

***MGOUN1* Encodes an *Arabidopsis* Type IB DNA Topoisomerase Required in Stem Cell Regulation and to Maintain Developmentally Regulated Gene Silencing**^W

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Maintenance of stem cells in the *Arabidopsis thaliana* shoot meristem is regulated by signals from the underlying cells of the organizing center, provided through the transcription factor *WUSCHEL* (*WUS*). Here, we report the isolation of several independent mutants of *MGOUN1* (*MGO1*) as genetic suppressors of ectopic *WUS* activity and enhancers of stem cell defects in hypomorphic *wus* alleles. *mgo1* mutants have previously been reported to result in a delayed progression of meristem cells into differentiating organ primordia (Laufs et al., 1998). Genetic analyses indicate that *MGO1* functions together with *WUS* in stem cell maintenance at all stages of shoot and floral meristems. Synergistic interactions of *mgo1* with several chromatin mutants suggest that *MGO1* affects gene expression together with chromatin remodeling pathways. In addition, the expression states of developmentally regulated genes are randomly switched in *mgo1* in a mitotically inheritable way, indicating that *MGO1* stabilizes epigenetic states against stochastically occurring changes. Positional cloning revealed that *MGO1* encodes a putative type IB topoisomerase, which in animals and yeast has been shown to be required for regulation of DNA coiling during transcription and replication. The specific developmental defects in *mgo1* mutants link topoisomerase IB function in *Arabidopsis* to stable propagation of developmentally regulated gene expression.

INTRODUCTION

Unlike animals, plants form new organs throughout their life by the activity of the apical shoot and root meristems. The shoot meristem center harbors pluripotent stem cells that are maintained undifferentiated by signals from neighboring niche cells, named the organizing center (OC) (Mayer et al., 1998). Stem cell daughter cells that leave the niche are recruited into leaf and floral primordia at the periphery of the meristem and into the plant axis underneath the niche. Stem cells express the signal peptide *CLAVATA3* (*CLV3*), and their identity is maintained by expression of the homeodomain protein *WUSCHEL* (*WUS*) in the OC (Mayer et al., 1998; Schoof et al., 2000). Outside the niche, *CLV3* and *WUS* expression are turned off, and a cascade of genes governing organ formation becomes expressed.

During development, cells undergo changes in their gene expression program as they adopt specific fates and stably maintain their expression patterns once final fates have been reached. Genetic studies indicate a central role for epigenetic regulation of cell fate in plants and animals. In the shoot meristems of *Arabidopsis thaliana*, *FASCIATA1* (*FAS1*) and *FAS2* genes, which en-

code subunits of the Chromatin Assembly Factor-1 (CAF-1) complex, are required to maintain the organization of shoot and root meristems (Kaya et al., 2001), and the chromatin remodeling factor *SPLAYED* (*SYD*) is required for correct *WUS* expression in the OC (Kwon et al., 2005). Polycomb group proteins, as part of the Polycomb Repressive Complex 2 (PRC2), act in mitotically stable silencing of genes during cell differentiation and patterning. For example, expression of the floral regulator gene *AGAMOUS* (*AG*) is silenced outside flowers, and release of silencing in mutants of the PRC2 component *CURLY LEAF* (*CLF*) results in abnormal growth of leaves (Goodrich et al., 1997; Kim et al., 1998).

Previously, it has been shown that in *mgoun1* (*mgo1*) mutants, the transition from meristematic to organ cell fates appears to be delayed, resulting in a gradual increase in meristem size (Laufs et al., 1998). Here, we identified several independent *mgo1* alleles as enhancers of hypomorphic *wus* alleles, and our genetic studies indicate that *MGO1* functions together with *WUS* in stem cell regulation. Furthermore, expression patterns of several developmentally regulated genes are disturbed in *mgo1* mutants, and genetic analyses reveal that *MGO1* functions synergistically with chromatin regulators. Positional cloning revealed that *MGO1* encodes a putative *Arabidopsis* type IB topoisomerase, linking regulation of DNA topology to stabilizing developmental control of gene expression.

RESULTS

Genetic Modifiers of *wus* Mutants Are Allelic to the *mgo1* Mutant

We searched for mutations affecting shoot meristem development in two separate sensitized ethyl methanesulfonate (EMS)

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mutant screens in the Landsberg *erecta* (*Ler*) ecotype. First, from a screen for mutations enhancing the shoot meristem defects of the weak *wus-6/jam* allele (Hamada et al., 2000), hereafter named *wus-6* for brevity, we isolated the recessive mutant *a185*. In a second mutant screen, we isolated two independent mutants, *s3801* and *s15670*, which restored organ formation inhibited by ectopic *WUS* expression from a *35S:WUS-GR* transgene (Schoof et al., 2000) (Figures 1A to 1C). Complementation tests done by crossing siblings of plants with enhanced stem cell defects revealed that *a185*, *s3801*, and *s15670* were allelic to each other (data not shown).

After outcrossing to the wild type, we analyzed the *s3801* single mutant. The first leaves of *s3801* seedlings appeared about 2 d delayed and were pointed compared with the wild type (Figures 1D and 1E). Mutant shoot apices lacked the layered organization and contained slightly larger cells in comparison to the wild type (Figures 1F and 1G; see Supplemental Figure 1 online), suggesting that the cells of the shoot meristem have partially lost their undifferentiated state. In mature *s3801* embryos, the number of cells in the shoot apex was smaller than in the wild type (see Supplemental Figure 1 online; *s3801* 5.2 ± 0.2 , wild type 11.2 ± 0.7). Postembryonically, however, the shoot apex of the *s3801* mutant gradually enlarged and became

fragmented into multiple apices (Figures 2A to 2D). *WUS:GUS* (for β -glucuronidase) and *CLV3:GUS* reporter genes (Mayer et al., 1998; Fletcher et al., 1999) were expressed in a linear array of domains of the fragmented apex (Figures 2E to 2H), suggesting that each fragment contains a separate stem cell niche. Phyllotaxis and internode spacing was variable in *s3801* unlike the stereotypic arrangement in the wild type (Figures 2A and 2B). A similar phenotype has previously been described for the *mgo1* mutant, whose molecular nature was unknown (Laufs et al., 1998). Genetic crosses revealed that *s3801*, *s15670*, and *a185* were allelic to *mgo1* and therefore were renamed *mgo1-4*, *mgo1-5*, and *mgo1-6*, respectively. All novel alleles displayed very similar developmental defects; thus, here, we will focus on our analysis of mainly *mgo1-4*, which likely is a null allele (see below). In addition to shoot meristem defects, we also detected defective root meristem architecture in *mgo1* mutants. In contrast with the stereotypic architecture of wild-type roots, *mgo1* roots appeared disorganized and contained crushed cells that appeared to be dead (arrow, Figures 2I and 2J). To assess whether cell division patterns were affected, we monitored expression of the G2-M transition reporter gene *CYCB1;1:GUS* (de Almeida Engler et al., 1999). In 5-d-old *mgo1* seedlings, the number of root cells expressing *CYCB1;1:GUS* was strongly

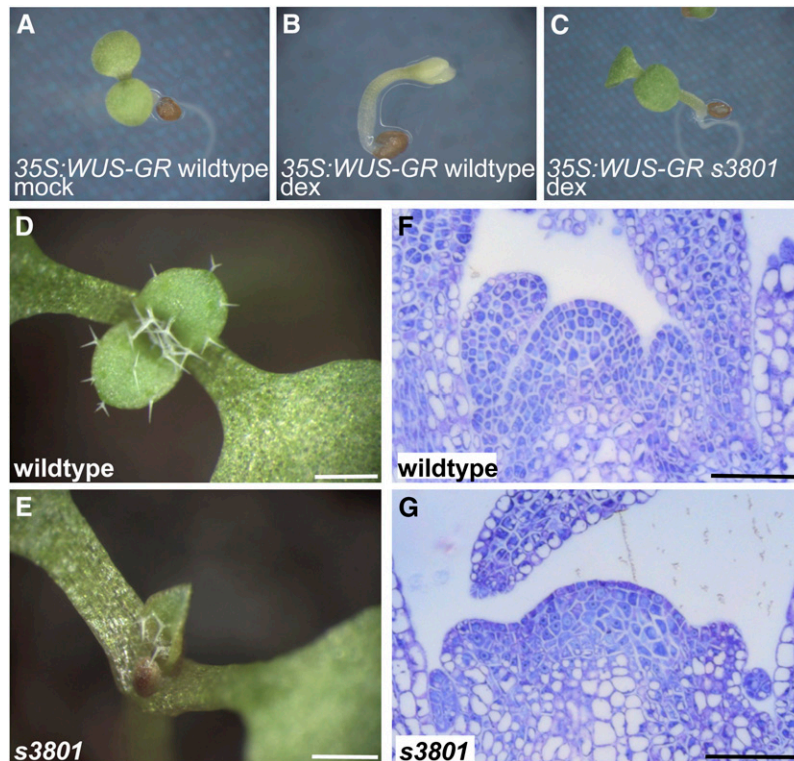


Figure 1. *s3801* Suppresses *35S:WUS-GR* Induced Inhibition of Differentiation.

