DNA Topoisomerase I Affects Polycomb Group Protein-Mediated Epigenetic Regulation and Plant Development by Altering Nucleosome Distribution in Arabidopsis

Xigang Liu, Lei Gao, Thanh Theresa Dinh, Ting Shi, Dongming Li, Ruozhong Wang, Lin Guo, Langtao Xiao, and Xuemei Chen

INTRODUCTION

By transiently nicking DNA, DNA topoisomerases relieve the torsional stress in DNA introduced during replication or transcription. Topoisomerases are classified as Type I or II, depending on whether the enzyme generates a single-stranded or double-stranded cleavage site (Wang, 2002). Genome-wide analyses in fission yeast suggest that Top1 (a type I enzyme) and Top2 (a type II enzyme) are needed for transcription initiation and termination, probably by promoting nucleosome disassembly at promoters and transcription termination sites (Durand-Dubief et al., 2010, 2011). The effects of DNA topoisomerases on gene expression in multicellular eukaryotes remain unknown.

Genetic studies in Arabidopsis thaliana uncovered surprisingly specific developmental functions of topoisomerase I. A type I topoisomerase gene, TOP1, was first isolated in Arabidopsis through homology to yeast Top1 (Kieber et al., 1992). Mutants in this gene, also referred to as MGOUN1, were later found to exhibit defects in stem cell homeostasis and phyllotaxy (Laufs et al., 1998; Takahashi et al., 2002; Graf et al., 2010). Because of the presence of a paralog that is tandemly arrayed in the genome with TOP1, TOP1 is referred to as TOP1α, whereas its paralog is named TOP1β (Takahashi et al., 2002). Seedlings harboring both the top1α-1 mutation and a TOP1β RNA interference construct are lethal (Takahashi et al., 2002), consistent with the critical functions of topoisomerase I in nucleic acid-based processes. However, it is intriguing that mutations in TOP1α should give rise to fairly specific developmental defects such as meristem malfunction.

The shoot apical meristem (SAM) is responsible for producing all above ground structures of a plant by generating new primordia appropriate for various developmental stages. The SAM is partitioned into several functional zones with the stem cells localized in the central zone. WUSCHEL (WUS), which encodes a homeodomain transcription factor, is expressed in the rib zone located beneath the central zone and instructs the overlying cells to maintain their stem cell identity (Mayer et al., 1998). After floral transition, the SAM produces floral meristems on its flanks. WUS also plays a key role in the maintenance of floral stem cells and its expression is turned off at stage 6 of flower development.
(Smyth et al., 1990) to result in the programmed termination of the floral meristem, which is also referred to as floral determinacy (Laux et al., 1996; Mayer et al., 1998). The temporally precise repression of WUS expression requires AGAMOUS (AG) (Laux et al., 1996; Lenhard et al., 2001; Lohmann et al., 2001), which encodes a MADS domain transcription factor (Yanofsky et al., 1990). AG promotes the expression of KNUCKLES (KNU), encoding a C2H2-type zinc finger protein, which in turn represses WUS expression (Sun et al., 2009). KNU activation by AG is associated with reduced levels of the repressive mark histone 3 lysine 27 trimethylation (H3K27me3) and the eviction of Polycomb Group (PcG) proteins that establish this mark at KNU (Sun et al., 2009, 2014). AG also represses WUS expression by directly binding to WUS and recruiting PcG proteins to deposit H3K27me3 (Liu et al., 2011).

The execution of various developmental programs in plants and animals entails precisely regulated establishment and/or override of PcG-mediated repression. PcG consists of a few subcomplexes, of which Polycomb Repressor Complex 2 (PRC2) deposits H3K27me3 at target genes and PRC1 recognizes this histone mark to establish repressive chromatin. In Arabidopsis, CURLY LEAF (CLF) and SWINGER encode H3K27 methyl-transferases and have overlapping functions during vegetative and reproductive development (Chanvivattana et al., 2004; Schubert et al., 2006). The H3K27me3 mark is recognized by LIKE HETEROCHROMATIN PROTEIN1 (LHP1; also known as TERMINAL FLOWER2 [TFL2] in the PRC1 complex (Turck et al., 2007; Zhang et al., 2007a; Exner et al., 2009). How PcG is recruited to targets in plants is not well understood, but a few studies have implicated transcription factors, such as AG and SEPALLATA3, as well as a noncoding RNA in PcG recruitment to specific targets (Liu et al., 2009; Heo and Sung, 2011; Liu et al., 2011). Overcoming PcG’s repressive effects to turn on gene expression requires chromatin remodeling proteins such as SPLAYED (SYD) and BHRAMA (BRM), which bind to PcG targets to presumably loosen chromatin to allow gene expression (Wu et al., 2012).

A top1a mutant (mgo1-4) was found to enhance hypomorphic wus alleles to result in the premature termination of the SAM and the production of adventitious inflorescence meristems and floral meristems (Graf et al., 2010). mgo1-4 interacts synergistically with mutations in several chromatin factors, implying a role for TOP1a in chromatin-based gene regulation (Graf et al., 2010). In our own genetic screen (see below), we isolated a top1a allele (top1a-2) as an enhancer of a weak ag allele in terms of its floral determinacy defects, again linking TOP1a to meristem activities. Furthermore, we found that top1a-2 strongly enhances the phenotypes of mutants in CLF and LHP1/TFL2, subunits of the PcG (Goodrich et al., 1997; Schubert et al., 2006). These genetic studies reveal connections among TOP1a, chromatin-based gene regulatory mechanisms, and meristem homeostasis. However, how TOP1a influences chromatin-based gene regulation and why mutations in a general factor in DNA topology give rise to fairly specific developmental defects have been enigmatic.

Here, we report the mechanistic link between TOP1a and PcG-based gene regulation, which may underlie the specific roles of TOP1a in plant development. We show that among genes whose expression is altered (increased or decreased) in the top1a-2 mutant, PcG targets are overrepresented. TOP1a promotes the expression of many PcG target genes and is also required for H3K27me3 deposition at, and repression of, some PcG targets including WUS. The mechanism underlying the seemingly opposite effects of TOP1a in the expression of PcG target genes lies in TOP1a’s role in reducing nucleosome density. Whole-genome nucleosome occupancy studies show an overall increase in nucleosome density at genes in top1a-2. The increased nucleosome density in top1a-2 may occlude the binding of factors that either recruit PcG to genes or help overcome the repressive effects of PcG on gene expression. In the floral meristem, the binding of AG to WUS is reduced in top1a-2, which results in reduced H3K27me3 levels at WUS and prolonged WUS expression, and consequently loss of floral determinacy. The whole-genome nucleosome occupancy studies also reveal a lack of a 5’ nucleosome-free region in PcG targets but not nontargets. This feature may condition a particular requirement for TOP1a for the expression of PcG target genes.

