DNA Topoisomerase Iα Affects the Floral Transition

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DNA topoisomerases modulate DNA topology to maintain chromosome superstructure and genome integrity, which is indispensable for DNA replication and RNA transcription. Their function in plant development still remains largely unknown. Here, we report a hitherto unidentified role of Topoisomerase Iα (TOP1α) in controlling flowering time in Arabidopsis (Arabidopsis thaliana). Loss of function of TOP1α results in early flowering under both long and short days. This is attributed mainly to a decrease in the expression of a central flowering repressor, FLOWERING LOCUS C (FLC), and its close homologs, MADS AFFECTING FLOWERING4 (MAF4) and MAF5, during the floral transition. TOP1α physically binds to the genomic regions of FLC, MAF4, and MAF5 and promotes the association of RNA polymerase II complexes to their transcriptional start sites. These correlate with changes in histone modifications but do not directly affect nucleosome occupancy at these loci. Our results suggest that TOP1α mediates DNA topology to facilitate the recruitment of RNA polymerase II at FLC, MAF4, and MAF5 in conjunction with histone modifications, thus facilitating the expression of these key flowering repressors to prevent precocious flowering in Arabidopsis.

The floral transition responds to multiple internal and external cues, which involve a spectrum of genetic pathways, including photoperiod, autonomous, vernalization, GA, and ambient temperature signaling pathways (Mouradov et al., 2002; Srikanth and Schmid, 2011). In Arabidopsis (Arabidopsis thaliana), these flowering pathways eventually regulate the expression of two major floral pathway integrators, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) and FLOWERING LOCUS T (FT), which in turn activate floral meristem identity genes, such as APETALA1 (API) and LEAFY (LFY), to transform a vegetative shoot meristem into an inflorescence meristem.

The expression of SOC1 and FT is partially regulated by a central flowering repressor, FLOWERING LOCUS C (FLC), and its five close homologs, MADS AFFECTING FLOWERING1 (MAF1) to MAF5, all of which belong to the family of MADS box transcription factors. FLC expression is activated mainly by a transcription activator complex consisting of FRIGIDA (FRI), FRI ESSENTIAL1, SUPPRESSOR OF FRI4, and FLC EXPRESSOR (Johanson et al., 2000; Michaels et al., 2004; Schmitz et al., 2005; Kim et al., 2006; Andersson et al., 2008; Choi et al., 2011). In contrast, FLC expression is suppressed by multiple regulators in vernalization and autonomous pathways that respond to prolonged cold treatment and developmental status, respectively (Sheldon et al., 2000; Michaels and Amasino, 2001). The expression of MAF genes also is affected by the vernalization pathway (Ratcliffe et al., 2001, 2003). The regulation of FLC and MAF genes is associated with various chromatin modifications at their loci, such as changes in histone H3 Lys-27 trimethylation (H3K27me3; Amasino, 2004; Henderson and Dean, 2004; Kim et al., 2009; He, 2012; Shen et al., 2014).

DNA topoisomerases are essential enzymes that mediate proper DNA topology by resolving unfavorable DNA supercoils, knots, and other over-wound intermediates accumulated during replication and transcription (Vos et al., 2011). These topoisomerases are classified into types I and II, which cleave and reseal single-strand and double-strand breaks, respectively (Wang, 2002; Ashour et al., 2015). Topoisomerase I (TOP1) plays a specific role in relaxing both positive and negative supercoiling by creating a single-strand DNA break and rotating the broken strand around the TOP1-bound DNA strand. Under physiological conditions, TOP1-mediated religation is favored after the generation of cleavage intermediates (Stewart et al., 1998). Several genome-wide studies have shown that TOP1 activity is required for proper gene expression. For example, TOP1 acts with the chromatin-remodeling protein Hrp1 to maintain an open chromatin state via
nucleosome disassembly at promoter regions to regulate gene transcription in fission yeast (Schizosaccharomyces pombe; Durand-Dubief et al., 2010). TOP1 also facilitates the transcription of long genes associated with autism and other neurodevelopmental disorders in mammals (King et al., 2013).

In Arabidopsis, the only two type I topoisomerase genes, TOP1α and TOP1β, are tandemly linked loci on chromosome 5, and their encoded proteins share more than 60% similarity. Down-regulation of TOP1β does not display observable phenotypes (Takahashi et al., 2002; Dinh et al., 2014), whereas loss of function of TOP1α affects primordium initiation in shoot apical meristems and floral meristems, resulting in abnormal phyllotaxis and plant architecture (Laufs et al., 1998; Takahashi et al., 2002). Consistently, TOP1α has been found to act together with WUSCHEL in stem cell maintenance in shoot and floral meristems, which might be due to a possible role of TOP1α in stabilizing epigenetic states (Graf et al., 2010). Furthermore, a recent study has suggested that TOP1α function in flowering development is associated with its effect on altering nucleosome distribution and the deposition of H3K27me3 mediated by Polycomb group (PcG) proteins (Liu et al., 2014). In addition, TOP1α also plays a role in the epigenetic silencing of transposable elements through DNA methylation and histone K9 dimethylation (Dinh et al., 2014).

