

The *ULTRAPETALA1* Gene Functions Early in Arabidopsis Development to Restrict Shoot Apical Meristem Activity and Acts Through *WUSCHEL* to Regulate Floral Meristem Determinacy

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Manuscript received March 15, 2004
Accepted for publication April 21, 2004

ABSTRACT

Shoot and floral meristem activity in higher plants is controlled by complex signaling networks consisting of positive and negative regulators. The Arabidopsis *ULTRAPETALA1* (*ULTI*) gene has been shown to act as a negative regulator of meristem cell accumulation in inflorescence and floral meristems, as loss-of-function *ult1* mutations cause inflorescence meristem enlargement, the production of extra flowers and floral organs, and a decrease in floral meristem determinacy. To investigate whether *ULTI* functions in known meristem regulatory pathways, we generated double mutants between *ult1* alleles and null alleles of the meristem-promoting genes *SHOOTMERISTEMLESS* (*STM*) and *WUSCHEL* (*WUS*). We found that, although the *ult1* alleles have no detectable embryonic or vegetative phenotypes, *ult1* mutations restored extensive organ-forming capability to *stm* null mutants after germination and increased leaf and floral organ production in *stm* partial loss-of-function mutants. Mutations in *ULTI* also partially suppressed the *wus* shoot and floral meristem phenotypes. However, *wus* was epistatic to *ult1* in the center of the flower, and *WUS* transcriptional repression was delayed in *ult1* floral meristems. Our results show that during the majority of the Arabidopsis life cycle, *ULTI* acts oppositely to *STM* and *WUS* in maintaining meristem activity and functions in a separate genetic pathway. However, *ULTI* negatively regulates *WUS* to establish floral meristem determinacy, acting through the *WUS-AG* temporal feedback loop.

HIGHER plants continuously produce organs such as leaves and flowers from small groups of cells at their growing tips, called apical meristems. During the development of *Arabidopsis thaliana*, the shoot apical meristem (SAM) provides all of the cells for above-ground organ formation while simultaneously maintaining a reservoir of pluripotent stem cells (STEEVES and SUSSEX 1989). The stem cell population resides at the very apex of the meristem and replenishes those cells that are lost during organogenesis on the meristem flanks. The SAM forms during embryonic development, but generates the vast majority of the lateral organs after germination. The SAM generates leaves during the vegetative phase of development, followed by stem tissue, axillary meristems, and an indeterminate number of flowers during the reproductive phase. Flowers arise from floral meristems, which sequentially produce sepals, petals, stamens, and carpels in a whorled pattern from the outside to the inside of the flower. Unlike SAMs, floral meristems are determinate structures that

terminate stem cell activity at the time of carpel formation.

Overlapping networks of meristem-promoting and meristem-restricting factors regulate shoot apical and floral meristem activity during Arabidopsis development. One key meristem-promoting factor is *SHOOTMERISTEMLESS* (*STM*). Plants carrying strong *stm* alleles fail to maintain a functional SAM during embryogenesis (BARTON and POETHIG 1993), while plants carrying weaker *stm* alleles have reduced shoot and floral meristem function (CLARK *et al.* 1996). *STM* encodes a *Knotted1-like homeobox* (*KNOX*) gene that is expressed only in shoot and floral meristem cells (LONG *et al.* 1996). *STM* activity prevents SAM cells from undergoing differentiation by restricting the expression of the *ASYMMETRIC LEAVES1* (*ASI*) and *AS2* genes to organ primordia, thereby preventing the inappropriate development of leaves across the shoot apex (BYRNE *et al.* 2000, 2002). Thus, *STM* provides an environment in which stem cell derivatives can become amplified to the appropriate extent prior to their incorporation into organ primordia.