RESULTS

TOP1a Is Required for the Termination of Floral Stem Cell Maintenance

The initial goal of the study was to identify genes that regulate the termination of the floral stem cell fate. We performed an ethyl methanesulfonate mutagenesis screen in the ag-10 background. Compared with ag null mutants with severe defects in both floral determinacy and floral organ identity specification (Bowman et al., 1989, 1991b), ag-10 is the weakest ag mutant known to date with only a mild floral determinacy defect (Ji et al., 2011). ag-10 flowers have a normal complement of floral organs as in the wild type (Figures 1A and 1B; Supplemental Table 1); however, a few siliques on an ag-10 plant are short and bulged, with additional organs growing inside, an indication of loss of floral determinacy (Ji et al., 2011). In the genetic screen, we focused on mutants with bulged siliques throughout the plant or with a flowers-within-flower phenotype reminiscent of ag null mutants.

One isolated mutant differed from the wild type in all four floral whorls but its floral determinacy defect was most notable as reflected by the presence of floral organs inside the primary, unfused, and sepalloid carpels (Figures 1C and 1F; Supplemental Table 1). Longitudinal sections of flowers revealed the presence of a floral meristem in stage 6 and older flowers in this mutant (Figure 1H, arrow) but not in ag-10 (Figure 1G). Genetic studies showed that the enhanced floral determinacy defects were caused by a single, recessive mutation. We performed map-based cloning and found a C-to-T mutation in the second exon of TOP1a (Kieber et al., 1992; Takahashi et al., 2002; Graf et al., 2010; Supplemental Figure 1A). This mutant, hereafter referred to as top1a-2, is likely a null allele since the mutation caused a stop codon that would eliminate the conserved central domain of the protein (Takahashi et al., 2002). The floral determinacy defect of ag-10 top1a-2 appeared to be weaker than that of ag-1, as judged by the number of floral organs produced by the floral meristem (Figures 1H and 1I). However, the floral meristem in ag-10 top1a-2 was indeterminate and continued to generate sepalloid carpels or sepalloid stamens.
An complex to stabilize the transient intermediate and block DNA topoisomerase I by binding to the DNA topoisomerase I-DNA.

The plant alkaloid camptothecin (CPT) inhibits the activities of topoisomerase I (TOP1α) (Table 1).

Flowers of ag-10 single mutant were more severe than those of ag-10 top1α-2 (Figures 1A and 1B). Thus, the topoisomerase activity of TOP1α was responsible for the enhanced floral determinacy defects.

By outcrossing ag-10 top1α-2 to the wild type, we isolated the top1α-2 single mutant. Top1α-2 plants displayed a similar spectrum of developmental defects as top1α-1, such as pointed rosette leaves, fasciated stems, abnormal phyllotaxy, and an enlarged SAM (Laufs et al., 1998; Takahashi et al., 2002; Graf et al., 2010). Top1α-2, like ag-10 top1α-2, differed from the wild type in the numbers of floral organs in all four whorls (Supplemental Table 1). The floral determinacy defects of ag-10 top1α-2 flowers were more severe than ag-10 or top1α-2 single mutant flowers, as reflected by the increased carpel number and the presence of secondary floral organs internal to the carpels (Supplemental Table 1).

**Chemical Inhibition of Topoisomerase I Activity Compromises Floral Stem Cell Termination**

The plant alkaloid camptothecin (CPT) inhibits the activities of topoisomerase I by binding to the DNA topoisomerase I-DNA complex to stabilize the transient intermediate and block DNA religation (Hertzberg et al., 1989). If the topoisomerase activity of TOP1α was required for floral stem cell termination, we would expect the application of CPT to ag-10 inflorescences to result in phenotypes similar to those of ag-10 top1α-2. We treated different side branches of ag-10 plants with 10 μM CPT and the control chemical DMSO on the same plant. After 10 d of single-dose, daily treatments, CPT-treated inflorescences produced bulged siliques with elongated gynophores, phenotypes observed when floral determinacy is compromised (Supplemental Figures 1C and 1E; Ji et al., 2011; Liu et al., 2011). Dissection of the siliques revealed the presence of additional carpelloid or staminoid organs inside (Supplemental Figure 1G), indicating compromised floral determinacy. DMSO treatments did not produce these phenotypes (Supplemental Figures 1B, 1D, and 1F). Therefore, the topoisomerase activity of TOP1α is required for the proper termination of floral stem cells during flower development.

**TOP1α Is Required for the Repression of WUS Expression in Flower Development**

To investigate the molecular basis of TOP1α’s role in floral meristem determinacy, we performed in situ hybridization to determine the temporal and spatial expression patterns of WUS...
and AG, two key regulators of floral stem cells. In the wild type, WUS expression commences in the rib zone at stage 1 of flower development and is shut off by stage 6 (Figure 2A; Mayer et al., 1998). In the ag-10 single mutant, ~10% of flowers have WUS expression in the rib zone persisting until stage 7 at the latest (Liu et al., 2011), while most flowers exhibit a normal temporal pattern of WUS expression (Figure 2B). In the top1a-2 single mutant, we did not observe prolonged WUS expression beyond stage 6 (Figure 2C). In ag-10 top1a-2, WUS expression was detected in the rib zone in all examined stage 7 and older flowers (Figure 2D). Thus, TOP1a is required for the repression of WUS expression.

As AG is required to shut off WUS expression at stage 6 (Laux et al., 1996; Lenhard et al., 2001; Lohmann et al., 2001), we next evaluated the effects of top1a-2 on AG expression. AG expression starts at stage 3 in the inner two whorls and persists till late stages of flower development (Yanofsky et al., 1990; Bowman et al., 1991a; Drews et al., 1991). A number of genes influence floral determinacy by promoting AG expression in the center of the floral meristem (Schultz et al., 1991; Alvarez and Smyth, 1999; Carles and Smyth, 2005; Prunet et al., 2008; Das et al., 2009; Maier et al., 2009). Therefore, it is possible that TOP1a confers floral determinacy by promoting AG expression. In wild-type, ag-10, and top1a-2 flowers, AG signals were detected by in situ hybridization in the inner two whorls at stages 6-7 (Figures 2E to 2G). In ag-10 top1a-2 flowers, the indeterminate floral meristem inside the fourth whorl also expressed AG (Figure 2H).

In situ hybridization cannot provide a quantitative assessment of gene expression, we performed laser capture microdissection to determine the effect of top1a-2 on AG expression in floral meristems. This was done also because AG was one of the downregulated genes in top1a-2 in microarray-based profiling of gene expression using whole inflorescences (Supplemental Data Set 1). Stage 3 and stage 6 floral meristems were dissected from Landsberg erecta (Ler) and top1a-2 flowers. Real-time RT-PCR on RNAs isolated from these samples showed that WUS RNA levels were higher in top1a-2 than in Ler not only at stage 6 but also at stage 3 (Supplemental Figure 2), suggesting that TOP1a probably represses WUS expression throughout stages 3-6. In Ler, WUS RNA levels were reduced in stage 6 flowers as compared with stage 3 flowers, consistent with the previously known temporal changes in WUS expression. In top1a-2, such a reduction was not observed, consistent with the prolonged WUS expression shown by in situ hybridization. AG RNA levels were also higher in top1a-2, especially at stage 6 (Supplemental Figure 2). Therefore, the effects of top1a-2 on floral determinacy could not be explained by reduced AG expression in floral meristems.