In this study, we report that TOP1α plays a hitherto unknown role in controlling the floral transition through directly affecting the expression of FLC and its closest homologs, MAF4 and MAF5. We show that mRNA expression of TOP1α remains at relatively stable levels during the floral transition regardless of changes in environmental and endogenous signals. TOP1α binds to the genomic regions of FLC, MAF4, and MAF5 and promotes the recruitment of RNA polymerase II complexes to the transcriptional start sites of these loci. The TOP1α effect on the expression of FLC, MAF4, and MAF5 correlates with the changes in histone modifications but is not directly relevant to nucleosome occupancy at these loci. Our results indicate that TOP1α could serve as a unique regulator that determines the DNA topology of FLC, MAF4, and MAF5 to recruit RNA polymerase II downstream of environmental and developmental signals, thus facilitating the expression of these key repressors to prevent precocious flowering in Arabidopsis.

RESULTS

Loss of Function of TOP1α Accelerates Flowering

To assess the function of TOP1α in plant development, we examined two T-DNA insertion mutants, top1a-7 (SALK_112625, also known as nsg01-7; Graf et al., 2010; Dinh et al., 2014) and top1a-10 (SALK_013164). top1a-10 and top1a-7 contained a T-DNA insertion in the first and ninth introns, respectively (Fig. 1A). There were no detectable N- and C-terminal TOP1α transcripts in top1a-10, while the N-terminal TOP1α transcript was still expressed at low levels in top1a-7 (Fig. 1B). Thus, we mostly used top1a-10 for subsequent analyses. top1a-10 and top1a-7 exhibited significantly early flowering compared with wild-type plants under both long days and short days (Fig. 1, C–E), suggesting that TOP1α inhibits the floral transition independently of the daylength conditions. In contrast, knockdown of TOP1β, the closest homolog of TOP1α, did not display a flowering defect under both long days and short days (Supplementary Fig. S1).

To confirm whether the early-flowering phenotype of top1α-10 is attributed to the loss of TOP1α, we transformed top1α-10 with a genomic construct (gTOP1α-4HA) that contains a 5.6-kb TOP1α genomic fragment including the 1.3-kb 5′ upstream sequence and the entire coding sequence plus introns fused in frame with a 4HA tag. Fourteen out of the 16 top1α-10 gTOP1α-4HA transformants in the T1 generation exhibited comparable flowering time to wild-type plants, indicating that lack of TOP1α activity contributes to the early-flowering defect of top1α-10. One fully rescued top1α-10 gTOP1α-4HA line (Fig. 1, C and D), which could contain only one T-DNA insertion site based on the segregation ratio, was selected for subsequent experiments.

TOP1α Is Expressed Ubiquitously in Arabidopsis

To examine the temporal and spatial expression patterns of TOP1α during the floral transition, we generated a gTOP1α-GUS reporter construct in which the TOP1α genomic region used for the complementation assay was fused to the GUS gene. Almost all gTOP1α-GUS transgenic lines examined displayed a ubiquitously strong staining pattern in whole seedlings at the vegetative phase and during the floral transition occurring 9 to 13 d after germination under our growth conditions (Fig. 2A). Consistently, quantitative analysis of TOP1α expression in wild-type plants grown under long days revealed only slight fluctuations in its expression before and during the floral transition (Fig. 2B). These observations indicate that TOP1α could be constitutively active in developing Arabidopsis seedlings.

Since TOP1α affects flowering time, we further examined whether various flowering genetic pathways affect its expression during the floral transition. In contrast to the dramatic down-regulation of FLC in FRI-Col (for Columbia), vernalization treatment did not alter TOP1α expression regardless of the FRI allele (Fig. 2C), indicating that neither the FLC-dependent nor the FLC-independent vernalization pathway affects TOP1α expression. TOP1α expression was not affected by CONSTANS (CO), a key regulator in the photoperiodic pathway (Fig. 2D), and did not display a diurnal oscillation under long days (Fig. 2E). TOP1α expression also was not affected in the autonomous pathway
mutants fld-3 and fve-4 (Fig. 2F). These observations suggest that neither the photoperiod pathway nor the autonomous pathway affects TOP1a expression. TOP1a expression in wild-type plants grown under short days was not responsive to GA treatment (Fig. 2G). Consistently, TOP1a expression remained unchanged in GA-deficient ga1-3 mutants and wild-type plants grown under short days (Fig. 2H), indicating that TOP1a is not regulated by the GA pathway. Collectively, these results demonstrate that mRNA expression of TOP1a is maintained at relatively stable levels during the floral transition regardless of changes in environmental and endogenous signals. In addition, we also compared the rate of rosette leaf production in developing wild-type and top1a-10 plants and found that there was no difference in leaf production rate before top1a-10 bolted (Fig. 2I), indicating that the plastochron lengths of wild-type and top1a-10 plants are comparable. In addition, the expression of two SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) family genes, SPL3 and SPL9, in the age-related pathway (Wu and Poethig, 2006; Wang et al., 2009; Wu et al., 2009; Wang, 2014) remained almost unchanged in top1a and wild-type plants (Fig. 2J). These observations imply that TOP1a also is not involved directly in the age-related pathway to affect flowering time.