The homeodomain transcription factor *WUSCHEL* (*WUS*; MAYER *et al.* 1998) maintains stem cell identity as part of a spatial negative feedback loop that functions in both shoot and floral meristems. *WUS* is expressed in a small group of cells in the interior of these meristems (MAYER *et al.* 1998), called the organizing center, where

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it is required to specify the overlying cells as stem cells (SCHOOF *et al.* 2000). In floral meristems, *WUS* is also required to induce the expression of its own repressor, *AGAMOUS* (*AG*; LENHARD *et al.* 2001; LOHMANN *et al.* 2001). Repression of *WUS* by *AG* is necessary to terminate stem cell activity at the appropriate time during flower development to permit the cells in the center of the flower to differentiate into carpels.

The stem-cell-promoting activity of *WUS* is modulated by the *CLAVATA* (*CLV*) signaling pathway. The function of the *CLV* pathway is to restrict excess stem cell accumulation by limiting the size of the *WUS* expression domain (BRAND *et al.* 2000; SCHOOF *et al.* 2000). The *CLV3* gene is expressed in the stem cell population of shoot and floral meristems and encodes a small, secreted signaling molecule (FLETCHER *et al.* 1999; ROJO *et al.* 2002). *CLV3* protein spreads through the extracellular space to the interior regions of the meristems, where it is proposed to interact with a receptor complex (TROTOCHAUD *et al.* 1999) consisting of the leucine-rich-repeat (LRR) receptor kinase *CLV1* (CLARK *et al.* 1997) and the LRR receptor-like protein *CLV2* (JEONG *et al.* 1999). *CLV3* represses *WUS* transcription in the stem cells and their lateral neighbors; however, the movement of *CLV3* protein into the meristem interior is limited by *CLV1*, allowing *WUS* to be transcribed in the organizing center (LENHARD and LAUX 2003).

An additional negative regulator of meristem cell accumulation is the *ULTRAPETALA1* (*ULT1*) gene. *ULT1* encodes a novel cysteine-rich protein with a B box-like domain and is expressed throughout shoot and floral meristems and in developing stamens and carpels (C. CARLES, D. CHOFFNES-INADA, K. REVILLE, K. LERTPIRIY-APONG and J. FLETCHER, unpublished results). The loss-of-function *ult1-1* and *ult1-2* mutations cause the reproductive (inflorescence) meristems to produce more floral meristems than normal and the floral meristems to produce extra organs (FLETCHER 2001). These phenotypes correlate with an increase in the size of *ult1* inflorescence and floral meristems and an enlargement of the *CLV1* expression domain in the interior of the *ult1* inflorescence meristem. *ult1* mutations also lead to the partial loss of floral meristem determinacy, such that supernumerary whorls of carpels, stamens, and/or undifferentiated tissue are observed in the center of *ult1* gynoecia.

The relationship between *ULT1* and the *CLV* loci, all of which negatively regulate shoot and floral meristem cell accumulation, was investigated using genetic analysis (FLETCHER 2001). Double mutants generated between *ult1* alleles and strong *clv1* or *clv3* alleles showed synergistic effects on inflorescence meristem size, indicating that these genes have overlapping functions in controlling SAM growth but act in separate genetic pathways. In addition, the central tissue of some *ult1 clv1* and *ult1 clv3* double-mutant flowers developed into entirely new inflorescence meristems, revealing that *ULT1* and

the *CLV* genes also interact to regulate floral meristem determinacy. One point at which the *ULT1* pathway and the *CLV* pathway might intersect is at the level of *WUS* regulation, as limiting *WUS* activity is essential for both proper SAM maintenance and floral meristem determinacy.

ULT1 acts to restrict shoot and floral meristem cell accumulation, while *STM* and *WUS* both function to promote meristem activity. To further investigate the role of *ULT1* in meristem growth control, we have analyzed the interactions between *ULT1* and the *STM* and *WUS* genes at the genetic and molecular levels. We find that *ULT1* functions in a genetic pathway separate from *STM* and *WUS* in restricting shoot and floral meristem size, but that *ULT1* and *WUS* act antagonistically in the same pathway to control floral meristem determinacy.