**TOP1a Functions in the AG Pathway to Regulate Floral Meristem Determinacy**

Genetic studies on top1a-2, ag-10, and ag-1 indicated that TOP1a acts in the AG pathway and that mutations in the two genes interact in a dosage-dependent manner. Flowers of the ag null mutant ag-1 exhibit both the transformation of stamens to petals and loss of floral determinacy in that carpels are replaced by another flower (Figure 3A). We crossed ag-10 top1a-2 with ag-1 heterozygous plants and obtained various genetic combinations in the F2 population by genotyping all three mutations. The top1a-2 ag-1 double mutant was indistinguishable from the ag-1 single mutant in terms of floral determinacy defects (Figure 3B), suggesting that TOP1a acts in the AG pathway. Interestingly, top1a-2 interacted with the ag alleles in a dose-dependent manner. First, top1a-2 enhanced the floral determinacy defect of ag-10/ag-1. ag-10/ag-1 plants had a stronger floral determinacy defect than ag-10 plants. While only a few flowers in an ag-10 plant had bulged gynoecia containing internal floral organs, all flowers in ag-10/ag-1 plants had bulged gynoecia that only occasionally

![Figure 2. WUS and AG Expression as Determined by in Situ Hybridization.](image-url)

(A) to (D) WUS expression in various genotypes.
(B) Stage 7 Ler (A) and ag-10 (B) flowers with no WUS expression.
(C) A stage 6 top1a-2 flower with no WUS expression.
(D) A stage 9 ag-10 top1a-2 flower. WUS expression is indicated by the arrow. Note that a dome-shaped floral meristem is present in (D).
(E) to (H) AG expression in various genotypes. AG expression was observed in the two inner whorls in Ler (E), ag-10 (F), and top1a-2 (G) flowers. AG expression was also detected in the indeterminate floral meristem (marked by the arrow) in ag-10 top1a-2 (H). Bars = 50 μm.
produced seeds (Figures 3C and 3D). top1a-2 enhanced ag-10/ ag-1 in that top1a-2ag-10-ag-1 flowers had a flowers-within- flower phenotype (Figure 3E). Second, we found that even TOP1α/top1a-2 ag-10/ag-1 plants had more severe floral determinacy defects than ag-10/ag-1 plants. TOP1α/top1a-2 ag-10/ ag-1 flowers had secondary stamens and sepaloid carpels growing inside the primary, unfused, sepaloid carpels (Figure 3F). In summary, the genotypes could be ordered based on their floral determinacy defects from weak to severe as TOP1α ag-10, TOP1α ag-10/ag-1, TOP1α/top1a-2 ag-10/ag-1, top1a-2 ag-10, and top1a-2 ag-10/ag-1, the last being similar to top1a-2 ag-1 or ag-1. Therefore, TOP1α and AG regulate floral meristem determinacy in a dosage-dependent manner in the same genetic pathway. This implies that the two genes may act in the same molecular process to confer floral determinacy.

**top1a-2 Drastically Enhances the Developmental Defects of PcG Mutants**

Our previous studies demonstrated that AG represses WUS expression in part by binding the WUS locus to promote PRC2-mediated H3K27me3 deposition as well as TFL2/LHP1 occupancy at WUS (Liu et al., 2011). Mutations in either CLF or TFL2/LHP1 enhance the ag-10 allele to result in bulged gynoecia containing ectopic floral organs inside, as does top1a-2 (Liu et al., 2011). This prompted us to evaluate whether the role of TOP1α in floral determinacy may be attributable to its involvement in PcG-mediated regulation of WUS expression.

We examined the effects of combining top1a-2 with mutations in CLF or LHP1/TFL2. We crossed ag-10 top1a-2 with ag-10 clf-47, which we had previously shown to exhibit bulged gynoecia containing ectopic floral organs inside (Figure 4A; Supplemental Table 2; Liu et al., 2011). The triple mutant was more severe than either ag-10 clf-47 or ag-10 top1a-2 in terms of floral determinacy defects (Figures 4A to 4C). Flowers of the triple mutant consisted of numerous sepals or petaloid sepals in the outer whorls and contained secondary organs inside the unfused sepaloid carpels (Figure 4C; Supplemental Table 2). As CLF and its paralog SWINGER share overlapping functions (Chanvivattana et al., 2004), the clf-47 loss-of-function allele can be considered a weak PRC2 mutant; therefore, the enhancement of clf-47 by top1a-2 could reflect a role of TOP1α in PcG-mediated gene regulation. More strikingly, combination of top1a-2 with tfl2-2, a loss-of-function mutation in LHP1/TFL2 (Kotake et al., 2003), resulted in loss of floral determinacy even in the wild-type AG background (Supplemental Table 2). The top1a-2 tfl2-2 double mutant, but not the tfl2-2 single mutant, had unfused sepaloid carpels with ectopic floral organs inside (Figures 4G and 4H). Top1a-2 clf-47 double mutant flowers had an internal flower within the fourth whorl organs and failed to form siliques (Figure 4I). These genetic results suggest that the role of TOP1α in floral determinacy requires PcG activities.

We next evaluated the link between TOP1α and PcG in other aspects of plant development. The seedlings of clf-47 resemble clf loss-of-function mutants in that they are severely dwarfed with true leaves curling upward (Figure 4D; Goodrich et al., 1997). clf loss-of-function mutants have weaker developmental defects than mutants of EMBRYONIC FLOWER2, another subunit of the vegetative PRC2, probably because of the existence of the CLF paralog SWINGER that compensates for the loss of CLF activity (Yang et al., 1995; Chanvivattana et al., 2004). The top1a-2 clf-47 double mutant was much smaller and dwarfed compared with either single mutant (Figures 4D to 4F). While clf- 47 and top1a-2 plants produced an average of 5.2 ± 0.8 and 7.1 ± 0.9 rosette leaves, respectively, top1a-2 clf-47 plants only produced an average of 3.1 ± 0.7 rosette leaves before bolting. These phenotypes were similar to those of emf2-3 (Chanvivattana et al., 2004). Therefore, the top1a-2 clf-47 phenotypes were reminiscent of loss of the vegetative PRC2. The inflorescence of the top1a-2 clf-47 double mutant terminated in a few disorganized and fused flowers (Figure 4F). In this respect, the double mutant resembled mutants in TFL2/LHP1. Therefore, top1a-2 enhances the phenotypes of clf-47 toward those reminiscent of severe reduction in PcG activity. This raises the possibility that TOP1α participates in PcG-mediated regulation of gene expression.