Expression of FLC and Its Close Homologs MAF4/5 Is Affected by TOP1a

To understand how TOP1a affects flowering time, we examined the temporal expression of two major floral pathway integrators, SOC1 and FT, and their common upstream repressor FLC as well as its close homologs MAF1 to MAF5, in wild-type and top1a-10 seedlings. Both SOC1 and FT were up-regulated in developing top1a-10 seedlings, particularly during the floral transition under long days (Fig. 3, A and B), which is in agreement with the early-flowering phenotype of top1a-10. Notably, FLC expression was reduced dramatically in top1a-10 compared with wild-type seedlings throughout the vegetative phase and floral transition under long days (Fig. 3C). Among MAF1 to MAF5, only MAF4 and MAF5 were obviously and consistently down-regulated in top1a-10 under long days (Fig. 3, D–H). Under short-day conditions, FLC and MAF4 also were greatly down-regulated in top1a mutants, while there was only a slight decrease in MAF5 expression.
Figure 2. Expression analysis of TOP1α during the floral transition. A, GUS staining of a representative gTOP1α-GUS line at the vegetative phase (3 and 6 d old) and during the floral transition (8 and 10 d old). Bars = 1 mm. B, Temporal expression of TOP1α in developing wild-type seedlings (5–11 d old) grown under long days. C, TOP1α expression is not affected by vernalization. Seeds were sown on Murashige and Skoog medium and vernalized at 4°C under low-light conditions for 8 weeks. The 9-d-old seedlings grown under long days were harvested for expression analysis. FLC expression was analyzed as a control for vernalization treatment. D, TOP1α expression is not altered in the CO mutant co-9. Expression analysis was performed on 9-d-old wild-type (WT) and co-9 seedlings grown under long days. E, TOP1α expression does not exhibit a circadian rhythm. Diurnal oscillation of TOP1α expression is shown in 9-d-old wild-type seedlings under long days. Samples were harvested at 4-h intervals over a 24-h period. Sampling time is expressed in hours as Zeitgeber time (ZT), which is the number of hours after the onset of illumination. F, TOP1α expression is not affected by the autonomous pathway. Expression analysis was performed on 9-d-old wild-type, fld-3, and fve-4 seedlings grown under long days. G, Effect of GA treatment on TOP1α expression in wild-type plants grown under short days. Exogenous GA (100 μM) or 0.1% ethanol (Mock) was applied weekly onto wild-type plants grown under short days. Seedlings treated from week 2 (W2) to week 3 (W3) were collected for expression analysis. H, Comparison of TOP1α expression in the GA-deficient mutant ga1-3 and wild-type plants. Seedlings grown under short days from week 2 (W2) to week 4 (W4) were collected for expression analysis. I, Comparison of rosette leaf number in developing wild-type and top1α-10 seedlings (9–18 d old) grown under long days. Values were scored from 30 plants of each genotype. Asterisks indicate statistically significant differences in leaf number of top1α-10 plants compared with that of wild-type plants (two-tailed paired Student’s t test, \( P < 0.001 \)). J, Expression of SPL3 and SPL9 is not altered in 9-d-old top1α mutants versus wild-type plants grown under long days. Gene expression in B to H and J was determined by quantitative real-time PCR and normalized to TUB2 expression. Error bars indicate SD.
Thus, the expression of *FLC*, *MAF4*, and *MAF5* is concomitantly down-regulated in *top1a-10* under both long days and short days. In contrast, the mRNA expression of other important flowering regulators, such as CO, *AGAMOUS-LIKE24*, *TERMINAL FLOWER1*, *LFY*, and *AP1*, was not obviously altered in *top1a* mutants during the floral transition (Supplemental Fig. S3).
We further performed a detailed examination of the genetic interaction between TOP1a and FLC using two approaches. First, we crossed top1α-10 with 35S:FLC and found that top1α-10 35S:FLC exhibited a comparable late-flowering phenotype to 35S:FLC (Fig. 3I). Consistently, FLC was highly expressed at similar levels in top1α-10 35S:FLC and 35S:FLC (Fig. 3J). Thus, overexpression of FLC completely suppresses the early flowering of top1α-10, implying that FLC acts downstream of TOP1a to control the flowering transition. Second, we crossed top1α-10 with FRI-Col, fld-3, and fve-4, in which FLC expression was greatly elevated (Fig. 3J) due to a promotive effect of FRI (Michaels and Amasino, 2001) and an increased histone H3/H4 acetylation at the FLC locus (He et al., 2003; Ausín et al., 2004; Kim et al., 2004), respectively. Although top1α-10 FRI-Col, top1α-10 fld-3, and top1α-10 fve-4 flowered later than wild-type and top1α-10 plants, they flowered significantly earlier than FRI-Col, fld-3, and fve-4 single mutants, respectively (Fig. 3I). In agreement with this observation, FLC expression in top1α-10 FRI-Col, top1α-10 fld-3, and top1α-10 fve-4 was higher than that in wild-type and top1α-10 plants but significantly lower than that in FRI-Col, fld-3, and fve-4, respectively (Fig. 3J). These results not only substantiate that FLC controls flowering downstream of TOP1a but also imply that TOP1α could affect the role of FRI, FLD, and FVE in mediating FLC expression levels.