(A) to (C) Induction of *WUS-GR* by dexamethasone (dex) results in arrest of differentiation in the wild type background (B), compare with mock treatment in (A). This developmental arrest is suppressed by the *s3801* mutation (C).

(D) and (E) Eight-day-old *s3801* seedlings (E) display retarded and pointed leaves in comparison to the *Ler* wild type (D).

(F) and (G) The vegetative *s3801* shoot meristem (G) is disorganized in comparison to the wild type (F).

Bars = 0.5 mm in (D) and (E) and 200 μ m in (F) and (G).

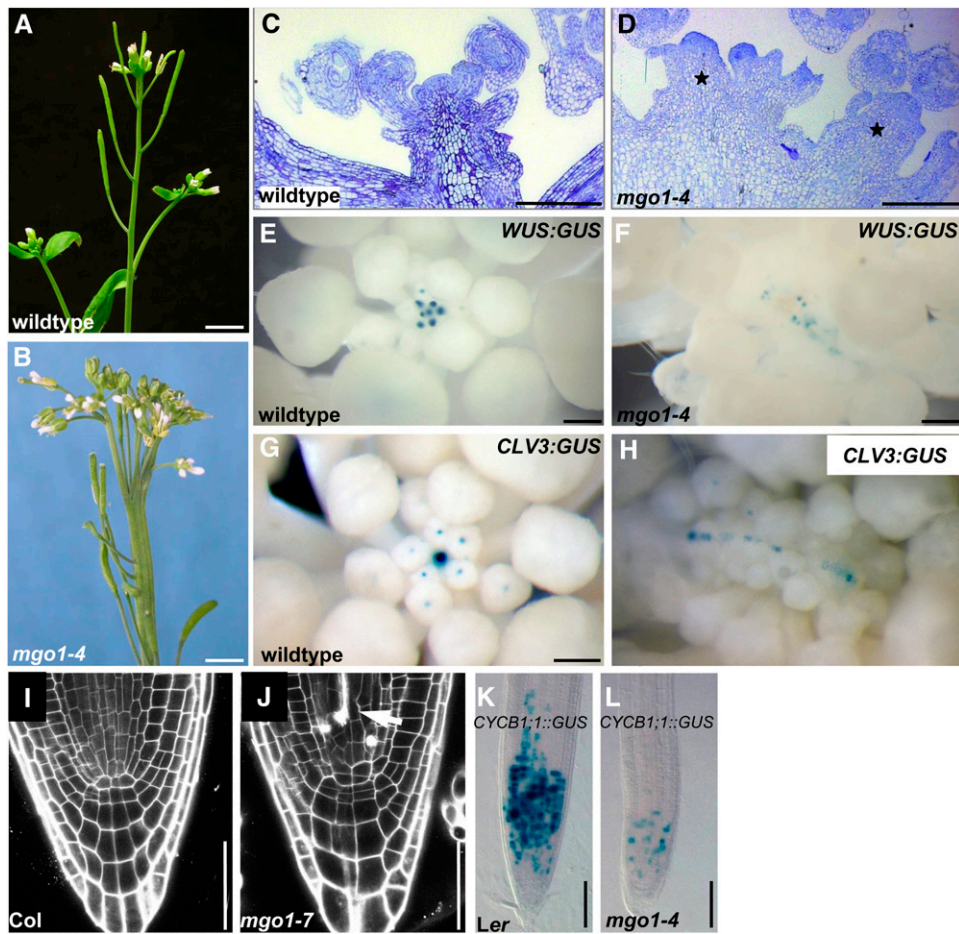


Figure 2. *mgo1* Meristem Phenotypes.

(A) and (B) Inflorescences of wild-type (A) and *mgo1-4* plants (B). The mutant displays fasciation and fragmentation of the apex. Phyllotaxis and internode spacing is variable in *mgo1-4* (B) in contrast with the stereotypic arrangement in the wild type (A).

(C) and (D) Inflorescence shoot meristems of wild-type (C) and *mgo1-4* plants (D). The *mgo1-4* inflorescence meristem displays multiple independent meristems (asterisks), each of which is generating floral primordia at its flanks.

(E) to (H) *WUS:GUS* and *CLV3:GUS* expression. In *mgo1-4* mutants [(F) and (H)], expression of both stem cell niche markers is fragmented into multiple domains arranged in a line, in contrast with the wild type [(E) and (G)].

(I) and (J) Confocal image of root meristems of 5-d-old seedlings of Columbia (Col) wild type (I) and *mgo1-7* (J) plants. Dead cells, which accumulate propidium iodide, are marked by an arrow.

(K) and (L) The number of cells expressing the *CYCB1:1:GUS* reporter is strongly reduced in 5-d-old primary roots of *mgo1-4* (L) versus *Ler* (K).

Bars = 5 mm in (A) and (B), 200 μ m in (C) and (D), 50 μ m in (E) to (H), 200 μ m in (I) and (J), and 100 μ m in (K) and (L).

reduced in comparison to the wild type (Figures 2K and 2L; see Supplemental Table 1 online), consistent with a smaller fraction of cells being in G2/M.

Thus, *mgo1* mutations affect cellular development and organization of both the shoot and the root meristem.

MGO1 Genetically Interacts with WUS in Stem Cell Regulation

To investigate in detail how in shoot meristem development *MGO1* and *WUS* genetically interact, we analyzed double mutants between *mgo1-4* and an allelic series of *wus* mutations.

wus-1 represents a null allele (Mayer et al., 1998), and mutant seedlings lack the primary shoot meristem (Figure 3C, Table 1) and contain partially differentiated cells instead (Laux et al., 1996). Postembryonically initiated adventitious shoot meristems terminated prematurely (Figure 3F) and only rarely gave rise to flowers (Table 1), which terminated after the formation of a single stamen (Figure 3J), compared with six stamens and a gynoecium in the wild type (Figure 6A). *wus-1 mgo1-4* double mutant shoot meristems terminated indistinguishably to *wus-1*. However, the frequency of *wus-1 mgo1-4* plants forming an adventitious shoot was strongly reduced compared with *wus-1* alone (Table 1, Fisher test, $P < 0.0001$). This indicates that in the absence of

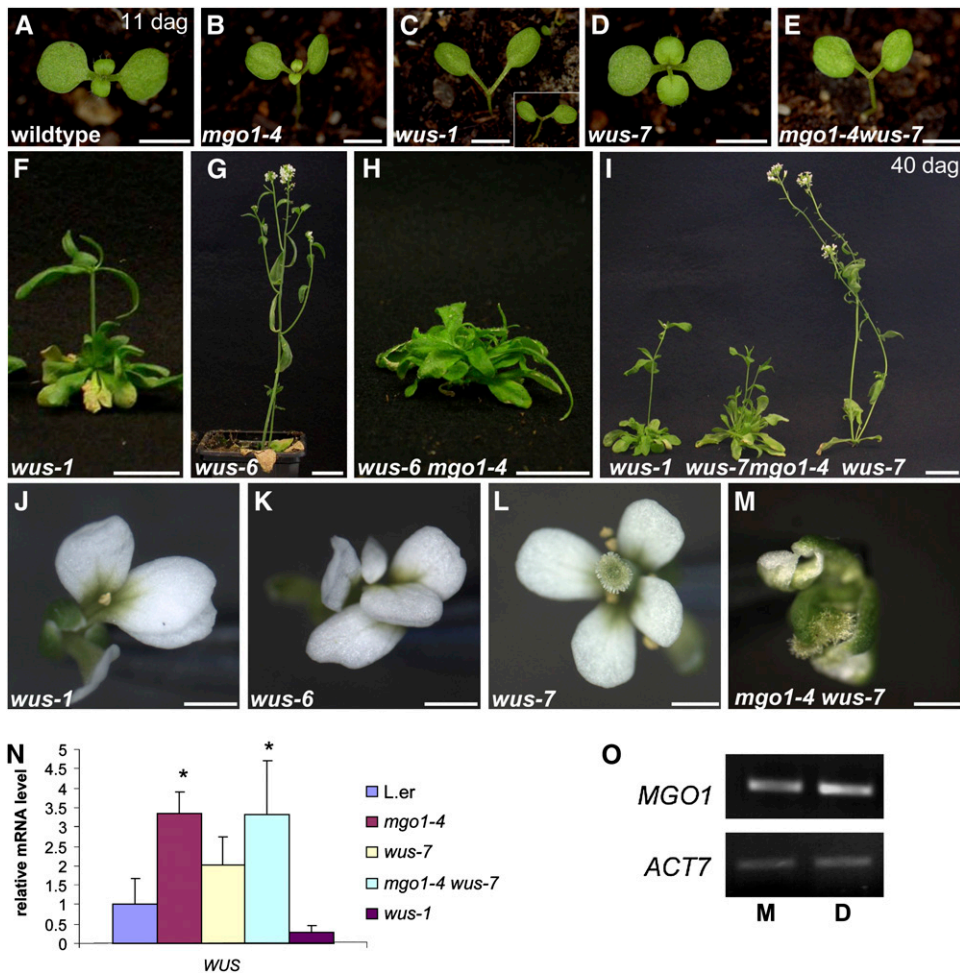


Figure 3. Genetic Interactions of *MGO1* and *WUS*.