**H3K27me3 Enrichment and AG Occupancy at WUS Are Decreased in top1a-2 Inflorescences**

Findings from our genetic studies prompted us to determine whether TOP1α acts directly at the WUS locus to promote H3K27me3 deposition. We first performed chromatin immunoprecipitation (ChIP) experiments to address whether TOP1α binds the WUS locus using the top1a-2 TOP1α:TOP1α-HA transgenic
line in which the top1a-2 morphological defects were rescued by the functional transgene. In inflorescence tissues, all four tested regions from the WUS locus were enriched in the anti-HA immunoprecipitation from top1a-2 TOP1a:TOP1a-HA relative to the Lcr or the no antibody controls (Figures 5A and 5B). TOP1a occupancy was relatively high in the promoter region (WUSp1) and WUSp6, which is 3’ to the WUS gene. This was similar to the observation in fission yeast that Top1 binds mainly to intergenic regions (Durand-Dubief et al., 2010). ChIP experiments in TOP1α:TOP1α-HA and TOP1α:TOP1α-HA ag-1 inflorescences revealed that TOP1α occupancy at WUS did not require AG (Supplemental Figure 3A).

Next, we examined the status of H3K27me3 at WUS in Lcr and top1a-2 inflorescences by ChIP. The entire WUS locus (from WUSp1 to WUSp6) was previously shown to harbor H3K27me3 (Zhang et al., 2007b; Liu et al., 2011). The levels of H3K27me3 at all tested regions of the WUS locus were decreased in top1a-2 compared with Lcr (Figure 5C). This was also true when ag-10

Figure 4. Synergistic Interactions between top1α-2 and Mutations in PcG Genes.

(A) An ag-10 clf-47 flower. Note the bulged gynoecium, an indicator of floral determinacy defects. Some sepals, petals, and stamens were removed to expose the gynoecium.
(B) A top view of an ag-10 top1α-2 flower. Note that the gynoecium is replaced by sepalloid organs and additional floral organs are present inside the fourth whorl sepalloid organs.
(C) An inflorescence of ag-10 top1α-2 clf-47 with a few terminal flowers; each flower has a flowers-within-flower phenotype.
(D) A clf-47 plant with curled rosette leaves and a short inflorescence stem.
(E) A top1α-2 clf-47 plant displaying stem fasciation and abnormal phyllotaxy.
(F) A top1α-2 clf-47 plant with a reduced number of rosette leaves, an extremely short stem, and an inflorescence that terminates in two fused flowers (arrow).
(G) A top1α-2 clf-47 plant with a reduced number of rosette leaves, an extremely short stem, and an inflorescence that terminates in two fused flowers (arrow).
(H) A top1α-2 clf-47 plant with a reduced number of rosette leaves, an extremely short stem, and an inflorescence that terminates in two fused flowers (arrow).
(I) A representative silique from ag-10 top1α-2 35S:TFL2-3HA plants showing that overexpression of TFL2 can partially rescue the ag-10 top1α-2 floral determinacy defect (cf. with [B]).

Bars = 1 mm in (A) to (C) and (G) to (I) and 5 mm in (D) to (F).
top1α-2 was compared with ag-10 (Supplemental Figure 3C). To rule out that the differences in H3K27me3 levels in the above comparisons were caused by the differences in morphological phenotypes between the genotypes, we examined the effects of CPT treatments on H3K27me3 levels at WUS. Wild-type inflorescences were treated twice a day with either DMSO or CPT and samples were collected at day 2, when no morphological alterations from CPT treatments were visible. Reduction in H3K27me3 levels at WUS was found in the CPT-treated samples (Supplemental Figure 3B).

Our previous studies show that AG binds the WUSp2 and WUSp6 regions (Figure 5A) to promote H3K27me3 deposition and TFL2/LHP1 occupancy at WUS (Liu et al., 2011). We examined AG occupancy at WUS in top1α-2 and wild-type inflorescences by ChIP using anti-AG antibodies. AG occupancy at WUSp2 and WUSp6 regions was significantly reduced in top1α-2 (Figure 5D). Therefore, TOP1α promotes the binding of AG to WUS in vivo. Since the binding of AG to the WUS locus is required for the recruitment of PcG to WUS (Liu et al., 2011), the reduction in H3K27me3 levels in the top1α-2 mutant could be...
explained by the reduction in AG occupancy. We next asked whether all AG target genes require TOP1α for H3K27me3 deposition. No reduction in H3K27me3 levels was found for five genes known to be bound by AG in vivo (ÓMaoiléidigh et al., 2013; Supplemental Figure 3D). In fact, H3K27me3 levels were increased at SHATTERPROOF2 (SHP2) and AG in top1a-2 (Supplemental Figure 3D).

**PcG Target Genes Are Enriched in TOP1α-Affected Genes**

While our studies in the floral stem cell model uncovered a requirement for TOP1α in PcG-mediated repression of WUS expression, we sought to determine whether this is applicable to other PcG targets. We performed microarray analysis to determine the effects of the top1a-2 mutation on genome-wide gene expression in inflorescences. We found 794 genes whose expression was altered (194 increased and 600 reduced) in a statistically significant manner in top1a-2 relative to the wild type (Supplemental Data Sets 1 and 2). Genes involved in “developmental process” and “reproduction” were enriched in the group showing reduced expression in top1a-2 (Supplemental Figure 4A). Intriguingly, 282 of the 794 genes (36%) are PcG targets in that they harbor the H3K27me3 mark in seedlings as revealed by a genome-wide analysis of H3K27me3 (Zhang et al., 2007b; Supplemental Data Sets 1 and 2; Figure 6A). Of the more than 4000 genes that are PcG targets in Arabidopsis (Zhang et al., 2007b), 3360 were represented and constituted 16% of all genes on the microarray (Figure 6A). TOP1α-affected genes were enriched in PcG targets (P value < 2.2e-16).

Genes on the microarray were classified into three groups according to their expression levels (high, medium, and low) in wild-type inflorescences (see Methods), and each group was further separated into PcG targets and nontargets. Each of the six groups was then examined for altered expression in top1a-2 relative to the wild type. An overwhelming enrichment for PcG targets was found for the high and medium expression groups (Figure 6B). Since genes in the “low expression” category may not even be expressed in the samples, this analysis revealed a strong enrichment for PcG targets among expressed genes that are affected by top1a-2. We chose to validate changes in gene expression with real-time RT-PCR for eight genes showing higher expression in top1a-2 (Supplemental Figure 4B) and another eight genes showing lower expression in top1a-2 (Supplemental Figure 4C). All 16 genes were validated to be expressed at higher or lower levels in top1a-2 as expected. We also examined the expression of KNU, which was not represented in the microarray, as it is a target of both AG and PcG and is involved in floral determinacy. KNU expression was not significantly affected by top1a-2 (Supplemental Figure 4B).

We next evaluated the levels of H3K27me3 at these genes by ChIP. Nearly all genes with the exception of KNU and two other genes, regardless of the effects of TOP1α on their expression, showed a reduction in H3K27me3 levels in the top1a-2 mutant (Supplemental Figure 4D). This reveals a requirement for TOP1α in PcG-mediated H3K27me3 deposition at many genes. However, the reduction in H3K27me3 levels in top1a-2 did not always correlate with increased expression (Supplemental Figures 4B to 4D). In fact, among the 282 PcG target genes whose

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**Figure 6.** Genes Altered in Expression in top1a-2 Are Enriched in PcG Targets.

(A) Pie charts showing the percentage of PcG targets among genes represented by the ATH1 microarray (top) and among genes altered in expression in top1a-2 relative to the wild type (bottom). Yellow and gray represent PcG targets and nontargets, respectively.

