples were selected manually.

The data for the genotoxic stress response were obtained from the AtGenExpress data set (29). Briefly, 16–days old WT seedlings (Col) were treated with bleomycin (1.5 μg/ml) and mitomycin (22 μg/ml) for 0.5, 1, 3, 6, 12, and 24 h. Gene expression values measured on ATH1 microarrays were normalized with GCRMA. Replicate measurements were averaged and normalized to time zero.

Cytological Analysis and Detection of GUS Activity

Sample preparation was performed as described (20). Nuclei from petals were spread on microscopic slides and stained with 4′,6′-diamidino-2-phenylindole (DAPI). The fluorescence patterns were examined with a Zeiss Axioplan microscope, and images were recorded with a MagnaFire® charge-coupled device camera (Optronics, Goleta, CA). Digital images were quantified using ImageJ 1.27Z (rsb.info.nih.gov/ij/). Immunostaining was performed as described (30). Slides were incubated overnight at 4 °C with antibodies against dimethyl H3K9 (1:100) or dimethyl H3K4 (1:500, both from Upstate) in 1% bovine serum albumin in phosphate-buffered saline. Detection was performed with an anti-rabbit Alexa 488-coupled antibody (1:1000, room temperature, 1 h; Molecular Probes) in 0.5% bovine serum albumin in phosphate-buffered saline. DNA was counterstained with DAPI in Vectashield mounting medium (Vector Laboratories). The nuclei were analyzed with a Deltavision deconvolution microscope, with images deconvoluted with SoftWORX software; single layers of the deconvoluted image stack are shown. Staining for GUS activity was performed as described previously (22).

RESULTS AND DISCUSSION

Silencing of Most Transposons and Heterochromatic Genes Is Independent of CAF-1—Heterochromatin has repressive effects on gene expression (31), and spurious transcriptional activation of silent transposons and heterochromatic genes has already been reported in the DNA hypomethylation mutant, ddm1 (32). Silencing of one such gene, TSI, was also lost in fas1 and fas2 (6). We therefore examined if silencing of other heterochromatin genes was affected in CAF-1 mutants as well. In all subsequent experiments, we used null alleles of the genes for the two larger CAF-1 subunits, FAS1/CAC1 and FAS2/CAC2. Because loss of the third CAF-1 subunit, MS1/CAC3, is lethal (21), we used transgenic plants in which protein levels of MS1 were reduced to 50% by expression of an antisense construct (msi1-as). Of four genes in pericentromeric heterochromatin that were activated in ddm1, only TSI was expressed in fas1 and fas2, but the other three genes remained silent in the CAF-1 mutants (Fig. 1A). Thus, transcriptional repression of several silenced pericentromeric loci persists in CAF-1 mutants.

To test the effect of CAF-1 on transcriptional repression of genes in heterochromatic regions of the chromosomes on a genome-wide scale,
we analyzed the transcriptome of CAF-1 mutants and msi1-as plants using Affymetrix ATH1 microarrays (see supplemental Tables S2–S7 for details and the complete data set). Experiments were performed with seedlings because they show either no or only minor visible developmental defects (10). We also reasoned that secondary transcriptional changes caused by CAF-1-dependent gene repression would be minimal at this early developmental stage. We examined whether up-regulation of transcription in CAF-1 mutants was stronger for genes in heterochromatic chromosomal regions than for genes located elsewhere. In telomeric heterochromatin, the amplitude of differences in gene expression between wild type and the CAF-1 mutants did not depend on the distance from the chromosome ends (Fig. 1, B–D), suggesting that Arabidopsis CAF-1 is not required to maintain transcriptionally silent states at telomeres. We also examined the effect of CAF-1 mutations on gene expression in pericentromeric regions, which normally have very low transcriptional activity. If CAF-1 was required for transcriptional repression of pericentromeric genes, loss of CAF-1 function should cause increased transcription of such genes. However, loss of CAF-1 function did not cause significantly increased expression of pericentromeric genes (Fig. 1E). Moreover, the distribution of signal log ratios (SLR), which are a measure for differential expression between wild-type and mutants, was similar for pericentromeric genes and for all chromosomal genes (Fig. 1E). Thus, transcriptional silencing of genes in telomeric and pericentromeric heterochromatin is maintained in the absence of CAF-1 function in Arabidopsis.