MATERIALS AND METHODS

Plant materials and growth conditions: Plants were grown in a 1:1:1 mixture of perlite:vermiculite:topsoil under 140 mmol/m² sec of continuous constant illumination at 22° and watered daily with a dilute (1:1500) solution of Miracle-Gro 20-20-20 fertilizer. Putative double-mutant plants were identified in the F₂ generation and confirmed through segregation analysis in the F₃ generation. All plants were in the Landsberg *erecta* (*Ler*) ecotype. The *stm-11* allele was provided by Jeff Long and Kathy Barton, and the *wus-1* allele by Thomas Laux.

Microscopy: Confocal laser scanning microscopy was performed as described previously (RUNNING *et al.* 1995) using a Zeiss 510 confocal microscope. Scanning electron microscopy was performed as described previously (BOWMAN *et al.* 1989) using a Hitachi 4700 electron microscope with digital imaging capability.

Histology: Tissues were fixed for histological analysis by formaldehyde acetic acid vacuum infiltration, dehydrated, processed through paraffin wax, sectioned at 8 μm thickness, stained with toluidine blue, and visualized using a Zeiss Axio-phot microscope. Images were acquired with a 12-bit MicroMax cooled CCD camera operated by IPLab software.

In situ hybridization: A *WUS* antisense probe for *in situ* hybridization was generated as described previously (MAYER *et al.* 1998) using a digoxigenin labeling mix (Roche). Tissue fixation and *in situ* hybridization were performed as described previously (JACKSON 1991). Slides were prehybridized at 55° for 5 hr, and 200 μg of probe per slide was then added for an overnight incubation at 55°. After two washes in 0.2× SSC 0.1% SDS of 10 min each at 55°, the slides were treated with 10 μg/ml RNase A for 30 min at 37°. Two more washes (0.2× SSC 0.1% SDS, 10 min each at 55°) were then performed. For signal detection the NBT/BCPIP reagents (Roche) were applied for 42 hr.

RESULTS

***ult1* embryonic and vegetative development:** Prior analysis revealed that *ult1* mutants accumulate excess SAM cells during the reproductive phase of development—both inflorescence and floral meristems are larger in *ult1-1* and *ult1-2* mutant plants than in wild-type plants, and the flowers produce more whorls and floral organs than normal (FLETCHER 2001). The *ult1-1*