(B) Pie charts showing the percentage of PcG nontarget (top) or target (bottom) genes altered in expression in top1a-2 relative to the wild type. Red and blue represent genes affected and unaffected, respectively, by top1a-2. Genes with low, medium, and high expression levels in the wild type (see Methods) were separately interrogated. The P values in (A) and (B) were calculated by Fisher’s exact test.
expression was altered in top1a-2, 58 were increased in expression and 224 were decreased in expression. Therefore, while TOP1α is required for the repression of a subset of PcG target genes, it also promotes the expression of many PcG target genes.

**TOP1α Affects Nucleosome Distribution**

We sought to determine how TOP1α assists in PcG-mediated repression and, more importantly, to understand how TOP1α also acts to promote the expression of many PcG target genes. In fission yeast, Top1 resides in intergenic regions flanking genes, and histone H3 occupancy is increased in intergenic regions, especially those flanking highly expressed genes in strains lacking Top1/Top2 activities (Durand-Dubief et al., 2010, 2011). This suggests that Top1 and Top2 reduce nucleosome density in gene regulatory regions to enable active transcription. Does TOP1α have similar effects on nucleosome density in Arabidopsis as in yeast? If so, do PcG targets have a particular requirement for TOP1α in maintaining normal nucleosome density? We sought to address these questions by profiling nucleosomes from wild-type and top1a-2 inflorescences at the genomic scale. Nuclei from wild-type and top1a-2 inflorescences were digested with micrococcal nuclease in a reaction time course, and no gross difference was observed in the micrococcal nuclease digestibility of the chromatin in the two genotypes (Supplemental Figure 5A). DNA protected by nucleosomes was subjected to high-throughput sequencing.

We performed a metagene analysis involving all genes 1 kb or longer annotated in the Landsberg genome to examine nucleosome density. Regions from −1 to +1 kb centered on the transcription start site (TSS) or transcription termination site (TTS) were separately interrogated. An increase in nucleosome density was found in top1a-2 relative to Ler in both biological replicates (Figures 7A and 7B; Supplemental Figures 5B and 5C). Unlike in fission yeast, for which an increase in nucleosome density in strains lacking Top1/Top2 activity is largely restricted to the intergenic region (IGR), in Arabidopsis, the increase in nucleosome density in top1a-2 was found in the transcribed region as well as the 5’ and 3’ IGRs (Figures 7A and 7B; Supplemental Figures 5B and 5C). Also unlike fission yeast Top1/Top2, Arabidopsis TOP1α’s role in reducing nucleosome density was not restricted to highly expressed genes. Genes with low, medium, and high levels of expression in inflorescences all showed enhanced nucleosome density in the transcribed region as well as the 5’ and 3’ IGRs in top1a-2 (Supplemental Figure 5D). Next, we examined the effects of the top1a-2 mutation on nucleosome density at PcG target and nontarget genes. Of 4979 PcG targets (Zhang et al., 2007b), 3739 that are 1 kb or longer were used in the analysis together with the same number of randomly chosen nontargets. For both PcG targets and

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**Figure 7. TOP1α Reduces Nucleosome Density.**

(A) and (B) A metagene analysis of nucleosome density in top1a-2 and the wild type (Ler). The 2-kb regions centering on the TSS (A) or TTS (B) for all genes that are no shorter than 1 kb in length were separately interrogated. Average nucleosome density at each base pair in these genes is displayed. (C) and (D) A metagene analysis for PcG target and nontarget genes. 3739 PcG target genes and the same number of randomly selected nontargets (gene length ≥1 kb) were used in the analysis as in (A) and (B). Note that the region immediately upstream of the TSS is low in nucleosome density compared with nearby regions for non-PcG targets, and such a 5’ NFR appears missing in PcG targets. (E) and (F) A Z-score-based statistical analysis of the 5’ NFR for all genes (E) or for PcG targets and nontargets (F). The genes used were the same as in (A) to (D). The x axis represents distance from the TSS in base pairs. The Z-score represents the number of standard deviations from the mean for the nucleosome number at any position in the region. Note that the results indicate differences in nucleosome density along the interrogated region (~1 to +1 kb) within each genotype; therefore, Ler and top1a-2 cannot be compared with each other. The P values were derived from Z-test for the position in the 5’ NFR that had the lowest number of nucleosomes. Note that results from biological replicate two were displayed in (A) to (F), and biological replicate one gave consistent results (Supplemental Figures 5B and 5C; data not shown).
nontargets, overall nucleosome density was increased in top1a-2 in the transcribed region and the 5’ and 3’ IGRs (Figures 7C and 7D). Therefore, top1a-2 leads to a similar increase in nucleosome density at all genes.

To further document the apparent increase in nucleosome density in top1a-2, we examined the distance between any two adjacent nucleosomes in the 5’ IGR (from −1 kb to the TSS) and thegenic region (from the TSS to +1 kb) in the wild type and top1a-2. There was indeed a shift of the distribution of adjacent nucleosome pair distance toward shorter distances in top1a-2 (Supplemental Figures 5E and 5F).

We confirmed the observed effects of top1a-2 on nucleosome density through histone H3 ChIP at individual loci. Among eight tested loci, four showed increased H3 occupancy (Supplemental Figure 6A). At the WUS locus, histone H3 occupancy was also higher in top1a-2 (Supplemental Figure 6B). In addition, histone H3 occupancy at WUS was drastically increased by CPT treatment (Supplemental Figure 6C).

We sought to identify features in nucleosome distribution that might distinguish PcG targets from nontargets. One common feature of nucleosome distribution in many organisms is the organization of nucleosomes around the TSS; a +1 nucleosome is positioned 3’ to the TSS, which is embedded in the 3’ end of a region in the 5’ IGR with low nucleosome density, referred to as the 5’ nucleosome-free region (5’ NFR) (Yuan et al., 2005; Lee et al., 2007; Mavrich et al., 2008; Schones et al., 2008). We found that a region of low nucleosome density immediately upstream of the TSS was obvious when all genes were examined in both the wild type and top1a-2 (Figure 7A). Intriguingly, when the 3739 PcG targets (Zhang et al., 2007b) and the same number of randomly chosen nontargets were examined, the PcG targets appeared to lack a 5’ NFR (Figure 7C). To further document this difference, we quantified nucleosomes in the region (~1 to +1 kb; with TSS being 0) and performed the statistical Z-test for the presence/absence of the 5’ NFR (see Methods). For 24,959 genes longer than 1 kb, a statistically significant 5’ NFR was found in the region in both the wild type and top1a-2 (Figure 7E). When this analysis was applied to PcG targets and nontargets, only the nontargets had a statistically significant 5’ NFR in the wild type (Figure 7F). top1a-2 did not affect the presence/absence of the 5’ NFR in PcG targets or nontargets (Figure 7F).