(A) to (E) Eleven-day-old seedlings. In wild-type **(A)**, *mgo1-4* **(B)**, and *wus-7* **(D)** seedlings, the first leaves have been formed. In the severe *wus-1* mutant **(C)**, intermediate *wus-6* mutant (inset), and in the *mgo1-4 wus-7* double mutant **(E)**, the primary shoot meristem has terminated without leaf formation.

(F) and (G) Comparison of *wus-1* **(F)** and *wus-6* **(G)** 45-d-old plants. Adventitious shoots in *wus-1* terminated prematurely in an aerial rosette, whereas *wus-6* produces an indeterminate shoot with many flowers.

(H) *wus-6 mgo1-4* double mutants rarely formed adventitious shoots and never produced flowers.

(I) Comparison of *wus-1*, *wus-7*, and *mgo1-4 wus-7* 40-d-old plants. *mgo1-4 wus-7* double mutants formed determinate adventitious shoots with a few defective flowers similar to *wus-1*, whereas *wus-7* single mutants produced indeterminate shoot with many flowers.

(J) to (M) Flower phenotype of *wus-1* **(J)**, *wus-6* **(K)**, *wus-7* **(L)**, and *mgo1-4 wus-7* **(M)** plants.

(N) Quantitative RT-PCR experiment showing mRNA expression levels of *WUS*. Asterisk represents significant difference of *WUS* mRNA compared with the wild type ($P < 0.05$).

(O) No changes in *MGO1* transcript levels are detectable by RT-PCR after induction of 35S:WUS-GR plants with dexamethasone **(D)** compared with mock treated plants **(M)**. *ACT7* (*actin7*) transcript was used as control.

Bars = 2.5 mm in **(A) to (E)**, 2 cm in **(F) to (H)**, 1 cm in **(I)**, and 2 mm in **(J) to (M)**.

WUS function, *MGO1* does not have an appreciable effect on embryonic shoot development but is required for postembryonic initiation of adventitious shoot meristems.

In the intermediate *wus-6* allele, where the *WUS* expression level is strongly reduced (Hamada et al., 2000; see Supplemental Figure 2B online), the seedling shoot meristems (inset in Figure 3C, Table 1) and the floral meristems (Figures 3J and 3K) terminated indistinguishably from *wus-1*. In contrast with *wus-1*, however, the postembryonically initiated adventitious shoot

meristems in *wus-6* were indeterminate and gave rise to numerous flowers (Figure 3G, Table 1). This suggests that stem cell maintenance in inflorescence meristems is less sensitive to reduction of *WUS* activity than it is in seedling and floral meristems. Unlike in either single mutant, the inflorescence shoot meristems in *mgo1-4 wus-6* double mutants terminated prematurely (Table 1, Fisher test, $P < 0.0001$), thereby mimicking the null allele *wus-1*. This indicates that residual *WUS* activity in *wus-6* requires *MGO1* to maintain the inflorescence meristem. Similar

Table 1. Shoot Meristem Defects in *wus mgo1* Plants

Seedling Meristems			Inflorescence Shoots					
Genotype of Parent Plants	<i>n</i>	Terminated (%)	Genotype	<i>n</i>	None (%)	Det. (%)	Indet. (%)	No. of Flowers on Main Shoot
<i>mgo1-4</i>	19	0	<i>mgo1-4</i>	16	0	0	100	28.4 ± 1.7
<i>wus-1/+</i>	128	28	<i>wus-1</i>	25	0	100	0	2.5 ± 1.5
<i>mgo1-4 wus-1/+</i>	116	28	<i>mgo1-4 wus-1</i>	15	80	20	0	0
<i>wus-6/+</i>	361	24	<i>wus-6</i>	13	0	0	100	26.2 ± 4.3
<i>mgo1-4 wus-6/+</i>	237	28	<i>mgo1-4 wus-6</i>	30	56	44	0	0
<i>wus-7/+</i>	99	0	<i>wus-7</i>	15	0	0	100	35 ± 9.8
<i>mgo1-4 wus-7/+</i>	51	10	<i>mgo1-4 wus-7</i>	36	0	100	0	2.7 ± 1.8

Primary shoot meristems were analyzed in 11-d-old seedlings. Inflorescence shoots were examined at d 45. det., shoot terminated prematurely; indet., indeterminate shoot.

to *wus-1 mgo1-4*, the frequency of plants forming an adventitious shoot was strongly reduced in *mgo1-4 wus-6* (Figure 3H, Table 1, Fisher test, $P < 0.001$).

Since *wus-1* and *wus-6* already have strong stem cell defects in the seedling shoot meristem and in floral meristems, addressing whether *mgo1* could also enhance stem cell defects at these developmental stages was not feasible in combinations with these alleles. To resolve this question, we isolated a novel allele, named *wus-7*, from an EMS screen. *wus-7* carries a missense mutation in the homeodomain and represents the weakest *wus* allele known to us (see Supplemental Figure 2A online). In contrast with *wus-1* and *wus-6* single mutants, *wus-7* seedlings initially displayed a normal-looking shoot meristem that gave rise to three to four true leaves before it terminated (Figure 3D, Table 1). Subsequently established adventitious shoots grew indeterminately and formed many flowers (Figure 3I, Table 1). *wus-7* flowers generated more organs than *wus-1* and *wus-6*, and a fraction displayed even a complete set of organs, including six fertile stamens and a fruit (Figure 3L), which was not observed in *wus-1* or *wus-6*. Therefore, the residual *WUS* activity in *wus-7* appears to be sufficient for seedling shoot meristem formation, indeterminate inflorescence development, and normal floral meristem development. Notably, this allelic series of *wus* mutants reveals that *WUS* function is variably required for stem cell maintenance during development, with highest to lowest requirement in the maintenance of the seedling shoot meristem, embryonic meristem formation and floral meristems, maintenance of the inflorescence meristem, and postembryonic initiation of adventitious shoot meristems.

Unlike the both single mutants, *mgo1-4 wus-7* double mutant seedlings variably lacked the primary shoot meristem and any leaf primordia (Figure 3E, Table 1), phenocopying *wus-1* seedlings. One possible way to explain this finding is that in the double mutant, leaf primordia were initiated but were delayed in growth, consistent with the delayed leaf development in *mgo1-4* single mutants. However, histological analysis showed that no signs of leaf initiation were noticeable in *mgo1-4 wus-7*, thus phenocopying *wus-1* (see Supplemental Figure 3 online). During postembryonic development, double mutant adventitious inflorescence meristems terminated prematurely (Figure 3I, Table 1, Fisher test, $P < 0.005$), again indistinguishably from the *wus-1*

single mutant. Floral organ formation was also reduced in *mgo1-4 wus-7*, compared with *wus-7*, although not to the extent of *wus-1* flowers (Figure 3M). Thus, *mgo1-4* can also enhance hypomorphic *wus* stem cell defects in seedling and floral meristems. Notably, however, adventitious shoots were still initiated in all *wus-7 mgo1-4* seedlings, in contrast with the reduced frequency in *wus-1 mgo1-4* or *wus-6 mgo1-4* (Figure 3I, Table 1, Fisher test, $P < 0.0001$), indicating that the compromised *WUS* activity of *wus-7* is sufficient for postembryonic shoot meristem initiation even in the absence of *MGO1* function.

To address whether *mgo1-4* enhanced hypomorphic *wus* alleles because *MGO1* promoted *WUS* expression, we analyzed *WUS* mRNA levels in 4-d-old *mgo1-4* seedlings. However, *WUS* mRNA level was elevated in *mgo1-4* mutants, arguing that *MGO1* does not stimulate *WUS* expression (Figure 3N). Notably, *WUS* mRNA level was also increased in *wus-7* mutants (Figure 3N), consistent with a negative autoregulation as proposed by the *WUS-CLV3* feedback model (Schoof et al., 2000), an effect that is not visible in *wus-1*, where the apex cells have undergone differentiation and terminated expression of *WUS* and *CLV3* altogether. The suppression of phenotypic effects of *35S:WUS-GR* activity by *mgo1* mutations could also be explained by activation of *MGO1* expression through *WUS*. However, we find that *MGO1* mRNA levels were unaltered in induced *35S:WUS-GR* plants (Figure 3O). Therefore, the genetic interactions do not appear to be due to *MGO1* regulating *WUS* expression or vice versa.

Together, our data show that *WUS* and *MGO1* functions converge at a common process in stem cell maintenance at all stages of shoot and floral meristem development.