Although expression of genes in heterochromatic regions of the chromosomes was not significantly affected in CAF-1 mutants, expression of other genes was changed. The small number of affected genes (fas1 = 2.1%, fas2 = 0.9%, msi1-as = 0.9%) demonstrates an unexpected robustness of the Arabidopsis genome against loss of CAF-1. Expression of 87 genes was altered significantly in both fas1 and fas2, which therefore could be direct CAF-1 targets (Fig. 2A and Table S2 in the supplemental material). The number of differentially expressed genes is considerably larger in fas1 than in fas2 or msi1-as. It has been shown that the largest CAF-1 subunit can interact with several proteins, and it is possible that some of these interactions do not involve the other two CAF-1 subunits (11, 33). To exclude possible effects caused by interactions of fas1, fas2, and msi1-as with different genetic backgrounds or by specific functions of FAS1, FAS2, and MSI1 in other protein complexes, we subsequently focused on changes that are common to fas1 and fas2. This set of 87 genes contains no genes for transposable elements, and only the transposon At1g36460 was significantly derepressed in fas1. Because silencing of more than 300 transposable elements probed by the microarray was not significantly affected, we conclude that most Arabidopsis transposons do not require CAF-1 for silencing. This is in striking contrast to DNA methylation, which is needed for the silencing of many transposons (34).

Histone H3.3 and Genes for DNA Repair Are Up-regulated in CAF-1 Mutants—Genes that show a significant change in their expression in CAF-1 mutants encode proteins for diverse biological functions (Fig. 2B, supplemental Table S3), but only genes for proteins that function in chromatin regulation, cell organization, and stress responses were significantly enriched among up-regulated genes in both fas1 and fas2. The genes for proteins involved in chromatin maintenance include three histone genes (At1g09200, encoding an H3.1; At1g13370, encoding an H3.3; At3g45930, encoding an H4), but only the gene for the replacement histone H3.3 was induced more than 5-fold (Fig. 3, A and B). Histone H3.3 is a replacement histone variant that is incorporated into nucleosomes independently of CAF-1. Another histone H3.3 gene probed by the ATH1 microarray (At5g10980) was up-regulated 1.2–1.8-fold as well. Because this gene is one of the most highly expressed genes in Arabidopsis, it is likely that even the small fold-change results in the production of a large amount of additional H3.3 histones. The preferential up-regulation of genes for H3.3 histones suggests that chromatin assembly in the absence of CAF-1, which incorporates H3.1 into chromatin (1), relies on replication-independent incorporation of H3.3. In yeast, mutants for CAF-1 show strong synergistic interactions with ASF1 or HIR, which are the dominant histone chaperones of the replication-independent pathway (for review see Ref. 1). Transcript levels of the two Arabidopsis ASF1 homologs and the one HIR homolog were not changed in the CAF-1 mutants, suggesting that their transcription is not limiting for replication-independent chromatin assembly.
In addition to histones, several genes coding for proteins involved in DNA repair were up-regulated in both fas1 and fas2 (Fig. 2A and Table 1). The increased expression of genes for DNA repair complexes in the Arabidopsis CAF-1 mutants is consistent with previous observations in yeast (35, 36). The up-regulated genes encode homologs of BRCA1, Rad51, RNA helicase A, PARP1, and others. Homologs of these proteins in other organisms often function together or interact physically or genetically with CAF-1 (37–43). Our data suggest that these proteins participate in a DNA-structure surveillance complex that interacts with CAF-1 subunits and that is required for recovery of stalled replication forks, as suggested previously for the BRCA1-associated genome surveillance complex (BASC) (37). Because several of the genes that have altered expression in CAF-1 mutants function in DNA repair, we next asked whether these genes are similarly regulated by genotoxic stress. We used publicly available microarray data from the AtGenExpress consortium that include response kinetics of seedlings to bleomycin, a drug that induces single and double strand breaks. Indeed, 32 of the 69 genes that have altered expression in CAF-1 mutants are repressed by genotoxic stress, demonstrating that transcriptional effects of CAF-1 mutations are not restricted to the simple activation of a DNA damage response. Notably, the poly(ADP-ribose) polymerase (PARP) and BRCA1 homologs belong to the DNA repair regulon, but the replacement histone H3.3 does not. Despite the increased expression of genes for DNA repair, fas1 and fas2 seedlings are hypersensitive to the DNA-damaging agent methylmethane sulfonate (6), suggesting that replication defects in fas1 and fas2 might lead to DNA damage that largely diminishes the available capacity of the repair machinery.