TOP1α Directly Controls FLC Expression

To examine whether TOP1α directly controls the expression of FLC, MAF4, and MAF5, we performed chromatin immunoprecipitation (ChIP) experiments to test TOP1α binding to these loci using the established top1α-10 gTOP1α-4HA line (Fig. 1, C and D), in which the expression of TOP1α-4HA could be detected specifically by the anti-HA antibody (Fig. 4A). ChIP assays of 9-d-old wild-type and top1α-10 gTOP1α-4HA seedlings showed that 10 fragments covering the FLC locus

Figure 4. TOP1α binds physically to FLC and MAF genomic regions. A, Expression of TOP1α-4HA fusion proteins in top1α-10 gTOP1α-4HA examined by western-blot analysis using anti-HA antibody. Expression of histone H3 was used as an internal control. B, Schematic diagrams show the genomic regions of FLC, MAF4, and MAF5. Exons and untranslated regions are represented by black and gray boxes, respectively, while introns and other genomic regions are represented by black lines. Translation start sites and stop codons are indicated. DNA fragments amplified in ChIP assays are labeled beneath the genomic regions. C and D, ChIP analysis of TOP1α-4HA binding to FLC (C) and MAF (D) genomic regions in 9-d-old wild-type (WT) and top1α-10 gTOP1α-4HA seedlings. ChIP enrichment using anti-HA antibody was compared with enrichment without anti-HA antibody. Asterisks indicate statistically significant differences in ChIP enrichment between wild-type and top1α-10 gTOP1α-4HA seedlings (two-tailed paired Student’s t test, P < 0.001). Error bars indicate SD.
and another 12 fragments covering MAF4 and MAF5 loci (Fig. 4B) were all enriched for TOP1α occupancy to different extents in the anti-HA immunoprecipitation from top1α-10 gTOP1α-4HA versus wild-type seedlings (Fig. 4, C and D). In contrast, ChIP assays without anti-HA antibody did not reveal obvious TOP1α occupancy at these loci. In addition, ChIP assays using anti-HA antibody also did not show TOP1α occupancy at the loci of several flowering time genes (MAF1, MAF2, and CO; Supplemental Fig. S4B), whose expression was not altered in top1α mutants under long days (Fig. 3, D and E; Supplemental Fig. S3A). These data suggest that TOP1α specifically occupies FLC, MAF4, and MAF5 loci (Fig. 4, C and D), indicating a role of TOP1α in directly affecting the expression of these genes. Furthermore, we found that the TOP1α association with FLC, MAF4, and MAF5 loci was not greatly changed in 6-, 9-, and 15-d-old developing seedlings (Fig. 4, C and D; Supplemental Fig. S4C). Thus, TOP1α may consistently promote the expression of these genes before, during, and after the floral transition.

In addition to a direct effect of TOP1α on FLC expression, we also investigated whether TOP1α could affect FLC indirectly via other known FLC upstream regulators. FLC expression is regulated by a complex network involving epigenetic regulators, such as Polycomb Repressive Complex (PRC) components, in response to flowering signals in autonomous, vernalization, and other vernalization-independent pathways (Clarke and Dean, 1994; Johanson et al., 2000; Zhang and van Nocker, 2002; Michaels et al., 2004; Simpson, 2004; Kim et al., 2009; He, 2012; Zografos and Sung, 2012; Kim and Sung, 2014). Thus, we examined the expression of those known upstream regulators of FLC in 9-d-old wild-type and top1α-10 seedlings. Neither the autonomous pathway regulators, including FCA, FPA, LD, FLK, FY, FLD, and FVE (Supplemental Fig. S5A), nor vernalization-dependent and -independent factors, such as FRI, FRL1, VRN2, HOS1, VIP3, VIP4, and VIP6 (Supplemental Fig. S5, B and C), were affected by the loss of function of TOP1α. Similarly, we found that the expression of PRC2 components, including CURLY LEAF, FERTILIZATION-INDEPENDENT ENDOSPERM, and EMBRYONIC FLOWER2, and the PRC1-like regulator TERMINAL FLOWER2/LIKE HETEROCHROMATIN PROTEIN1, remained almost unchanged in 9-d-old wild-type and top1α-10 seedlings (Supplemental Fig. S5D). These analyses preclude an indirect effect of TOP1α on FLC expression through other known FLC upstream regulators.