**DISCUSSION**

**TOP1α in Nucleosome Density or Positioning**

Given the general role of topoisomerases in relieving torsional stresses in DNA during replication or transcription, it is surprising that genetic studies in Arabidopsis, including this work, implicate a role for TOP1α in specific developmental processes. Our findings on the effects of TOP1α on nucleosome density provide a reasonable explanation for this paradox. Although the role of TOP1α in decreasing nucleosome density applies to most, if not all, genes, the lack of a 5’ NFR in PcG targets probably makes them particularly sensitive to a further increase in nucleosome density caused by TOP1α loss of function. As PcG target genes are crucial players in various developmental processes, it is reasonable that TOP1α loss of function tends to affect plant development. However, the role of TOP1α in decreasing nucleosome density suggests that TOP1α also influences other chromatin-based gene regulation. In fact, genetic interactions between top1a mutations and mutations in chromatin regulators support this notion (Graf et al., 2010). We also uncovered a role of TOP1α in transposon silencing in a separate study (Dinh et al., 2014).

It should be noted that, due to the fact that mixed cell types were used in the nucleosome profiling, the observed apparent increase in nucleosome density in top1a could be due to two possible scenarios or a combination of the two. First, nucleosome density is increased across all cell types in top1a. Second, nucleosome density is not affected in top1a, but the positioning of nucleosomes along a gene is altered in some but not all cell types. Therefore, TOP1α could be affecting nucleosome density, positioning, or both. In addition to the measurements of nucleosome density, most measurements (such as gene expression, H3K27me3 levels, etc.) in this study were conducted with mixed cell types. Such comparisons between the wild type and top1a could not capture differences in individual cell types. Cell type-specific measurements will be necessary to interrogate molecular events associated with developmental decisions occurring in a small number of cells.

**TOP1α Promotes Floral Determinacy through AG**

Loss of function in TOP1α enhances the weak ag-10 mutation to result in prolonged WUS expression and failure to properly terminate floral stem cells. Genetics studies placed TOP1α in the AG pathway, acting in a dose-dependent manner with AG to confer floral determinacy. Previous studies show that AG directly represses WUS expression by binding to the WUS locus to recruit PcG to WUS (Liu et al., 2011). AG also represses WUS expression indirectly through promoting the expression of KNU by reducing H3K27me3 levels at KNU (Sun et al., 2009, 2014). We did not observe an effect of top1a-2 on KNU expression or H3K27me3 levels. We found that AG occupancy and the levels of H3K27me3 at WUS were reduced in top1a-2, which suggests that TOP1α assists AG in recruiting the PcG to WUS. Consistent with this, mutations in TOP1α and PcG subunits show synergistic interactions and overexpression of LHP1/TFL2 partially rescues the floral determinacy defects of ag-10 top1a-2. We propose that, by facilitating the binding of AG to WUS, TOP1α allows for the recruitment of PcG, which in turns deposits H3K27me3 at WUS to silence its expression.

**TOP1α in Establishing PcG-Mediated Repression at PcG Targets**

Genetic studies show strong synergistic interactions between top1a alleles and mutations in genes encoding PcG subunits (Graf et al., 2010; this study), which suggests that TOP1α is required for PcG-mediated repression of gene expression. Consistent with this notion, AG, which is repressed in vegetative organs by PcG in the wild type (Goodrich et al., 1997), is ectopically expressed in leaves and stems in mgoun1 alleles (Graf et al., 2010). In this study, we show that TOP1α promotes the
deposition of H3K27me3 at WUS and 14 other loci (Supplemental Figure 4D).

In plants, how PcG is recruited to specific target genes is largely unknown. Two studies have implicated transcription factors in PcG recruitment (Liu et al., 2009; Liu et al., 2011). If this is a general mechanism for PcG recruitment, then our findings that TOP1α alters nucleosome density or positioning shed light on the involvement of TOP1α in PcG-mediated gene regulation. By reducing nucleosome density or altering nucleosome positioning at regulatory regions, TOP1α may allow transcription factors to bind to their target genes, which would lead to the recruitment of PcG.

**TOP1α in Overriding PcG-Mediated Repression**

PcG-mediated repression of genes is dynamically regulated. Genes that are repressed during vegetative development by PcG-mediated repression of genes is largely unknown. Two studies have implicated transcription factors in PcG recruitment (Liu et al., 2009; Liu et al., 2011). If this is a general mechanism for PcG recruitment, then our findings that TOP1α alters nucleosome density or positioning shed light on the involvement of TOP1α in PcG-mediated gene regulation. By reducing nucleosome density or altering nucleosome positioning at regulatory regions, TOP1α may allow transcription factors to bind to their target genes, which would lead to the recruitment of PcG.

**METHODS**

**Plant Materials**

All Arabidopsis thaliana strains used in this study are in the Ler background except for ag-10Col (Liu et al., 2011), in which ag-10 in Ler was introgressed into the Columbia (Col) background by five backcrosses, tfll2-2 (Larsson et al., 1998), an allele in Col that was backcrossed twice into Ler, and 35S:TFLL2-3HA (Liu et al., 2009), which is in the Col background. Lines ag-1 (Bowman et al., 1989), ag-10 (Ji et al., 2011), and clf-47 (Liu et al., 2011) were previously described, and top1α-2 was isolated in this study from a genetic screen in the ag-10 background. It was backcrossed two times before being used in any analyses. All plants were grown at 23°C under continuous light.

To produce the top1α-2 clf-47 and ag-10 top1α-2 clf-47 mutant combinations, ag-10 top1α-2 plants were crossed with clf-47. In the F2 population, clf-47 homozygous plants were identified based on the vegetative phenotypes and genotyped for top1α-2 and ag-10 to identify top1α-2 clf-47 and ag-10 top1α-2 clf-47 plants. For top1α-2 genotyping, genomic DNA was amplified with the primers top1μMuF and top1μMuR and the PCR products were digested with Msel, which only cut the DNA from the mutant. The clf-47 genotype was confirmed by PCR amplification of genomic DNA with the primers clfμMuF and clfμMuR and digesting the products with Bstul, the site of which was lost in clf-47.

To generate the top1α-2 tfll2-2 and ag-10 top1α-2 35S:TFLL2-3xHA combinations, tfll2-2 and 35S:TFLL2-3xHA (Liu et al., 2009) were crossed with top1α-2 and ag-10 top1α-2/+ plants, respectively. In the F2 population of the top1α-2 × tfll2-2 cross, the tfll2-2 mutation was identified based on the terminal flower phenotype and top1α-2 was genotyped as described above. For the ag-10 top1α-2 35S:TFLL2-3xHA combination, F2 plants were selected with Basta to identify plants with the 35S:TFLL2-3xHA transgene, and ag-10 and top1α-2 were genotyped as described above.

To obtain plants with various combinations of top1α-2, ag-10, and ag-1 alleles, ag-10 top1α-2 was crossed with ag-1+/+ plants. In the F2 population, plants with floral determinacy defects were identified and genotyped for top1α-2, ag-10, and ag-1.

Information about the primers used for genotyping is provided in Supplemental Data Set 3.