In addition, transcript levels for the mitotic cyclin B1;1 were consistently up-regulated in CAF-1 mutants (Fig. 3, A and B), although the changes did not exceed the significance threshold (p = 0.27, fold change = 2.0) in fas1 in the original microarray experiment. Inspection of publicly available microarray data using the Genevestigator tool (44) revealed that CYCB1;1 is strongly induced by genotoxic stress, which is unlike all other mitotic cyclins in Arabidopsis. In mammals and yeast, mitotic cyclins and cyclin-dependent kinases are required for DNA damage checkpoint activation and recombination repair (45–48), and mammalian cdk/cyclin B1 and yeast Clb2-Cdk1 phosphorylate p60 and CAC1, respectively (49, 50). Thus, it is possible that Arabidopsis CYCB1;1 functions not only during mitosis but also in the response to replication stress to modify CAF-1 activity. Use of a CYCB1;1 β-glucuronidase reporter gene (18) confirmed the strong activation of CYCB1;1 expression in CAF-1 mutants. Because increased expression of CYCB1;1 was restricted to proliferating cells, lack of CAF-1 in Arabidopsis affects proliferating cells much more severely than it does non-dividing cells (Fig. 3C).

**Genomic Analysis of CAF-1 Mutants**

**Genes Active in Late S-phase Are Up-regulated in CAF-1 Mutants—** Human cells with a reduced CAF-1 function accumulate in S-phase (7, 8). Therefore, we examined whether S-phase-specific genes were significantly more up-regulated in Arabidopsis CAF-1 mutants than genes specific for other cell cycle stages. Using quantile-quantile plots we compared the expression maximum during the cell cycle of genes significantly deregulated in CAF-1 mutants with the expression maximum of all genes expressed in a cell cycle-specific pattern in synchronized Arabidopsis cells (51). Genes specifically expressed during late S-phase were enriched in the sets of genes significantly up-regulated in the CAF-1 mutants (Fig. 5, A and B). Similarly, averaged SLR values for fas1 and fas2 of 10,274 genes with known cell cycle phase-specific expression patterns (51) revealed a preferential up-regulation of late S-phase-spe-

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**TABLE 1**

| Probe set | Gene | Annotation | Family name   | Function                      |
|-----------|------|------------|---------------|-------------------------------|
| 246132_at | At5g20850 | Rad51-like protein | RecA/Rad51 | DNA binding, recombination |
| 24770K_at | At5g59480 | SSM1-like protein | SSMS1 | Pyrimidine 5’-nucleotidase |
| 25444S_at | At4g21070 | A1BRCA1 | BRCA1 | Repair, transcription |
| 255500_at | At5g2390 | NAD+ ADP-ribose transferase | PARP1 | Repair, transcription |
| 256832_at | At3g22880 | DMC1 | RecA/Rad51 | DNA binding, recombination |
| 260223_at | At1g74390 | Putative exonuclease | dinG | DNA-helicase and exonuclease |
| 264674_at | At1g99815 | DNA polymerase δ p12 | Pol δ p12 | DNA polymerase δ p12 |
| 266511_at | At2g47680 | Putative ATP-dependent RNA helicase A | RHA | Repair, transcription |