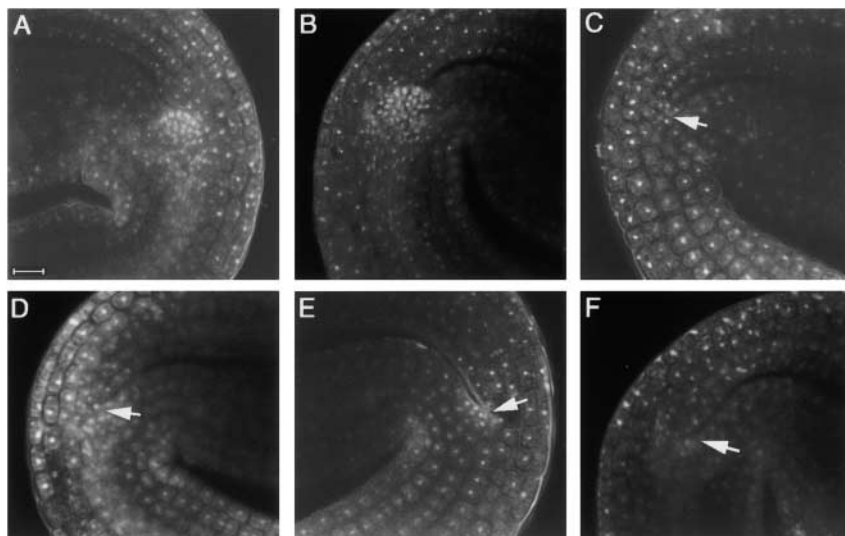


FIGURE 1.—Embryonic shoot apical meristem formation in wild-type, *ult1*, *stm*, and *wus* mutant plants. (A) Wild-type *Ler* embryonic SAM appears as a dome-shaped structure at the base of the two cotyledons. (B) *ult1-2* embryonic SAM resembles that of the wild type. (C) *stm-11* embryos lack a dome-shaped SAM structure at the base of the cotyledons (arrow). (D) *ult1-2 stm-11* embryos resemble *stm-11* single-mutant embryos. (E) *wus-1* embryos have only a few densely staining cells at the base of the cotyledons (arrow) and lack a dome-shaped SAM structure. (F) *ult1-2 wus-1* embryos resemble *wus-1* single-mutant embryos.

mutation has more severe effects on meristem size and on sepal and petal number than the *ult1-2* mutation. The phenotypes of plants carrying the *ult1-3* mutation, which is caused by a T-DNA insertion that abolishes *ULT1* transcription (C. CARLES, D. CHOFFNES-INADA, K. REVILLE, K. LERTPIRIYAPONG and J. FLETCHER, unpublished results), are statistically indistinguishable from those of *ult1-2* plants. Thus, *ult1-2* is a phenotypic null allele for the *ULT1* locus, while the *ult1-1* allele has slightly more severe effects on meristem size and sepal/petal number than the *ult1-3* knockout allele and is weakly semidominant (C. CARLES, D. CHOFFNES-INADA, K. REVILLE, K. LERTPIRIYAPONG and J. FLETCHER, unpublished results).

To determine at what stage plant development is first affected by mutations in *ULT1*, we examined wild-type *Ler*, *ult1-1*, and *ult1-2* embryos and seedlings. Shoot apical meristem cell layering, organogenesis, and organ morphology appear normal in *ult1-1* and *ult1-2* embryos and seedlings (Figure 1B and data not shown). Confocal laser scanning microscopy reveals that the *ult1-2* mature embryonic meristem size and cell number is not significantly different from that of wild-type meristems (Figure 1, A and B). *ult1-1* mutant embryonic meristems on average are $33.0 \pm 1.2 \mu\text{m}$ wide and $8.5 \pm 0.5 \mu\text{m}$ tall ($n = 7$), while *Ler* embryonic meristems on average are $31.2 \pm 1.2 \mu\text{m}$ wide and $9.3 \pm 0.4 \mu\text{m}$ tall ($n = 12$). Seven-day-old *ult1-1* seedlings ($52.4 \pm 2.0 \mu\text{m}$ wide, $22.4 \pm 1.1 \mu\text{m}$ tall, $n = 18$) likewise have approximately the same size shoot apical meristem as *Ler* seedlings ($48.9 \pm 2.3 \mu\text{m}$ wide, $23.9 \pm 1.8 \mu\text{m}$ tall, $n = 8$). *ult1-3* null mutant plants are also indistinguishable from wild-type plants during the embryonic and vegetative periods. Thus *ult1* mutant phenotypes are not detectable until the reproductive phase of development.

***ult1* interactions with *stm*:** Since *ult1* mutants accumulate excess meristem cells during reproductive develop-

ment, we hypothesized that the *ult1* alleles might restore shoot and/or floral meristem activity to *stm* mutants, which fail to maintain meristem cells in an undifferentiated state. This has been shown to be the case for the *clv1* and *clv3* mutants, which partially suppress *stm* mutant phenotypes in a dose-dependent manner (CLARK *et al.* 1996). To test this hypothesis, we generated double mutants between the *ult1* alleles and strong and weak *stm* alleles and followed their embryonic and postembryonic development.