**TOP1α Promotes the Association of RNA Polymerase II Complexes to FLC and MAF Loci**

As DNA topoisomerases may play a role in relaxing unfavorable DNA supercoils to facilitate transcription (Vos et al., 2011), we proceeded to investigate whether TOP1α binding to FLC and MAF4/5 loci affects the accessibility of RNA polymerase II complexes to these loci by ChIP analysis of wild-type and top1α-10 seedlings using the anti-RNA polymerase II C-terminal domain (CTD) antibody, which recognizes the CTD of the

![Figure 5](image-url)  
*Figure 5.* TOP1α promotes the association of RNA polymerase II complexes to FLC and MAF4/5 loci. A, Expression of RNA polymerase II (indicated by the largest subunit, NRPB1) in 9-d-old wild-type (WT) and top1α-10 seedlings detected by western-blot analysis using anti-RNA polymerase II (Pol II) CTD antibody. Expression of histone H3 was used as an internal control. B and C, ChIP assays show a reduced association of RNA polymerase II complexes with the regions flanking the transcription start sites of FLC (B) and MAF4/5 (C) in top1α-10 versus wild-type seedlings. ChIP enrichment using anti-RNA polymerase II CTD antibody was compared with enrichment without the antibody. Asterisks indicate statistically significant differences in ChIP enrichment between wild-type and top1α-10 seedlings (two-tailed paired Student’s t test, P < 0.05). Error bars indicate SD.
largest subunit of RNA polymerase II (NRPB1). While the expression of NRPB1 was comparable in 9-d-old wild-type and top1α-10 seedlings (Fig. 5A), its association with FLC, MAF4, and MAF5 loci was altered in top1α-10 (Fig. 5, B and C). ChIP assays revealed that, compared with the assays without the antibody, enrichment of NRPB1 was significantly higher in wild-type than in top1α-10 seedlings at the fragments FLC-1 to FLC-4, MAF4.1 to MAF4.3, and MAF5.2 to MAF5.4 (Fig. 5, B and C), which exactly flank the transcription start sites of FLC, MAF4, and MAF5, respectively (Fig. 4B). In contrast, enrichment of NRPB1 at the housekeeping gene ACTIN7 (ACT7) remained unchanged in wild-type and top1α-10 seedlings (Fig. 5B). These results demonstrate that recruitment of RNA polymerase II complexes to the transcriptional start sites of FLC, MAF4, and MAF5 loci was impaired in the absence of TOP1α, suggesting that TOP1α promotes the recruitment of RNA polymerase II at these loci during the floral transition.

**TOP1α Affects Histone Modifications at FLC, MAF4, and MAF5 Loci**

Many studies have reported the involvement of histone modifications in regulating the expression of FLC and MAFs (Ausín et al., 2004; Kim et al., 2004; Sung and Amasino, 2004; Greb et al., 2007; Jiang et al., 2008, 2009; Buzas et al., 2011; He, 2012; Jégou et al., 2014; Shen et al., 2014; Zhu et al., 2015). Posttranslational modifications of RNA polymerase II components, whose recruitment is mediated by TOP1α, also contribute to histone modifications as transcription proceeds (Egloff and Murphy, 2008; Spain and Govind, 2011). In addition, TOP1α function in gene regulation has been implicated to be relevant with histone modifications, such as H3K27me3 (Liu et al., 2014). Thus, we further investigated whether TOP1α affects H3K27me3 modification at FLC, MAF4, and MAF5 loci through measuring H3K27me3 levels at these loci by ChIP assays of 9-d-old wild-type and top1α-10 seedlings. Levels of H3K27me3 enrichment at these loci were all increased significantly in top1α-10 (Fig. 6, A and B). Notably, the increase occurred from the region near the transcriptional start site to the end of the gene body rather than other intergenic regions. On the contrary, H3K27me3 at the active housekeeping gene ACT7 remained at similarly low levels in both wild-type and top1α-10 seedlings (Fig. 6A). The observation of increased H3K27me3 levels at FLC, MAF4, and MAF5 is consistent with the down-regulation of FLC, MAF4, and MAF5 in top1α-10, since the repressive H3K27me3 mark is typically associated with inactively transcribed chromatin. As the H3K27me3 mark is deposited by PRC2 and bound by PRC1, we further tested whether TOP1α interacts with various components in Arabidopsis PRC2 and PRC1 complexes using yeast two-hybrid analysis. However, none of these PRC subunits showed obvious interaction with TOP1α in budding yeast (Saccharomyces cerevisiae; Supplemental Fig. S6), implying that TOP1α may indirectly affect H3K27me3 modification.