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**FIGURE 3.** Histone H3.3 and recombination genes RAD51 and CYCB1;1 are up-regulated in CAF-1 mutants. A, average signals from microarrays. Error bars indicate the range of the replicate measurements. B, semiquantitative reverse transcription-PCR reveals that microarray results are conservative and changes are often larger than seen on the arrays. C, GUS activity in wild-type (Col) and fas1 or fas2 mutant plants carrying a CYCB1;1::GUS reporter transgene. Note that consistent with the basipetal decrease in cell division (62), CYCB1;1::GUS expression is diminishing in the distal half of young developing leaves. GUS activity is also diminishing in the nondividing cells of older leaves. Bar = 1 mm.
cific genes in response to the loss of CAF-1 (Fig. 5C). Thus, unlike mammalian cells without CAF-1 activity, which arrest in S-phase (7, 9, 10), Arabidopsis CAF-1 mutants may only delay passage through late S-phase when heterochromatic DNA is typically replicated. In mammalian cells, DNA damage activates several checkpoint pathways including the p53 pathway (52), and lack of CAF-1 causes programmed cell death (9). Notably, some apoptosis-inducing pathways are restricted to animals. This could be a reason why yeast and plant cells but not mammalian cells can survive without CAF-1.

**CAF-1 Mutants Have Reduced Heterochromatin Contents**—Our results have established that Arabidopsis CAF-1 is not required for silencing of heterochromatic genes. However, heterochromatin was historically defined as chromatin remaining visibly condensed throughout the cell cycle, and condensation is the major hallmark of heterochromatin. Therefore, we tested whether Arabidopsis CAF-1 is required for normal heterochromatin condensation. Heterochromatin in Arabidopsis is densely clustered in chromocenters (53), which were visible in the nuclei of wild-type plants and also in CAF-1 mutants and msi1-as plants (Fig. 6, A–F). To quantify the heterochromatin content, we measured the amount of DNA condensed into visible heterochromatin using the relative fluorescence of chromocenters in DAPI-stained nuclei (53). Using this method we found that nearly 15% of the Arabidopsis genome is heterochromatic in wild-type plants (Fig. 6G). This heterochromatin fraction was reduced by 20–30% in the CAF-1 mutants. Because chromocenters are not completely lost in the Arabidopsis CAF-1 mutants, the reduced heterochromatin content most likely escaped detection in previous studies (6). Thus, CAF-1 contributes to complete heterochromatin compaction in Arabidopsis, but a
substantial fraction of heterochromatin can also be assembled in the absence of CAF-1. It is therefore possible that CAF-1 facilitates heterochromatin formation but itself is not directly involved in heterochromatin assembly. Alternatively, parallel pathways for heterochromatin formation may exist in Arabidopsis. Notably, replication-coupled H3.1 and H4 isoforms are enriched in heterochromatic regions (12), and H3.3 is more often associated with less condensed, actively transcribed DNA (54). Thus, increased expression of H3.3 in CAF-1 mutants and a possibly increased incorporation of H3.3 into chromatin could explain the decreased DNA condensation observed in the Arabidopsis CAF-1 mutants.