***MGO1* Acts Synergistically with Chromatin Remodeling Factors**

The defects in meristem and organ development of *mgo1* suggest that *MGO1* is involved in fundamental processes in development. Since the shoot and root meristem phenotypes in *mgo1-4* resembled those of the chromatin remodeling mutants *fas1-1* and *fas2-2* (Kaya et al., 2001), we asked whether chromatin regulation might be affected in *mgo1* mutants. Double mutants between *mgo1-4* and the putative null alleles *fas1-1* or

fas2-1 produced a large mass of apparently undifferentiated cells in place of the shoot meristem (see Supplemental Figure 4 online), similar to the previously reported double mutants between *fas* and the *mgo1-1* allele (Laufs et al., 1998). Being far more severe than the addition of the single mutant defects, these phenotypes suggested that *MGO1* and *FAS* genes affect the same downstream processes. In agreement with this, we found that *fas1-1* enhanced the defects in the *wus-7* allele in a similar way as *mgo1-4: wus-7 fas1-1* seedlings lacked a shoot meristem and either displayed an empty apex indistinguishable from the null allele *wus-1* or a central filament instead (Figures 4A to 4E; see Supplemental Table 2 online). *wus-7 fas1-1* plants were unable to form an indeterminate meristem and gave rise to small bushy plants (Figures 4F to 4I).

To investigate whether *MGO1* function is generally associated with chromatin regulation, we analyzed genetic interactions between *mgo1* and strong loss-of-function or null alleles of several chromatin remodeling mutants. The *PICKLE (PKL)* gene encodes a CHD3-type chromatin remodeling factor and mediates postembryonic repression of genes normally expressed in the embryo (Ogas et al., 1999; Rider et al., 2004). The *pk1-15* mutant (Eshed et al., 1999) looked normal at the seedling stage (Figure 5E) but displayed mild pleiotropic defects, such as reduced plant growth later in development (Figure 5H). In contrast with either single mutant, *pk1-15 mgo1-4* double mutants were extremely dwarfed (Figure 5F) and had a much more enlarged and fasciated shoot meristem (Figure 5G). By contrast, *pk1-15 wus-7* double mutants had an additive phenotype (see Supplemental Figure 5 online), indicating that *wus-7* was not generally enhanced by mutations in chromatin factors. The chromatin remodeling factor *SYD* promotes *WUS* expression and shoot apical meristem development (Wagner and Meyerowitz, 2002; Kwon et al., 2005). Sixteen-day-old *syd-2* seedlings looked largely normal but had reduced leaf size (Figure 5I). At later stages in development, *syd-2* mutants displayed reduced growth and upward curling leaves compared with the wild type (Figure 5L). By contrast, 16-d-old *mgo1-4 syd-2* double mutant seedlings had not formed any leaves (Figure 5J) and only later in development the shoot meristem gradually enlarged and gave rise to small and severely lobed leaves (Figure 5K). LIKE HETEROCHROMATIN PROTEIN 1 (*LHP1*) is involved in heterochromatin formation and has been proposed to maintain gene repression initiated by PRC2, in analogy to the function of PRC1 in animals (Gaudin et al., 2001; Kotake et al., 2003). *lhp1-3* (also referred to as *tf12-1*) mutant seedlings were strongly reduced in size and displayed downward curled leaves in comparison to the wild type (Figure 5M). *mgo1-7 lhp1-3* seedlings displayed severe defects with small, narrow leaves and shoot meristem arrest (Figure 5N). Seedlings mutant for the PRC2 component *CLF* (Goodrich et al., 1997) are smaller than the wild type and display small and upward curling leaves (Figure 5O). By contrast, *mgo1-4 clf-2* double mutants displayed a much more pronounced leaf curling than *clf-2* alone (Figure 5P), although *mgo1-4* single mutants do not show any leaf curling.

In summary, combinations of *mgo1* and all chromatin factor mutants tested displayed defects more severe than an expected additive phenotype. While we cannot exclude that *MGO1* and tested chromatin factors act in linear pathways, the use of strong

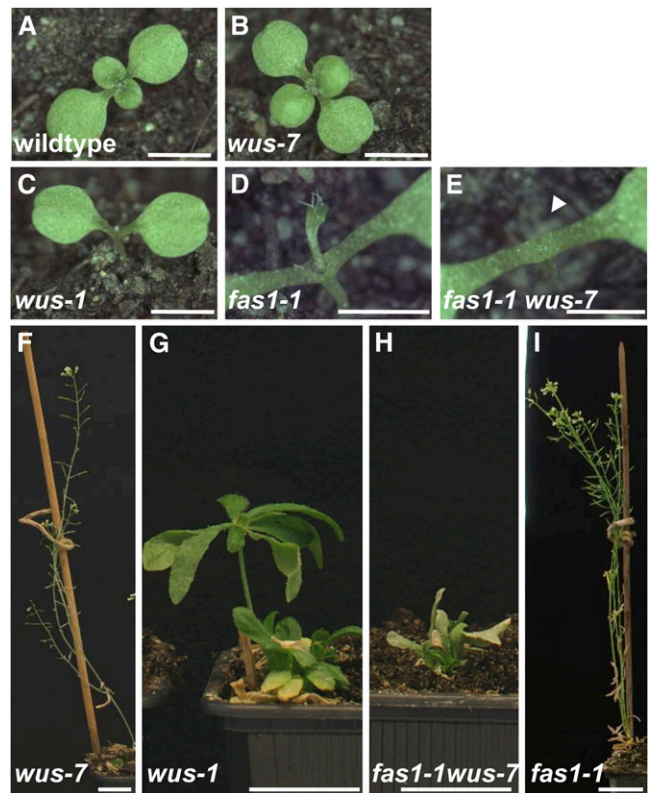


Figure 4. The *wus-7 fas1-1* Double Mutant.

(A) to (E) Phenotype of 10-d-old seedlings. In wild-type (A) and *wus-7* (B) seedlings, the first leaves have been formed. In *wus-1* (C), the primary shoot meristem has terminated without leaf formation. In *fas1-1* (D) seedlings, leaf formation is strongly delayed and leaves are malformed. *fas1-1 wus-7* (E) seedling displaying an empty apex (arrowhead) instead of a shoot meristem.

(F) to (I) Comparison of 45-d-old plants. Adventitious shoots in *wus-1* (G) terminated prematurely in an aerial rosette, whereas *wus-7* (F) and *fas1-1* (I) shoots are indeterminate. *fas1-1 wus-7* (H) plants terminated prematurely like *wus-1*.

Bars = 2.5 mm in (A) to (E) and 2 cm in (F) to (I).

loss-of-function or null alleles for all mutants analyzed suggests that *MGO1* and chromatin regulator functions converge at the same downstream processes.

***MGO1* Is Required to Maintain Developmentally Regulated Gene Repression**

Based on the observed genetic interactions, we hypothesized that *MGO1* affects a subset of the same genes that are targeted directly by chromatin remodeling factors. To test this, we analyzed expression of genes known to be direct targets of *CLF* (*PRC2*) regulation. Transcription of *AG*, which promotes carpel and stamen identity (Bowman et al., 1991; Drews et al., 1991; Liu and Meyerowitz, 1995), is normally repressed outside floral whorls 3 and 4 by a *CLF*-containing *PRC2* complex (Goodrich et al., 1997; Schubert et al., 2006). *mgo1-4* flowers displayed defects suggestive of ectopic *AG* activity (Bowman et al., 1991;

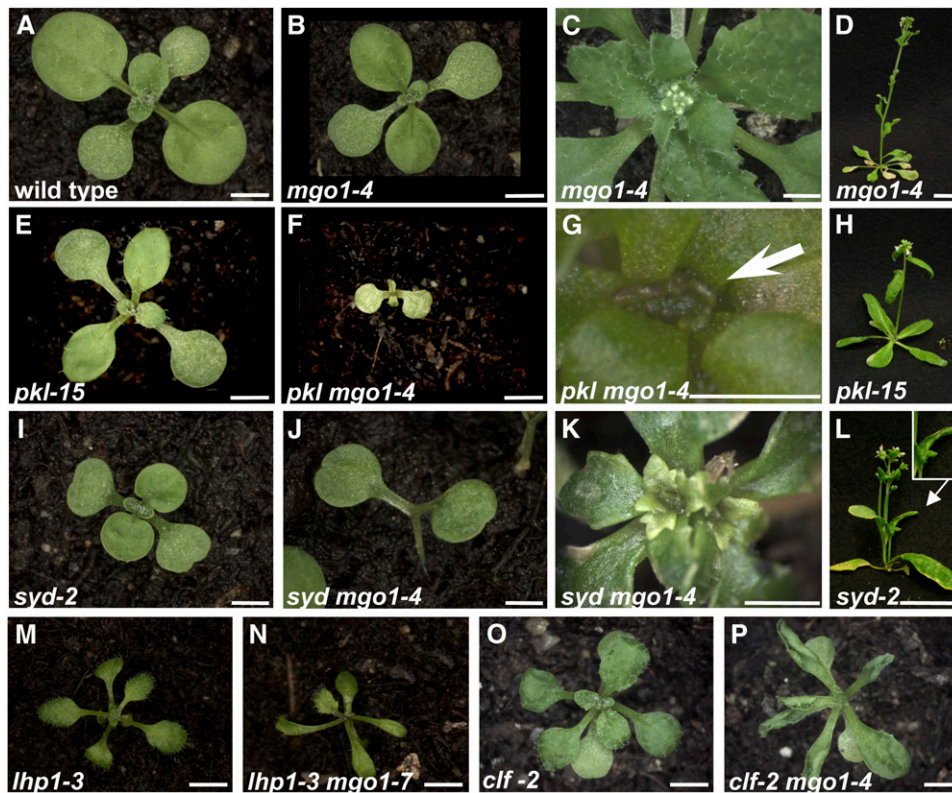


Figure 5. Combinations of *mgo1-4* with Chromatin Factor Mutants.