In addition to H3K27me3, we also examined the effect of TOP1α on histone acetylation levels at FLC by ChIP analysis using the anti-acetylated histone H3 Lys9/14 (H3K9/14ac) antibody. In wild-type plants, levels of H3K9/14 acetylation were generally low at the FLC loci
DISCUSSION

DNA topoisomerases are essential enzymes that relieve the supercoils generated in the processes of DNA replication, transcription, recombination, and chromatin remodeling by introducing temporary cleavage of DNA (Vos et al., 2011). Although many in vitro studies, such as those using cellular extracts and chromatin templates, have suggested a role of DNA topoisomerases in affecting gene expression, how they function in multicellular organisms in different developmental contexts is still largely unknown. In this study, we report that TOP1α, an Arabidopsis type IB topoisomerase that targets single-strand DNA breaks, plays an important role in controlling the transition from vegetative to reproductive development in Arabidopsis. Loss of function of TOP1α results in precocious flowering under both long and short days, which is due mainly to the down-regulation of a key flowering repressor, FLC, and its close homologs, MAF4 and MAF5 (Fig. 7).

Several lines of evidence suggest that TOP1α is required to directly facilitate FLC expression in Arabidopsis. First, FLC expression is substantially downregulated in developing top1α-10 seedlings under both long and short days. Notably, FLC is almost undetectable in top1α-10 during the floral transition under long days. Thus, among the flowering genes examined, FLC displays the most dramatic change in gene expression levels in response to TOP1α activity. Second, the expression of other known upstream regulators of FLC is not altered in top1α-10 during the floral transition.

TOP1α Does Not Affect Nucleosome Occupancy at FLC, MAF4, and MAF5 Loci

It has been suggested that the TOP1α ortholog in fission yeast, TOP1, facilitates nucleosome disassembly to maintain an open chromatin and promote active transcription (Durand-Dubief et al., 2010). In Arabidopsis, TOP1α affects nucleosome distribution and is required for PcG-mediated repression of gene expression (Liu et al., 2014). To clarify whether the TOP1α effect on FLC expression is relevant to changes in nucleosome occupancy, we examined the occupancy of the +1 nucleosome, which is located about 70 bp 5′ to the transcription start site of the FLC locus, as reported previously (Ashour et al., 2015; Finnegan, 2015). There was no obvious difference in nucleosome density at FLC between wild-type and top1α-10 seedlings evaluated by histone H3 ChIP (Supplemental Fig. S7, A and B). Consistently, the amount of nucleosome-protected DNA after micrococcal nuclease digestion also was not changed at FLC in top1α-10 (Supplemental Fig. S7, A and C). A similar pattern of nucleosome occupancy also was found at MAF4 and MAF5 loci in wild-type and top1α-10 seedlings (Supplemental Fig. S7, A–C). These results suggest that the TOP1α effect on the expression of FLC, MAF4, and MAF5 may not be directly relevant to nucleosome distribution at these loci during the floral transition.

Figure 7. Model of the TOP1α role in controlling the floral transition. In the absence of TOP1α, the condensed chromatin at FLC, MAF4, and MAF5 loci prevents the recruitment of RNA polymerase II, which is associated with increased H3K27me3 but decreased H3K9/14ac modifications. This suppresses the expression of these flowering repressors, thus derepressing the floral transition. In wild-type plants, TOP1α binds to FLC, MAF4, and MAF5 loci and removes DNA helical tension to create an open chromatin at these loci. These changes facilitate the recruitment of RNA polymerase II (Pol II), which correlates with decreased H3K27me3 but increased H3K9/14ac modifications, thus promoting the expression of these flowering repressors to prevent precocious flowering. Blue circles represent H3K27me3, and orange triangles represent H3K9/14ac.
Arabidopsis TOP1
MAF5
loci but does not affect the occupancy of RNA near the transcriptional start sites of Arabidopsis TOP1 regulates transcription initiation. In contrast, DNA topology is closely associated with the regulation of gene expression in both processes of transcriptional initiation and elongation (Wang, 2002; Vos et al., 2011; Kouzine et al., 2014). Formation of the transcription preinitiation complex involves multiple steps in order (Hahn, 2004). The TATA box binding protein, a subunit of the transcription initiation factor TFIIID, first binds to the promoter of a specific target and recruits TFIIA and TFIIIB. TFIIH then interacts with RNA polymerase II and TFIIA and TFIIIB and recruits them to the promoter. The growing complex further engages TFIIIE and TFIIH, whose subunits contain ATPase and helicase activities, to facilitate the formation of the transcription bubble. DNA topoisomerase I has been identified as a coactivator of the TFIIID complex (Kretzschmar et al., 1993; Merino et al., 1993) and demonstrated to enhance the formation of the active TFIIH-TFIIA complex on the promoter (Shykind et al., 1997). Consistently, inhibition of DNA topoisomerase I activity reduces the density of RNA polymerase II at promoter pause sites in human cells (Khobta et al., 2006). In budding yeast, DNA topoisomerases are required for the recruitment of RNA polymerase II but are dispensable for ongoing transcriptional elongation (Roedgaard et al., 2015). These observations demonstrate a pivotal role of DNA topoisomerase I in transcriptional initiation. In contrast, some other studies have suggested that DNA topoisomerase I also affects transcriptional elongation by resolving unfavorable supercoils generated in the course of the movement of RNA polymerase II (Mondal et al., 2003; García-Rubio and Aguilar, 2012; King et al., 2013). Our results demonstrate that TOP1α facilitates the recruitment of RNA polymerase II to the regions near the transcriptional start sites of FLC, MAF4, and MAF5 loci but does not affect the occupancy of RNA polymerase II in the other regions, suggesting that Arabidopsis TOP1α specifically affects transcriptional initiation rather than transcriptional elongation at target genes during the floral transition.