**Ethyl Methanesulfonate Mutagenesis and Mapping**

Ethyl methanesulfonate mutagenesis was performed in ag-10 as described (Liu et al., 2011). The ag-10 top1α-2 mutant was isolated and was backcrossed into Ler two times to obtain the top1α-2 single mutant and the ag-10 top1α-2 double mutant for further analysis. For map-based cloning, ag-10 top1α-2 was crossed into ag-10Col to create the mapping population. In the F2 generation, plants with floral determinacy defects were selected for rough mapping, which showed that the mutation was linked to the marker civ10 on chromosome 5. In fine mapping, we designed new simple sequence length polymorphism or cleaved-amplified polymorphic sequence makers based on the polymorphisms between Col and Ler (http://www.arabidopsis.org/Cereon) and located the mutation in the region represented by the BACs K18G13 and MCD7. TOP1α was selected as a candidate gene for sequencing.

**Plasmid Construction and Plant Transformation**

To generate TOP1α:TOP1α-HA, the TOP1α genomic region was amplified by PCR with the primer pair TOP1αgenoF/TOP1αgenoR (Supplemental Data Set 3) using Ler genomic DNA as the template. The PCR product was cloned into pENTR/D-TOPO (Invitrogen) and the resulting plasmid was sequenced to ensure the integrity of the gene. The plasmid was then linearized and the insert was recombinated into pEarleyGate301 (Earley et al., 2006) using a Gateway LR Clonase kit (Invitrogen). TOP1α:TOP1α-HA was transformed into ag-10 top1α-2/+ plants with the floral dip method (Bechtold and Pelletier, 1998).

**In Situ Hybridization and Histological Staining**

In situ hybridization was conducted as previously described (Liu et al., 2011). To generate the antisense AG probe, the plasmid pClT655 (Yanojfsky et al., 1990) was linearized with HindIII (New England Biolabs) and used as a template for in vitro transcription with T7 RNA polymerase (New England Biolabs). For the WUS probe, the coding region of WUS was amplified by RT-PCR and cloned into pGEM-T-easy (Promega). The plasmid was digested with SpeI and transcribed with T7 RNA polymerase to generate the WUS antisense probe.

For Toluidine Blue staining, inflorescences were fixed and embedded in wax as they would be prepared for in situ hybridization. The sections on slides were briefly dipped in 0.1% Toluidine Blue, which was dissolved in 0.1% sodium borate, and rinsed in water.

**Micrococcal Nuclease Digestion of Chromatin**

One gram of inflorescences containing unopened buds from Ler and top1α-2 plants was ground in liquid nitrogen, and the powder was transferred to M1 buffer (10 mM phosphate buffer, pH 7.0, 0.1 M NaCl, 10 mM mercaptoethanol, 1 M hexylene glycol, 1× protease inhibitor cocktail [Roche], and 1 mM phenylmethylsulfonyl fluoride). The suspension was filtered through four layers of Miracloth. The filtrate was centrifuged at
13,400g for 10 min at 4°C. The pellet (crude nuclei preparation) was re-
suspended and washed in M2 buffer (M1 buffer plus 10 mM MgCl₂ and
0.5% Triton X-100) three times and once in M3 buffer (10 mM phosphate
buffer, pH 7.0, 0.1 M NaCl, 10 mM mercaptoethanol, 1× protease inhibitor
cocktail [Roche], and 1 mM phenylmethylsulfonyl fluoride). After centri-
fugation at 16,400g for 1 min at 4°C, the nuclei were dissolved in digestion
buffer (50 mM Tris-HCl, 5 mM CaCl₂, 1× BSA, 20 μg/mL RNase A, and 1×
protease inhibitor cocktail [Roche]) containing 0.4 units/μL microccocal
nuclease (NEB) and incubated at 37°C for 30 min. DNA was extracted using
the CTAB method (Porebski et al., 1997) and resolved in a 2% agarose gel.
DNA bands corresponding to ~150 bp were excised and purified with a gel
purification kit (Qiagen). DNA libraries were constructed with a TruSeq DNA
Sample Preparation v2 kit (Illumina) and sequenced with Illumina’s
HiSeq2000 platform.

For the time course of microccocal nuclease digestion, 1 g of inflo-
rescences containing unopened buds from Ler and top1a-2 was collected
and nuclei were extracted as above. The nuclei were then dissolved
in 1.2 mL digestion buffer and incubated at 37°C. Then, 200 μL reaction
mixture was taken at different time points (0, 2, 4, 6, 8, and 10 min) and the
reaction was stopped by the addition of 10 μL 0.5M EDTA. DNA was extracted using the CTAB method and resolved in a 1.5% agarose gel.

Treatment of Plants with CPT

For observing the phenotypic effects of CPT, 10 μM CPT and DMSO were
applied to different side branches from the same ag-10 plants once daily.
After 10 d of treatment, the silicues were dissected open and photo-
graphed.

For ChIP assays after CPT treatment, plants were treated with 10 μM
CPT and DMSO once every 12 h for 2 d. Inflorescences (0.5 g) containing
unopened flowers were collected and ChIP was conducted as described
below with either anti-H3 or anti-H3K27me3 antibodies.

ChIP and Real-Time PCR

ChIP was performed as previously described (Liu et al., 2011). Inflo-
rescences were completely ground in liquid nitrogen and cross-linked
in 1% formaldehyde (Sigma-Aldrich) for 10 min on ice. The chromatin was
then pelleted by centrifugation and sonicated into DNA fragments of ~500 bp.
The lysate was precleared with 50 μL protein-A agarose beads/salmon
sperm DNA (Millipore) for 1 h and then incubated with anti-H3K27me3
(Abcam), anti-histone H3 (Abcam), anti-HA (Abcam), or anti-AG (Liu et al.,
2011) antibodies overnight. Anti-H3K27m3, anti-H3, and anti-HA anti-
odies were used at 5 μg per 0.5 g inflorescence tissue; anti-AG antibody
were used at 8 μg per 0.5 g inflorescence tissue. The bound chromatin was
precipitated with columns from the Qiagen plasmid extraction kit. Real-time PCR was conducted on the input, no antibody control and
antibody-bound DNA in triplicates. Three biological repeats were con-
ducted to ensure reproducibility.

For real-time RT-PCR analysis, inflorescences were ground and
extracted with the TRI reagent (Molecular Research Center). RNA was
precipitated by isopropanol and treated with DNaseI (Roche) to eliminate
contaminating DNA. Reverse transcription was performed with the M-
MLV reverse transcriptase (Promega). Quantitative real-time RT-PCR was
performed in triplicates using the ABI7500 and the SYBR RT-PCR kit (DBI
Bioscience) as described (Zheng et al., 2009).

DNA oligonucleotides used for these studies are listed in Supplemental
Data Set 3.

Laser Capture Microdissection

Laser capture dissection was performed as previously described (Liu et al.,
2011). Briefly, inflorescences from Ler and top1a-2 plants were fixed in
ethanol/acetic acid, dehydrated, and embedded in paraffin blocks. Ribbons
with 8-μm sections were loaded on a slide and dewaxed. Visual inspection
was conducted to identify the sections containing stage 3 or 6 flowers. The
central regions of stage 3 or 6 flowers were excised using the Arcturus™
Laser Capture Microdissection instrument (Applied Biosystems). Total RNA
was extracted with the Arcturus PicoPure RNA Isolation Kit (Applied Bio-
systems) according to the manufacturer’s instructions. Real-time RT-PCR
was performed as described above. CAP BINDING PROTEIN20 (CBP20;
At5g44200) was used as a loading control (see Supplemental Data Set 3 for
primer information). Three biological replicates were conducted.