Plants homozygous for the *stm-11* null allele (LONG and BARTON 1998) form a pair of normal embryonic leaves (cotyledons) during embryogenesis, but fail to develop a densely staining dome of meristematic cells at the base between them (Figure 1C). Among the progeny of *ult1-2* F₂ plants that segregated *stm-11*, we found that ~25% of embryos lacked a dome-shaped SAM between the cotyledons (Figure 1D). In addition, progeny of *ult1-1* F₂ plants that segregated *stm-11* also yielded ~25% embryos lacking a discernible SAM. Thus neither the *ult1-1* mutation nor the *ult1-2* mutation rescues the *stm-11* embryo shoot-meristemless phenotype.

Following germination, *stm-11* mutant seedlings do not produce postembryonic organs between the cotyledons (Figure 2, A and C) and completely lack a dome-shaped shoot apical meristem (Figure 2E). Double mutants generated between *stm-11* and either *ult1-1* or *ult1-2* initially resembled *stm-11* single-mutant plants. After 7 days of growth, the *ult1-1 stm-11* and *ult1-2 stm-11* double-mutant seedlings showed no evidence of organ formation between the cotyledons (Figure 2G). However, after 10 days ~15% of the *ult1-1 stm-11* and *ult1-2 stm-11* double-mutant plants began to develop leaves (Figure 2, B, D, and G). Scanning electron microscopy and sectioning revealed that the leaves produced by the double-mutant plants originated at the seedling shoot apex from the flanks of a dome of meristematic cells

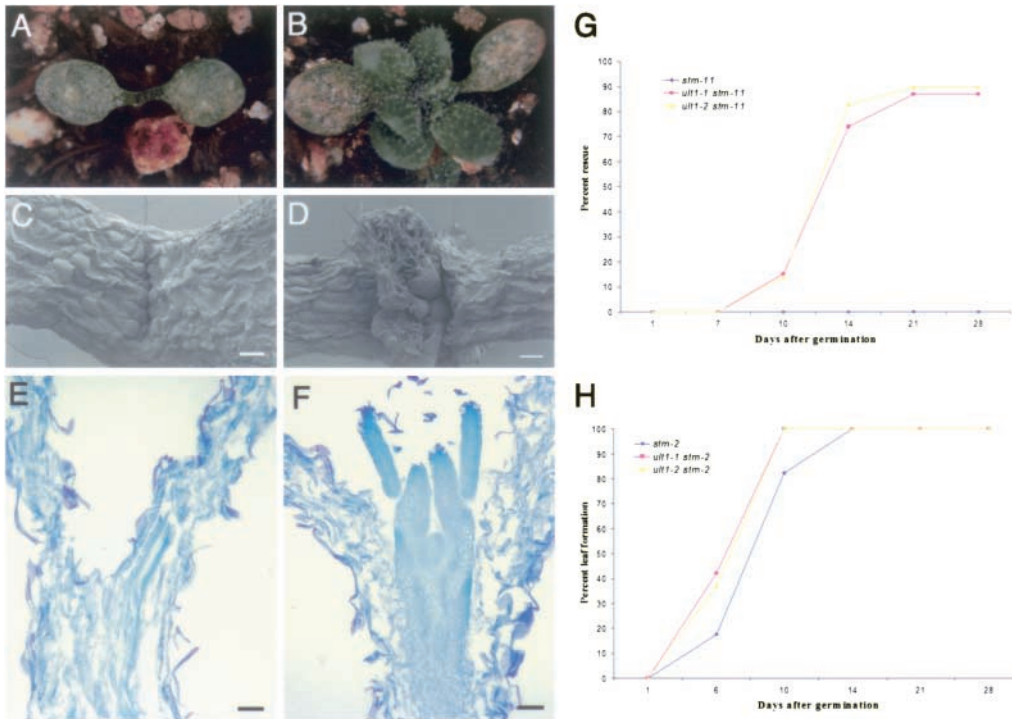


FIGURE 2.—Shoot apical meristem activity in *ult1 stm-11* seedlings. (A) *stm-11* seedling 14 days after germination. No organs have formed between the cotyledons. (B) *ult1-2 stm-11* seedling 14 days after germination. Leaves are developing from between the cotyledons. (C) SEM of an *stm-11* seedling 10 days after germination. (D) SEM of an *ult1-1 stm-11* seedling 10 days after germination. Leaves are forming between the cotyledons at the position where they are normally produced by the shoot apical meristem in wild-type plants. A pair of developing leaves arch over the SAM. (E) Section through an *stm-11* seedling 10 days after germination. There is no evidence of a dome-shaped SAM. (F) Section through an *ult1-2 stm-11* seedling 10 days after