It is noteworthy that TOP1α binding to FLC, MAF4, and MAF5 correlates both with the promotion of the association of RNA polymerase II complexes to the corresponding transcriptional start sites and changes in histone modifications, but it does not directly affect nucleosome occupancy at these loci, as reported recently for its function in floral meristem development (Liu et al., 2014). Posttranslational modifications of RNA polymerase II components, particularly phosphorylation of the CTD of the largest Rpb1 subunit, have been suggested to serve as an important means to recruit histone modifiers and chromatin-remodeling complexes to regulate transcription initiation, elongation, and termination (Spain and Govind, 2011; Hsin and Manley, 2012). We have found that TOP1α significantly affects H3K27me3 levels at FLC, MAF4, and MAF5 during the floral transition. Since, in budding yeast, TOP1α does not interact with PcG proteins that directly determine the deposition and recognition of the H3K27me3 mark, we reason that the effect of TOP1α on H3K27me3 levels may result from its recruitment of RNA polymerase II complexes at these loci, which in turn modulate histone modifications and affect transcriptional elongation. This process is likely to affect histone deacetylation mediated by FLD and FVE (He et al., 2003; Ausín et al., 2004; Yu et al., 2011) at FLC as well, since hyperacetylation of H3K9/14 at FLC in fl-3 and fve-4 mutants also is significantly suppressed by top1α-10 (Fig. 6C).

In contrast to the specificity of TOP1α in controlling the expression of FLC, MAF4, and MAF5 during the floral transition, TOP1α expression is constitutively present in developing seedlings and remains at relatively stable levels during the floral transition regardless of variations in environmental and endogenous flowering signals. We envisage two possible scenarios to explain these observations. TOP1α may act together with other specific upstream regulators of FLC, MAF4, and MAF5 to control their gene expression during the floral transition. These partners could either interact with TOP1α at the protein level or act with TOP1α in the same genetic pathway, as demonstrated by the interaction between TOP1α and AG in the control of floral meristem development (Liu et al., 2014). Alternatively, TOP1α could serve as a constitutive positive regulator of FLC, MAF4, and MAF5, whereas other upstream regulators of these genes may antagonize this provocative effect to suppress their expression in response to various flowering signals. Further identification of protein or genetic interacting partners of TOP1α will help to shed more light on the underlying mechanisms by which TOP1α promotes the expression of FLC, MAF4, and MAF5 to prevent precocious flowering in plants.

CONCLUSION
Our results demonstrate that TOP1α, an Arabidopsis type IB topoisomerase that targets single-strand DNA breaks, plays an important role in controlling the transition from vegetative to reproductive development in Arabidopsis by directly affecting the expression of FLC and its closest homologs, MAF4 and MAF5. TOP1α physically binds to the genomic regions of these genes and promotes the binding of RNA polymerase II complexes to their transcriptional start sites. These correlate with the changes in histone modifications but do not directly affect nucleosome occupancy at these loci. Therefore, TOP1α affects the recruitment of RNA polymerase II to the loci of key flowering repressors, thus facilitating their expression to prevent precocious flowering in Arabidopsis.
MATERIALS AND METHODS

Plant Materials and Growth Conditions

Arabidopsis (Arabidopsis thaliana) plants were grown on soil under long days (16 h of light/8 h of dark) or short days (8 h of light/16 h of dark). The mutants col-0, fld-3, fve-4, ColFR3722 (FRI-Col), top1a-10 (SALK_031364), top1a-7 (SALK_112625), and top1b (SALK_069847) are in the Col background, whereas gat-3 is in the Landsberg erecta background. Transgenic plants were generated through Agrobacterium tumefaciens-mediated transformation. Transformants containing gTOP1a-4HA, gTOP1a-GUS, and 35S:FLC were selected by Basta on soil.