In various species, including Arabidopsis, heterochromatin is enriched in histone H3K9 dimethylation (H3K9me2) and poor in H3K4me2 (55). To test whether decreased compaction of chromo-
Genomic Analysis of CAF-1 Mutants

Reduced chromocenter formation in the CAF-1 mutants was similar to the reduction reported for the *ddm1* DNA hypomethylation mutant (55). We therefore tested whether reduced heterochromatin formation in the CAF-1 mutants correlated with a reduction or loss of DNA methylation. In contrast to DNA in *ddm1*, pericentromeric DNA from CAF-1 mutants remained strongly methylated (Fig. 6F). Thus, reduced heterochromatin formation in CAF-1 mutants is not caused by global DNA hypomethylation. However, because mammalian p150 interacts with the methyl-CpG-binding protein MBD1 (33, 57) and because both CAF-1 and DNA methylation contribute to the duplication of heterochromatin (Ref. 55 and this study), our data support a model in which DNA methylation and CAF-1 cooperate in heterochromatin formation. Because heterochromatic gene silencing depends on CAF-1 only in yeast (2–4, 58), which has no genomic DNA methylation, but not CAF-1, is needed for the silencing of many transposons (34). Furthermore, DNA hypomethylation leads to increased expression of many genes in *Arabidopsis* (59), but no significant overlap with the set of genes up-regulated in *fas1* or *fas2* exists. The genes *At*2g14610 and *At*3g29650, for instance, which are strongly up-regulated by DNA hypomethylation and in *mom1*, *ddm1*, *met1*, *cmt3*, *drm1/drm2*, or *kyp* mutants remain silent in CAF-1 mutants. These data suggest that CAF-1 and DNA methylation could function in parallel, partially redundant pathways. This hypothesis was tested by assessing the effect of global DNA hypomethylation on CAF-1 mutants. Global DNA hypomethylation of the genome does not immediately interfere with *Arabidopsis* development (17), and we observed that wild-type seedlings developed normally in the presence of 5-aza-2’-deoxycytosine (aza-dC), which causes global DNA demethylation at the concentration used (Ref. 59, Fig. 8, and data not shown). In contrast, the majority of CAF-1 null mutant seedlings failed to form any postembryonic organs in the presence of aza-dC (Fig. 8). DNA demethylation had no obvious effects on *msi1-as* seedlings, which retain almost half of the wild-type MS11 levels (data not shown). In affected *fas1* and *fas2* seedlings, the root and the shoot apical meristems produced no or very few new postembryonic cells, indicating a complete arrest of meristem function in the absence of CAF-1 function and DNA methylation. In CAF-1 mutant seedlings grown on aza-dC, nuclei from most of the few cells produced postembryonically were microscopically normal, but some of these nuclei had no visible chromocenters. Although this might suggest that CAF-1 and DNA methylation are together required for heterochromatin formation, the low number of such nuclei prevented more detailed analysis. It will be important to investigate the relationship between CAF-1 and DNA methylation for chromocenter formation in future studies. CAF-1-mediated chromatin assembly does not function upstream of DNA methylation, but CAF-1 and DNA methylation might act in parallel pathways in *Arabidopsis*. Although loss of CAF-1 or DNA methylation alone has only mild effects on plant development, the combined loss of both completely abolishes cell proliferation and development. Recently, it was reported that SETDB1, which is required for histone H3 methylation at lysine 9 in mammalian heterochromatin, interacts with MBD1 (57). Because MBD1 can recruit H3K9 methyltransferase activity to sites of heterochromatin assembly either
via binding to methylated DNA or via binding to CAF-1, it is possible that the observed synergistic effects of DNA hypomethylation and loss of CAF-1 are mediated by MB1. Importantly, in Arabidopsis DNA methylation is required for chromocenter formation and gene silencing, but H3K9 dimethylation is not (55, 56, 60, 61).

Together, our data show that CAF-1 contributes to heterochromatin assembly in S-phase. Loss of CAF-1 activity in Arabidopsis might extend their life cycle even in the absence of CAF-1. Heterochromatin formation is a hierarchical process involving many factors, and our results suggest that in Arabidopsis CAF-1 is mainly necessary for the physical compaction of chromatin but not for transcriptional silencing of heterochromatic genes. DNA methylation could form an epigenetic backup system that enables development of Arabidopsis without CAF-1 function.

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