(A), (B), (E), (F), (I), and (J) Phenotype of 16-d-old seedlings of single and double mutants with the genotypes indicated.

(C), (D), (G), (H), (K), and (L) About 5-week-old single and double mutants.

(E) to (H) *pkl-15 mgo1-4* double seedlings are dwarfed plants (F) with sessile, foliage leaves, which later develop enlarged and misshapen shoot apices (arrow in [G]).

(I) to (K) *syd-2 mgo1-4* double mutant seedlings do not show primary leaves at 16 d (J), unlike each single mutant ([B] and [I]). At later stages, double mutants produce narrow, serrated leaves and enlarged shoot meristems (K).

(L) *syd-2* adult 40-d-old plant with curled leaf (arrow, inset).

(M) and (N) Nineteen-day-old *mgo1-7 lhp1-3* seedlings (N), in comparison with the *lhp1-3* single mutant (M).

(O) and (P) Phenotype of 19-d-old *mgo1-4 clf-2* double mutants (P): leaf curling is more severe than in either single mutant ([B] and [O]).

Bars = 2 mm in (A) to (C), (E), (F), (I), (J), and (Q) to (T), 2 cm in (D), (H), and (L), 0.5 mm in (G), and 1 mm in (K).

Drews et al., 1991; Liu and Meyerowitz, 1995), such as stigma-like protrusions on petals and ectopic ovules at the margins of sepals (Figure 6C) and multiple carpelloid organs (Figure 6D). These defects became progressively more severe as the plant matured (Figures 6B to 6D). Importantly, ectopic carpelloid tissue of the *mgo1-4* single mutant was suppressed by the *ag-1* mutation (Figure 6F) in *mgo1-4 ag-1* double mutants, consistent with these phenotypes being caused by ectopic activation of *AG* function. To investigate whether *AG* transcription is changed in *mgo1-4*, we monitored expression of a *pAG-I::GUS* reporter gene (Sieburth and Meyerowitz, 1997). In contrast with the wild type (Figures 6G, 6I, and 6K), in *mgo1-4* mutants, *pAG-I::GUS* was ectopically expressed in sectors of leaves (Figure 6H; 43% of *mgo1-4* seedlings; $n = 105$, Fisher test, $P < 0.001$), inflorescence stems (Figure 6J), and flowers (Figure 6L). Importantly, these sectors were at variable positions and of variable sizes, suggesting a stochastic origin. We detected six cases of revertant white

sectors that were completely encompassed by stained cells within a total of 75 *GUS* positive sectors, suggesting that reversion to the repressive state of the reporter gene also occurred in *mgo1-4* (Figure 6M). Thus, *MGO1* is required to stabilize expression states of the *AG* gene.

BREVIPEDICELLUS (*BP*) encodes a homeodomain protein involved in the development of the shoot meristem and the vasculature (Ori et al., 2000; Katz et al., 2004). In the *mgo1-4* mutant, we detected ectopic expression of a *BP::GUS* reporter gene in random patches of vascular cells of cotyledons and leaves (Figures 6N and 6O; 25/25 in *mgo1-4*, 0/93 in the wild type, Fisher test, $P < 0.0001$).

In contrast with these *PRC2* targets, we did not detect significant changes in expression levels in *mgo1-4* of the *L5 35S::GUS* transgene, which is repressed by transcriptional gene silencing (Morel et al., 2000; Probst et al., 2004; Ono et al., 2006), or of *TSI*, *MULE*, and *CACTA*-like repeats (Steimer et al., 2000; Takeda et al.,

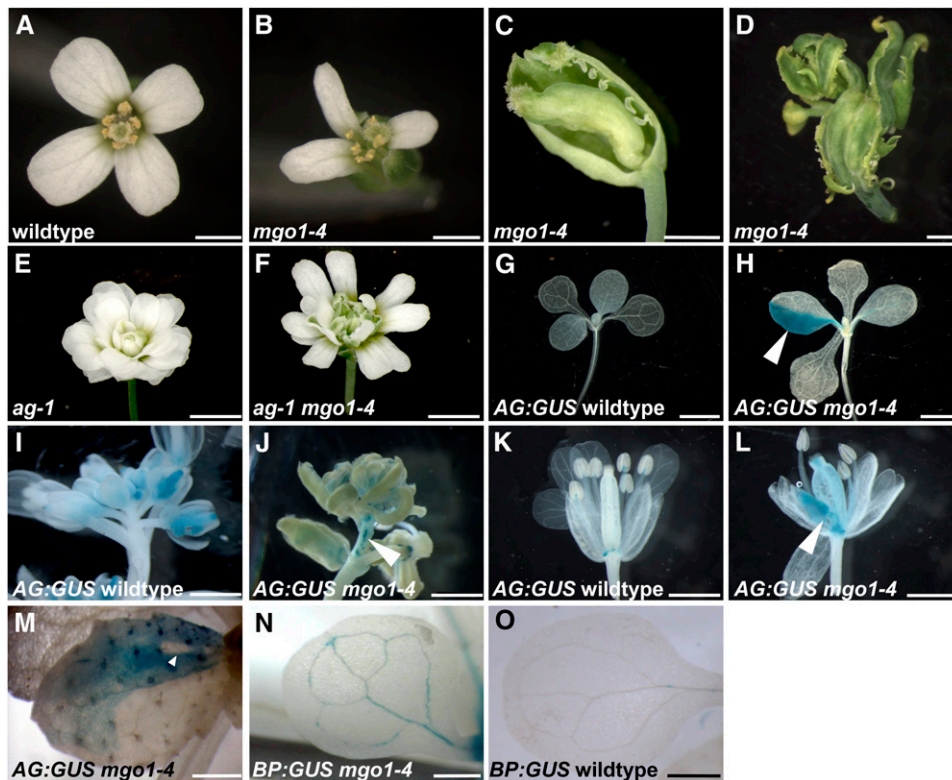


Figure 6. Deregulated Gene Expression in *mgo1*

(A) to (D) An early arising *mgo1-4* flower is shown in (B), a later arising flower with stigmatic tissue and ectopic ovules on outer whorl organs in (C), and a terminal *mgo1-4* flower in (D).

(E) and (F) Ectopic carpelloid tissue formation (cf. to [C] and [D]) is repressed in *ag-1 mgo1-4* (F) plants.

(G) to (M) Expression of *pAG::GUS* (blue) is ectopically activated in random sectors (arrowheads) in *mgo1-4* in contrast to the wild type in rosette leaves ([G] and [H]), inflorescences ([I] and [J]), and flowers ([K] and [L]). A revertant sector (arrowhead) is shown in (M).

(N) and (O) Expression of *pBP::GUS* (blue) is detected in cotyledonary vasculature and at the tips of the rosette leaves of *mgo1-4* (N) but not in the wild type (O).

Bars = 2 mm in (A) and (B), 1 mm in (C) and (D), 2 mm in (E) to (J), and 1 mm in (K) to (O).

2004), which are repressed mainly via DNA methylation in pericentromeric heterochromatin (see Supplemental Figure 6 online).

Taken together, these findings indicate that *MGO1* is required to maintain expression states of several, but not all, epigenetically regulated genes.

***MGO1* Encodes a Topoisomerase IB**

The *mgo1-4* mutation was mapped to a 64-kb region that spans the BACs MCO15 and MTE17 on chromosome 5. Sequencing candidate genes within this interval revealed a single gene (At5g55300) that carries a mutation in all *mgo1* alleles analyzed (Figure 7A). The reading frame encodes the predicted type IB topoisomerase *TOP1 α* (Kieber et al., 1992; Takahashi et al., 2002). The *mgo1-4* mutation results in the loss of most of the topoisomerase core domain and the catalytic C-terminal domain, which are highly conserved in eukaryotes, strongly suggesting that *mgo1-4* is a null allele (Figure 7A). *Arabidopsis* contains a second reading frame located in tandem to *MGO1*, which encodes the closely related type IB topoisomerase

TOP1 β . In contrast with the developmental defects observed in *mgo1-4*, a *top1 β* loss-of-function mutant isolated from the SALK T-DNA collection did not display any visible defects (see Supplemental Figure 7 online), consistent with previously published data of RNA interference-induced downregulation of *TOP1 β* (Takahashi et al., 2002). Thus, only *MGO1*, but not its homolog *TOP1 β* , is genetically indispensable for normal development.

To study the spatial pattern of *MGO1* transcript in shoot and floral meristems, we performed in situ hybridization experiments. *MGO1* mRNA was detected uniformly in inflorescence meristems and in all whorls until stage 3 of flower development (Figure 7B). Soon after sepal initiation, *MGO1* expression was downregulated in sepal primordia (Figure 7B) but remained expressed in petals, stamens, and carpel primordia, where it became further restricted to the inner side of carpels and eventually to the ovule primordia (Figures 7B and 7C). In mid-stage embryos, *MGO1* mRNA was also uniformly distributed (Figure 7F). Thus, *MGO1* expression appears to be ubiquitous and most abundant in actively dividing tissues.