Plasmid Construction

To construct gTOP1a-4HA, a 5.6-kb TOP1a genomic fragment was amplified with the Phusion Hot Start II High-Fidelity DNA polymerase (Finnzyme) using primers pTOP1a-Xhol-F and pTOP1a-Xmol-R. After enzyme digestion, the PCR fragment was cloned into a pENTR4-HA vector. The fused gTOP1a-4HA fragment was then recombined into the destination vector pGWB by LR recombination using Gateway LR Clonase II enzyme mix (Life Technologies). To construct gTOP1a-GUS, the same 5.6-kb TOP1a genomic fragment was cloned into pHY107 (Liu et al., 2007). To construct 35S:FLC, the FLC coding region was amplified and cloned into pGreen 0229-35S that contains 2× 35S promoters. Primer sequences used for plasmid construction are listed in Supplemental Table S1.

GUS Staining

GUS staining of gTOP1a-GUS transgenic plants was carried out as described previously (Tao et al., 2012). Seedlings were fixed in ice-cold 90% acetone for 20 min and then washed with the rinse solution (0.5 mM NaH2PO4, 0.5 mM Na2HPO4, 100 mM K2Fe(CN)6, and 100 mM K4Fe(CN)6) three times. After incubation, the solution was incubated at 37°C for 4 h. Chlorophyll in samples was removed by an ethanol series, and samples were observed subsequently with a light microscope.

Expression Analysis

Total RNA was extracted using the FavorPrep Plant Total RNA Mini Kit (Favorgen). cDNA was synthesized using iScript Reverse Transcription Supermix (Bio-Rad) according to the manufacturer’s instructions. Quantitative real-time PCR was performed in triplicate on three independently collected samples using the CFX384 Real-Time PCR Detection System (Bio-Rad) with iQ SYBR Green Supermix (Bio-Rad). The expression of TOP1a was normalized to At5g02780, At4g02780; At5g65060; and At1g65480, anti-RNA polymerase II CTD (Abcam), or anti-H3 (Abcam) antibody. Antibodies with their binding proteins and DNAs were recovered by Dynabeads (Life Technologies) according to the manufacturer’s instructions. After quantitative real-time PCR, DNA enrichment was calculated by the Percent Input Method (ChIP Analysis; Life Technologies). ChIP assays were repeated with three biological replicates. Primers used for ChIP assays are listed in Supplemental Table S1.

Measurement of Nucleosome Occupancy

The measurement of nucleosome occupancy at FLC, MAF4, and MAF5 loci was performed by both ChIP-quantitative PCR analysis using anti-h3 antibody (Abcam) and digestion of chromatin by micrococcal nuclease (Mnase; New England Biolabs). For Mnase digestion, extracted nuclei were resuspended in Mnase buffer (20 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM CaCl2, and 0.3 mM Suc). Half of the nucleus suspension solution was used as a control, and the other half was digested by Mnase (10,000 gel units per 200 μL) for 2 h at 37°C. After adding 1 μL of RNase (10 mg mL−1), the solution was incubated at 37°C for 1 h to digest RNA. After adding 10 μL of proteinase K (20 mg mL−1), the solution was further incubated at 45°C for another 1 h. Samples were subsequently treated by phenol/chloroform to remove protein. DNA was then precipitated with 2.5 volumes of 100% ethanol, one-tenth volume of 3 μL NaAc (pH 5.2), and 1 μL of glycogen (20 mg mL−1) overnight at −20°C. Quantitative real-time PCR was used subsequently to examine the DNA recovered from the sample after Mnase digestion as compared with that in the control without Mnase digestion. Measurement of nucleosome occupancy using both approaches was repeated with three biological replicates. Primers are listed in Supplemental Table S1.

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL databases under the following accession numbers: TOP1a, At5g55300; TOP1b, At5g55310; FLC, At5g10140; MAFI, At1g7080; MAF2, At5g6050; MAF3, At5g65060; MAF4, At5g65070; MAF5, At5g65080; CO, At5g15480; GA1, At4g02780; SOC1, At2g45660; FT, At1g65480; FLD, At3g10390; FVE, At2g19520; NRFB1, At4g35800; TUB1, At5g2690; ACT7, At5g09810; and MLI, At4g30870.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Loss of function of TOP1b does not affect flowering time.

Supplemental Figure S2. Expression of FLC and MAF1 to MAF5 in 18-d-old wild-type and top1a mutant plants grown under short days.

Supplemental Figure S3. Expression of other flowering-relevant genes in top1a mutants.

Supplemental Figure S4. Examination of TOP1a binding to various gene loci.

Supplemental Figure S5. Expression of other known upstream regulators of FLC in top1a mutants.

Supplemental Figure S6. TOP1a does not interact with Arabidopsis PR2C and PR1 components in yeast.

Supplemental Figure S7. Measurement of nucleosome occupancy at FLC, MAF4, and MAF5 loci.

Supplemental Table S1. List of primers used in this study.

ACKNOWLEDGMENTS

We thank J. Xu for providing top1a-10 and top1a-7 seeds from the Arabidopsis Biological Resource Center and members of the Hao Yu laboratory for critical reading of the article. Received October 14, 2016; accepted November 8, 2016; published November 11, 2016.
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