Microarray-Based Transcript Profiling

RNAs were isolated from wild-type and top1a-2 Arabidopsis inflo-
rescences as described above. Affymetrix ATH1 arrays were hybridized in
three biological replicates according to the manufacturer’s instructions.
The microarray data were analyzed using the R package affy (Gautier
et al., 2004) from BioConductor (http://www.bioconductor.org). The
MASS method was used for background correction and normalization of
intensity values. Differentially expressed genes were identified using the R
package limma (Smyth, 2004) from BioConductor using a false discovery rate
cutoff of ≤0.05. The false discovery rate was calculated from the three
biological replicates.

Genes were classified based on their expression values in the wild
type. There are 22,810 probes in the Affymetrix array, which correspond to
20,807 unique, nuclear loci (TAIR, http://www.Arabidopsis.org/). All genes
were assigned to one of three categories according to their log2 ex-
pression values in the wild type (low, <7; medium, 7 to 10; high, >10). In
total, 7901, 9762, and 3144 genes were found in low, medium, and high
expression categories, respectively.

Differentially expressed genes were imported to agriGO with all genes
represented on the Affymetrix ATH1 Genome Array (GPL198) as back-
ground genes (http://bioinfo.cau.edu.cn/agriGO analysis.php; Du et al.,
2010). For the first-level Gene Ontology term “biological process,” the
numbers of genes associated with Gene Ontology secondary level terms
were exported for the background genes and genes with increased and
reduced expression in top1a-2. Fisher’s exact test was employed to calculate the P value.

Whole-Genome Analysis of Nucleosome Density

The raw reads were filtered with Illumina’s quality control pipeline. The
samples were separated according to their barcodes (indexes). The reads
were mapped to the Arabidopsis Ler genome (Gan et al., 2011) using SOAP2
(Li et al., 2009) with two mismatches allowed. The nucleosome-calling
program NOrMAL (Polishko et al., 2012) was used to identify nucleosomes
and their positions in the genome. The nucleosome numbers were nor-
malized based on the number of total sequenced reads of each library.

For metagene analysis of nucleosome distribution, nucleosomes were
mapped to all genes annotated in the Landsberg genome (http://mus.
wel.com/19genomes/) (Gan et al., 2011) and their corresponding
IGRs. A 1-kb region upstream and downstream of each gene (the trans-
scribed region) was designated as the 5’ and 3’ IGR, respectively.
The metagene analysis of nucleosome distribution was performed as de-
scribed (Kaplan et al., 2009; Zhang and Pugh, 2011). In brief, every base
pair from −1 to +1 kb centering on the TSS or TTS was interrogated for
whether it is covered by a nucleosome. Then, the average nucleosome
signal per base pair was calculated as the average value across all genes.
Only genes equal to or longer than 1 kb were used for all the metagene
analysis (Chodavarapu et al., 2010).

The metagene analysis of nucleosome distribution was also performed
for specific groups of genes, such as PcG targets and genes with different
expression levels with the same method. A total of 3739 PcG targets that are
1000 bp or longer (Zhang et al., 2007b) and the same number of randomly
selected PcG nontargets were analyzed. Genes with high, medium, and low levels of expression were determined from our microarray-based transcript profiling (described above). For the metagene analyses, 2837 highly expressed genes \( (\geq 1\) kb only) and the same numbers of randomly selected genes expressed at medium and low levels were included.

For the 5’ NFR analysis, all nucleosomes were mapped to genes \( (\geq 1\) kb only) and the corresponding IGRs. The nucleosomes that were mapped to the 1-kb 5’ IGR and the first 1 kb of each gene were counted. Then the nucleosome number \( (n(i)) \) at each position was used for the calculation of Z-score. Z-scores at each position \( (i = [-1000,999]) \) were calculated by subtracting the average nucleosome number \( (\mu) \) from the nucleosome number \( (n(i)) \) at this position and dividing the difference by the standard deviation \( (\sigma) \) of the nucleosome numbers in the region.

\[
Z\text{ score}(i) = \frac{n(i) - \mu}{\sigma}
\]

The distance between adjacent nucleosomes was computed for regions upstream and downstream of the TSS separately. All genes with length \( \geq 1\) kb were included in the analysis. The middle position equidistant between the 5’ and 3’ ends of a nucleosome was used to represent the location of each nucleosome. The distance between two nucleosomes was calculated for all adjacent nucleosome pairs in each region. The distribution of distances among all adjacent nucleosome pairs was then calculated.

Data Deposition
The microarray and nucleosome mapping data have been deposited into National Center for Biotechnology Information Gene Expression Omnibus under the identification numbers GSE42403 and GSE53950, respectively.

Accession Numbers
Sequence data from this article can be found in the GenBank/EMBL libraries under the following accession numbers: AG, AT4G18960; AP3, AT3G54340; CBBP20, AT5G42200; CLF, AT2G23380; elf4A1, AT3G13920; KNU, AT5G14010; TFL2/LHP1, AT5G17890; TOP1α, AT5G55300; SEP3, AT1G24260; SHP2, AT2G42830; TOP1β, AT5G55310; and WUS, AT2G17950.

Supplemental Data
The following materials are available in the online version of this article.

**Supplemental Figure 1.** The TOP1α Gene and the Enzymatic Activity of the Protein Are Required for Floral Determinacy.

**Supplemental Figure 2.** AG and WUS Expression in Stages 3 and 6 Floral Meristems.

**Supplemental Figure 3.** Chromatin Immunoprecipitation to Determine TOP1α Occupancy and H3K27me3 Levels at WUS and Other Genes.

**Supplemental Figure 4.** TOP1α Is Required for H3K27me3 Deposition at PcG Target Genes.

**Supplemental Figure 5.** TOP1α Reduces Nucleosome Density.

**Supplemental Figure 6.** Determination of Nucleosome Density by ChIP Using Anti-H3 Antibodies.

**Supplemental Table 1.** Floral Organ Counts in Wild Type and Mutants.

**Supplemental Table 2.** Floral Organ Counts in Various Mutants.

**Supplemental Data Set 1.** Genes Downregulated in top1α-2.

**Supplemental Data Set 2.** Genes Upregulated in top1α-2.

**Supplemental Data Set 3.** Oligonucleotides Used in This Study.

**Supplemental Methods.**

**Supplemental References.**

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**AUTHOR CONTRIBUTIONS**

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DNA Topoisomerase I Affects Polycomb Group Protein-Mediated Epigenetic Regulation and Plant Development by Altering Nucleosome Distribution in Arabidopsis

Xigang Liu, Lei Gao, Thanh Theresa Dinh, Ting Shi, Dongming Li, Ruozhong Wang, Lin Guo, Langtao Xiao and Xuemei Chen

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