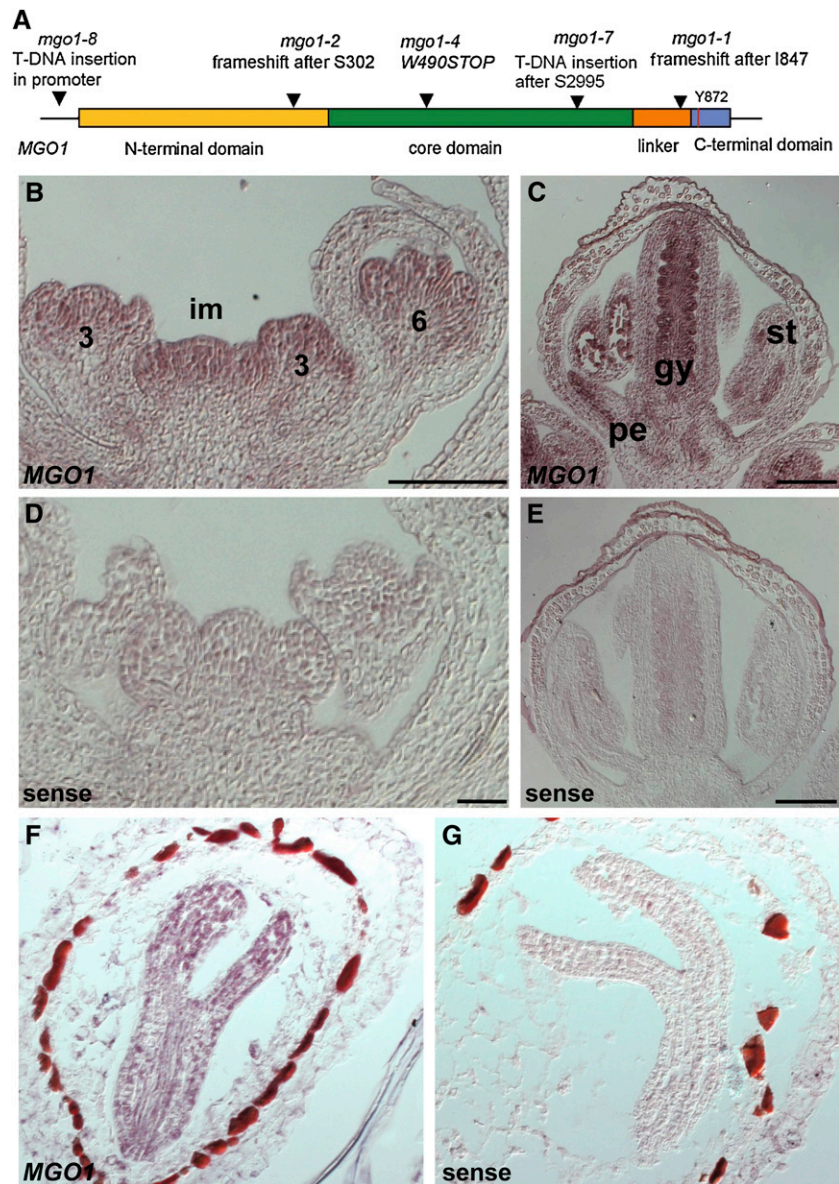


Figure 7. *MGO1* Gene Structure and Expression.

(A) Gene structure of *MGO1/TOP1 α* and identified mutations.

(B) to (E) Expression of *MGO1* examined by in situ hybridization on wild-type inflorescence tissues. *MGO1* transcript (brown-reddish color) preferentially accumulates in the inflorescence meristem (im) and in inner whorls of floral buds **(B)**, and in a stage 10 flower **(C)** in petals (pe), stamens (st), and inner margin of the gynoecium (gy). Sense controls are in **(D)** and **(E)**.

(F) and **(G)** *MGO1* transcript accumulates throughout mid-stage embryos **(F)**. Sense control is in **(G)**.

Bar = 100 μ m.

DISCUSSION

mgo1 mutants were first recognized for their delayed allocation of cells into organ primordia at the shoot meristem flanks, which results in a gradual enlargement of the meristem (Laufs et al., 1998). Here, we show that *MGO1* affects cellular decisions and gene expression states during development and genetically acts in cooperation with the transcription factor *WUS* and

chromatin regulators. We find that *mgo1* alleles carry lesions in the *Arabidopsis* type IB topoisomerase gene (*TOP1 α*) (Kieber et al., 1992; Takahashi et al., 2002), linking DNA topoisomerase function to developmental gene regulation.

Our results revealed that *WUS* function is differentially required for stem cell maintenance at different stages of meristem development and that at all stages *mgo1* caused premature termination of the shoot meristem in *wus* hypomorphs, contrary to the

enlargement of the shoot meristem in the *mgo1* single mutant. A plausible explanation for these apparently opposite effects of the *mgo1* mutation is that *MGO1* functions in two separate pathways in the shoot meristem: (1) stem cell maintenance in the center and (2) allocation of peripheral cells into organ primordia at the flanks of meristem. According to this model, in *mgo1* single mutants, shoot meristem formation is defective in the mature embryo, but subsequently the delayed exit of cells from the meristem determines the phenotype and the meristem gradually enlarges. By contrast, in combination with hypomorphic *wus* alleles, the loss of stem cells determines the phenotype and prevents meristem enlargement.

How could *mgo1* mutations enhance specific stem cell defects in hypomorphic *wus* alleles? One possibility is that *MGO1* promotes expression of *WUS*. However, this appears unlikely since we did not find reduced *WUS* mRNA levels in *mgo1* mutants. Furthermore, *mgo1* mutations suppressed the effects of ectopic *WUS* activity expressed from the 35S promoter. Vice versa, *WUS* activity seems not to affect *MGO1* mRNA levels in agreement with reported transcriptome data (Leibfried et al., 2005). Another possibility is that *mgo1* mutations directly affect the *WUS* protein, but the repression of adventitious shoot meristem formation in the null mutant *wus-1* by *mgo1-4* does not support this scenario. Therefore, our results argue for a model where *WUS* and *MGO1* functions converge at a common downstream process in stem cell regulation.

Topoisomerases transiently break one (type I) or both (type II) DNA strands to solve the topological problems associated with DNA replication and transcription (Champoux, 2001; Wang, 2002). Studies in yeast suggest that type IB topoisomerase is required for efficient chromatin assembly in mitotically cycling cells (Garinther and Schultz, 1997; Salceda et al., 2006), and a direct involvement in transcription as a positive or negative regulator has been discussed (Merino et al., 1993). It was previously shown that *WUS* functions as a transcriptional repressor of the cytokinin response regulators (Leibfried et al., 2005) and can interact with *TOPLESS* (Kieffer et al., 2006), which appears to mediate gene repression via histone H3 deacetylation (Long et al., 2006; Szemenyei et al., 2008). Thus, it is possible that *MGO1* is required for *WUS*-mediated gene repression via chromatin remodeling.

In addition to its effects on stem cell regulation, we find that *MGO1* is required to stabilize expression states of developmentally regulated genes against stochastic switches. The stochastic occurrence of ectopic *AG:GUS* expression in *mgo1-4* suggests that maintenance of chromatin marks is unstable in the absence of topoisomerase function, allowing occasional switches between on and off states (Ono et al., 2006; Henikoff, 2008). At least in the case of *AG*, once established, such a change of expression state appears to be passed on to the descendent cells during the majority of subsequent cell divisions but might be occasionally switched back to a repressed state as evidenced by revertant *AG:GUS* sectors. Our studies suggest that *MGO1* cooperates with chromatin regulators. Therefore, one plausible mechanism is that *MGO1* is part of the machinery required to copy chromatin marks during cell division, consistent with the involvement of topoisomerases in chromatin assembly during mitosis in yeast (Garinther and Schultz, 1997; Salceda et al., 2006) and a role of the replication machinery in cellular memory (McNairn and

Gilbert, 2003; Vermaak et al., 2003; Barrero et al., 2007; Yin et al., 2009). Supporting this model, mutations in the genes encoding the catalytic subunits of DNA polymerases α and ε result in similar phenotypes and genetic interactions as *mgo1* (Barrero et al., 2007; Yin et al., 2009).

Topoisomerase IB deficiencies in mouse, worm, and fly cause deleterious effects correlated to defective cell proliferation (Lee et al., 1993, 2001; Morham et al., 1996; Zhang et al., 2000). Similarly, reducing the activities of both *MGO1* and the closely related *TOP1 β* gene in *Arabidopsis* resulted in seedling lethality (Takahashi et al., 2002), indicating that these topoisomerases are redundantly necessary for survival. However, only *mgo1* single mutants display defects in specific developmental processes, indicating that either these processes are specifically sensitive to reduction of overall topoisomerase activity or that, in addition to more general functions overlapping with those of *TOP1 β* , *MGO1* might have a more specific role in stabilizing the expression state of a subset of genes. Future analysis will verify these models and address the underlying mechanism of *MGO1* action.

METHODS

Plant Materials and Growth Conditions

mgo1-4, *mgo1-5*, *mgo1-6*, and *wus-7* were isolated from EMS-mutagenized populations and were outcrossed to wild-type *Ler* plants three times before further analyses. The insertion allele *mgo1-7* (SALK_112625) in the Col background was identified from the SALK collection of T-DNA-tagged lines (Alonso et al., 2003). All other mutant alleles and transgenic lines used in this study are listed in Supplemental Table 3 online. Plant growth conditions were as previously described (Laux et al., 1996).

Mapping, Genetic Analysis, and PCR Genotyping

The *mgo1-4* mutation was localized by mapping recombination breakpoints in 923 progeny plants of a *mgo1-4* \times Col-0 cross, initially with simple sequence length polymorphism markers and later with PCR-based markers that were designed on the basis of published sequence information (Jander et al., 2002). The *mgo1-4* mutation resulted in a new *AluI* restriction site. Primers *mgo1-4* FOR (5'-CGGAGAAATTTCTGGAAT-GACTG-3') and *mgo1-4* REV (5'-CACACAGCTCCCATGGCTTAGTA-AAG-3') were used for PCR genotyping. *AluI* digest in the wild type results in two bands of 504 and 270 bp and in the mutant allele three bands (461, 270, and 43 bp). *syd-2* plants were genotyped using primer sequences kindly provided by Doris Wagner: *syd-2*_FOR (5'-GATTGC-TGTGGCTTCACTGGTCT-3') and *syd-2*_REV (5'-GTGATTTGATTA AAAA-CTTTGCCCTTCT-3'). Genotyping for presence of the T-DNA linked with *pk1-15* was performed using primers (5'-GAACTAATAACGTTCACTGA-AGGG-3') and (5'-TTAGGAATAAATCTTGCAACGG-3'). *ag-1* plants were genotyped using (5'-GGACAATTCTAACACCGGATC-3') and (5'-CTAT-CGTCTACCCATCAAAGC-3') primers. All reporter lines were crossed into respective mutant or transgenic lines, and expression analysis was performed in segregating F3 families. Genotyping for *wus-7* mutation was performed using primers *wus-7F* (5'-CCGACCAAGAAAGCGGCA-ACA-3') and *wus-7R* (5'-AGACGTTCTTGCCCTGAATCTTT-3') followed by *XmnI* digestion.

RT-PCR Analysis

Total RNA was extracted from the aboveground parts of seedlings or from inflorescence tissues using the RNeasy Plant mini kit (Qiagen). For normal

RT-PCR reaction, total RNA was reverse transcribed with SUPERScript III reverse transcriptase (Invitrogen) and oligo(dT) primer. Primers and PCR conditions used for RT-PCR in this study are listed in Supplemental Table 4 online. For quantitative RT-PCR, total RNA was reverse transcribed with SUPERScript III (Invitrogen). PCR was performed using LightCycler480 SYBR Green I Master kit (Roche) in the LightCycler480 machine (Roche) with primers listed in Supplemental Table 5 online. Three reference genes were chosen (Czechowski et al., 2005), and geNORM software was used to validate the reference genes prior to normalization. RNA extraction, reverse transcription, and primers used for analyzing the expression of heterochromatic genes were as previously described (Mathieu et al., 2005).

Microscopy

Confocal microscopy of propidium iodide-stained root tissues and mature embryos was done as described previously (Running et al., 1995; Sarkar et al., 2007) using the 543-nm argon laser of a Zeiss LSM510 microscope. The 4',6-diamidino-2-phenylindole staining of seedlings was performed as described previously (Hülkamp et al., 1994) using a UV laser and the Zeiss LSM510 microscope.

Histology

Preparation of histological sections from LR-White embedded material (Laux et al., 1996), GUS staining (Schoof et al., 2000), and in situ hybridization (Mayer et al., 1998) were previously described. For the *MGO1* riboprobe, *MGO1* was amplified from a *Ler* cDNA library using primers (5'-GAATTCATGGGACTGAAACAGTTTCAA-3') and (5'-CTC-GAGCTAACGGCGCAGAAATCTGTAC-3') and subcloned into pGEM-T (Promega) to yield PG3. An 888-bp N-terminal part of *MGO1* was excised from PG3 by *EcoRI-EcoRV* digest and subcloned into pBlue-script KS to yield PG14. For the antisense probe, PG14 was linearized with *Bam*HI and transcribed with T3 RNA polymerase (Promega) using a digoxigenin labeling kit (Roche Diagnostics); for the sense probe, PG14 was linearized with *Cla*I and transcribed with T7 RNA polymerase (Promega).

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under accession number At5g55300 (*MGO1/TOP1 α*). The Germplasm identification number from this article is SALK_112625 (*mgo1-7*).

Supplemental Data

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

- Supplemental Figure 1.** Embryo Shoot Apices of *mgo1* Mutants.
- Supplemental Figure 2.** Mutations in the *WUS* Gene.
- Supplemental Figure 3.** Comparison of Shoot Apices of *wus-1* and *wus-7 mgo1-4*.
- Supplemental Figure 4.** *mgo1-4 fas1-1* Double Mutant.
- Supplemental Figure 5.** *wus-7 pkl-15* Double Mutant.
- Supplemental Figure 6.** Heterochromatic Gene Expression in *mgo1* Mutants.
- Supplemental Figure 7.** Phenotype of the *top1 β* Mutant.
- Supplemental Table 1.** *CYCB1;1:GUS* Expression in *mgo1* Primary Roots (5 d).
- Supplemental Table 2.** Shoot Meristem Defects in *wus fas* Plants.

Supplemental Table 3. Plant Materials.

Supplemental Table 4. Sequences of Oligonucleotides Used for RT-PCR.

Supplemental Table 5. Sequences of Oligonucleotides Used for qRT-PCR.

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REFERENCES

- Alonso, J.M., et al. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**: 653–657.
- Barrero, J.M., Gonzalez-Bayon, R., del Pozo, J.C., Ponce, M.R., and Micol, J.L. (2007). INCURVATA2 encodes the catalytic subunit of DNA Polymerase alpha and interacts with genes involved in chromatin-mediated cellular memory in *Arabidopsis thaliana*. *Plant Cell* **19**: 2822–2838.
- Bowman, J.L., Drews, G.N., and Meyerowitz, E.M. (1991). Expression of the *Arabidopsis* floral homeotic gene AGAMOUS is restricted to specific cell types late in flower development. *Plant Cell* **3**: 749–758.
- Champoux, J.J. (2001). DNA topoisomerases: Structure, function, and mechanism. *Annu. Rev. Biochem.* **70**: 369–413.
- Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K., and Scheible, W.-R. (2005). Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiol.* **139**: 5–17.
- de Almeida Engler, J., De Vleeschauwer, V., Burssens, S., Celenza, J.L., Jr., Inzé, D., Van Montagu, M., Engler, G., and Gheysen, G. (1999). Molecular markers and cell cycle inhibitors show the importance of cell cycle progression in nematode-induced galls and syncytia. *Plant Cell* **11**: 793–808.
- Drews, G.N., Bowman, J.L., and Meyerowitz, E.M. (1991). Negative regulation of the *Arabidopsis* homeotic gene AGAMOUS by the APETALA2 product. *Cell* **65**: 991–1002.
- Eshed, Y., Baum, S.F., and Bowman, J.L. (1999). Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis*. *Cell* **99**: 199–209.
- Fletcher, J.C., Brand, U., Running, M.P., Simon, R., and Meyerowitz, E.M. (1999). Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems. *Science* **283**: 1911–1914.
- Garinther, W.I., and Schultz, M.C. (1997). Topoisomerase function during replication-independent chromatin assembly in yeast. *Mol. Cell. Biol.* **17**: 3520–3526.
- Gaudin, V., Libault, M., Pouteau, S., Juul, T., Zhao, G., Lefebvre, D., and Grandjean, O. (2001). Mutations in LIKE HETEROCHROMATIN PROTEIN 1 affect flowering time and plant architecture in *Arabidopsis*. *Development* **128**: 4847–4858.

- Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E., and Coupland, G. (1997). A Polycomb-group gene regulates homeotic gene expression in Arabidopsis. *Nature* **385**: 44–51.
- Hamada, S., Onouchi, H., Tanaka, H., Kudo, M., Liu, Y.G., Shibata, D., MacHida, C., and Machida, Y. (2000). Mutations in the WUSCHEL gene of *Arabidopsis thaliana* result in the development of shoots without juvenile leaves. *Plant J.* **24**: 91–101.
- Henikoff, S. (2008). Nucleosome destabilization in the epigenetic regulation of gene expression. *Nat. Rev. Genet.* **9**: 15–26.
- Hülkamp, M., Misra, S., and Jürgens, G. (1994). Genetic dissection of trichome cell development in Arabidopsis. *Cell* **76**: 555–566.
- Jander, G., Norris, S.R., Rounsley, S.D., Bush, D.F., Levin, I.M., and Last, R.L. (2002). Arabidopsis map-based cloning in the post-genome era. *Plant Physiol.* **129**: 440–450.
- Katz, A., Oliva, M., Mosquna, A., Hakim, O., and Ohad, N. (2004). FIE and CURLY LEAF polycomb proteins interact in the regulation of homeobox gene expression during sporophyte development. *Plant J.* **37**: 707–719.
- Kaya, H., Shibahara, K.I., Taoka, K.I., Iwabuchi, M., Stillman, B., and Araki, T. (2001). FASCIATA genes for chromatin assembly factor-1 in Arabidopsis maintain the cellular organization of apical meristems. *Cell* **104**: 131–142.
- Kieber, J.J., Tissier, A.F., and Signer, E.R. (1992). Cloning and characterization of an *Arabidopsis thaliana* topoisomerase I gene. *Plant Physiol.* **99**: 1493–1501.
- Kieffer, M., Stern, Y., Cook, H., Clerici, E., Maulbetsch, C., Laux, T., and Davies, B. (2006). Analysis of the transcription factor WUSCHEL and its functional homologue in *Antirrhinum* reveals a potential mechanism for their roles in meristem maintenance. *Plant Cell* **18**: 560–573.
- Kim, G.-T., Tsukaya, H., and Uchimiya, H. (1998). The CURLY LEAF gene controls both division and elongation of cells during the expansion of the leaf blade in *Arabidopsis thaliana*. *Planta* **206**: 175–183.
- Kotake, T., Takada, S., Nakahigashi, K., Ohto, M., and Goto, K. (2003). Arabidopsis TERMINAL FLOWER 2 gene encodes a heterochromatin protein 1 homolog and represses both FLOWERING LOCUS T to regulate flowering time and several floral homeotic genes. *Plant Cell Physiol.* **44**: 555–564.
- Kwon, C.S., Chen, C., and Wagner, D. (2005). WUSCHEL is a primary target for transcriptional regulation by SPLAYED in dynamic control of stem cell fate in Arabidopsis. *Genes Dev.* **19**: 992–1003.
- Laufs, P., Dockx, J., Kronenberger, J., and Traas, J. (1998). MGOUN1 and MGOUN2: Two genes required for primordium initiation at the shoot apical and floral meristems in *Arabidopsis thaliana*. *Development* **125**: 1253–1260.
- Laux, T., Mayer, K.F., Berger, J., and Jürgens, G. (1996). The WUSCHEL gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* **122**: 87–96.
- Lee, M.H., Park, H., Shim, G., Lee, J., and Koo, H.S. (2001). Regulation of gene expression, cellular localization, and in vivo function of *Caenorhabditis elegans* DNA topoisomerase I. *Genes Cells* **6**: 303–312.
- Lee, M.P., Brown, S.D., Chen, A., and Hsieh, T.S. (1993). DNA topoisomerase I is essential in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **90**: 6656–6660.
- Leibfried, A., To, J.P., Busch, W., Stehling, S., Kehle, A., Demar, M., Kieber, J.J., and Lohmann, J.U. (2005). WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* **438**: 1172–1175.
- Liu, Z., and Meyerowitz, E.M. (1995). LEUNIG regulates AGAMOUS expression in Arabidopsis flowers. *Development* **121**: 975–991.
- Long, J.A., Ohno, C., Smith, Z.R., and Meyerowitz, E.M. (2006). TOPLESS regulates apical embryonic fate in Arabidopsis. *Science* **312**: 1520–1523.
- Mathieu, O., Probst, A.V., and Paszkowski, J. (2005). Distinct regulation of histone H3 methylation at lysines 27 and 9 by CpG methylation in Arabidopsis. *EMBO J.* **24**: 2783–2791.
- Mayer, K.F., Schoof, H., Haecker, A., Lenhard, M., Jürgens, G., and Laux, T. (1998). Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* **95**: 805–815.
- McNairn, A.J., and Gilbert, D.M. (2003). Epigenomic replication: Linking epigenetics to DNA replication. *Bioessays* **25**: 647–656.
- Merino, A., Madden, K.R., Lane, W.S., Champoux, J.J., and Reinberg, D. (1993). DNA topoisomerase I is involved in both repression and activation of transcription. *Nature* **365**: 227–232.
- Morel, J.B., Mourrain, P., Beclin, C., and Vaucheret, H. (2000). DNA methylation and chromatin structure affect transcriptional and post-transcriptional transgene silencing in Arabidopsis. *Curr. Biol.* **10**: 1591–1594.
- Morham, S.G., Kluckman, K.D., Voulomanos, N., and Smithies, O. (1996). Targeted disruption of the mouse topoisomerase I gene by camptothecin selection. *Mol. Cell. Biol.* **16**: 6804–6809.
- Ogas, J., Kaufmann, S., Henderson, J., and Somerville, C. (1999). PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **96**: 13839–13844.
- Ono, T., Kaya, H., Takeda, S., Abe, M., Ogawa, Y., Kato, M., Kakutani, T., Mittelsten Scheid, O., Araki, T., and Shibahara, K. (2006). Chromatin assembly factor 1 ensures the stable maintenance of silent chromatin states in Arabidopsis. *Genes Cells* **11**: 153–162.
- Ori, N., Eshed, Y., Chuck, G., Bowman, J.L., and Hake, S. (2000). Mechanisms that control knox gene expression in the Arabidopsis shoot. *Development* **127**: 5523–5532.
- Probst, A.V., Fagard, M., Proux, F., Mourrain, P., Boutet, S., Earley, K., Lawrence, R.J., Pikaard, C.S., Murfett, J., Furner, I., Vaucheret, H., and Mittelsten Scheid, O. (2004). Arabidopsis histone deacetylase HDA6 is required for maintenance of transcriptional gene silencing and determines nuclear organization of rDNA repeats. *Plant Cell* **16**: 1021–1034.
- Rider, S.D., Jr., Hemm, M.R., Hostetler, H.A., Li, H.C., Chapple, C., and Ogas, J. (2004). Metabolic profiling of the Arabidopsis pkl mutant reveals selective derepression of embryonic traits. *Planta* **219**: 489–499.
- Running, M.P., Clark, S.E., and Meyerowitz, E.M. (1995). Confocal microscopy of the shoot apex. In *Methods in Cell Biology: Plant Cell Biology*, D.W. Galbraith, D.P. Burque, and H.J. Bohnert, eds (San Diego, CA: Academic Press), pp. 215–227.
- Salceda, J., Fernandez, X., and Roca, J. (2006). Topoisomerase II, not topoisomerase I, is the proficient relaxase of nucleosomal DNA. *EMBO J.* **25**: 2575–2583.
- Sarkar, A., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., Scheres, B., Heidstra, R., and Laux, T. (2007). Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* **446**: 811–814.
- Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F., Jürgens, G., and Laux, T. (2000). The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* **100**: 635–644.
- Schubert, D., Primavesi, L., Bishopp, A., Roberts, G., Doonan, J., Jenwein, T., and Goodrich, J. (2006). Silencing by plant Polycomb-group genes requires dispersed trimethylation of histone H3 at lysine 27. *EMBO J.* **25**: 4638–4649.
- Sieburth, L., and Meyerowitz, E.M. (1997). Molecular dissection of the AGAMOUS control region shows that cis elements for spatial regulation are located intragenetically. *Plant Cell* **9**: 355–365.
- Steimer, A., Amedeo, P., Afsar, K., Frasz, P., Mittelsten Scheid, O.,

- and Paszkowski, J. (2000). Endogenous targets of transcriptional gene silencing in *Arabidopsis*. *Plant Cell* **12**: 1165–1178.
- Szemenyei, H., Hannon, M., and Long, J.A. (2008). TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. *Science* **319**: 1384–1386.
- Takahashi, T., Matsuhara, S., Abe, M., and Komeda, Y. (2002). Disruption of a DNA topoisomerase I gene affects morphogenesis in *Arabidopsis*. *Plant Cell* **14**: 2085–2093.
- Takeda, S., Tadele, Z., Hofmann, I., Probst, A.V., Angelis, K.J., Kaya, H., Araki, T., Mengiste, T., Mittelsten Scheid, O., Shibahara, K., Scheel, D., and Paszkowski, J. (2004). BRU1, a novel link between responses to DNA damage and epigenetic gene silencing in *Arabidopsis*. *Genes Dev.* **18**: 782–793.
- Vermaak, D., Ahmad, K., and Henikoff, S. (2003). Maintenance of chromatin states: An open-and-shut case. *Curr. Opin. Cell Biol.* **15**: 266–274.
- Wagner, D., and Meyerowitz, E.M. (2002). SPLAYED, a novel SWI/SNF ATPase homolog, controls reproductive development in *Arabidopsis*. *Curr. Biol.* **12**: 85–94.
- Wang, J.C. (2002). Cellular roles of DNA topoisomerases: A molecular perspective. *Nat. Rev. Mol. Cell Biol.* **3**: 430–440.
- Yin, H., Zhang, X., Liu, J., Wang, Y., He, J., Yang, T., Hong, X., Yang, Q., and Gong, Z. (2009). Epigenetic regulation, somatic homologous recombination, and abscisic acid signaling are influenced by DNA polymerase {epsilon} mutation in *Arabidopsis*. *Plant Cell* **21**: 386–402.
- Zhang, C.X., Chen, A.D., Gettel, N.J., and Hsieh, T.S. (2000). Essential functions of DNA topoisomerase I in *Drosophila melanogaster*. *Dev. Biol.* **222**: 27–40.