germination. A correctly positioned SAM is producing leaves from its flanks, and the organization of the meristem cell layers is intact. (G) Percentage of *ult1-1 stm-11* (red, $n = 46$) and *ult1-2 stm-11* (yellow, $n = 29$) mutant seedlings that formed a shoot apical meristem and produce true leaves following germination and growth under constant light. No *stm-11* plants (blue, $n = 41$) grown at the same time under the same conditions produced any leaves or any sign of a shoot apical meristem. (H) Percentage of *stm-2* (blue, $n = 26$), *ult1-1 stm-2* (red, $n = 35$), and *ult1-2 stm-2* (yellow, $n = 27$) mutant seedlings that formed a shoot apical meristem and produced true leaves following germination and growth under constant light. Nearly all of the single and double-mutant plants produced organs from the shoot apex, but the *ult1-1* and *ult1-2* mutations increased the rate at which *stm-2* mutants developed postembryonic leaves. Bars: 100 μm , C and D; 30 μm , E and F.

between the cotyledons (Figure 2, D and F). In contrast, leaves that are occasionally produced by single-mutant plants carrying the weaker *stm-1* allele arise from the hypocotyl region (CLARK *et al.* 1996). After 21 days nearly 90% of *ult1-1 stm-11* and *ult1-2 stm-11* plants displayed this “restored” phenotype, while none of the *stm-11* single mutants showed signs of postembryonic organ formation (Figure 2G). Thus, a shoot apical meristem structure and organogenic capability is eventually restored to *stm-11* mutant plants when *ULT1* activity is absent. This experiment also reveals that *ULT1* is active during the vegetative phase of development, despite the fact that the *ult1* mutants lack a detectable vegetative phenotype.

Ultimately, 100% of the restored *ult1-1 stm-11* plants and 86% of the restored *ult1-2 stm-11* plants produced one or more abnormal inflorescence meristems bearing one to a few flowers (Figure 3). Compared to wild-type (Figure 3A) and *ult1-1* (Figure 3B) inflorescences, which produced flowers in a stereotypical spiral phyllotaxy, *ult1-1 stm-11* inflorescences consisted of disorganized aerial structures with abnormal phyllotaxy, consisting of leaves and reduced numbers of flowers (Figure 3C). The flowers produced by the double-mutant inflo-

rescences were also abnormal. Wild-type flowers normally form four sepals, four petals, five or six stamens, and two carpels that fuse to form the central gynoecium (SMYTH *et al.* 1990). In contrast, *ult1-1 stm-11* and *ult1-2 stm-11* flowers often contained fused and/or mosaic organs, such as fused stamens and sepal/petal, petal/stamen, and stamen/carpel mosaics (see supplemental table at <http://www.genetics.org/supplemental/>). Moreover, while sepal number was similar to that observed in the wild type, the petals, stamens, and carpels were either reduced in number or absent (Figure 4A). Although the center of the flower was the most severely affected, rare *ult1-1 stm-11* flowers with central structures bearing ovules and/or stigmatic tissue were observed (Figure 3D), indicating that *stm-11* plants are capable of forming all organ types when *ULT1* activity is lost. *ult1-1 stm-11* and *ult1-2 stm-11* plants displaying these inflorescence and floral phenotypes resembled plants homozygous for the weak *stm-2* allele (CLARK *et al.* 1996). Thus, significant inflorescence and floral meristem activity is restored to *stm* null mutant plants in the absence of *ULT1* function.

stm-2 mutant plants retain some meristematic activity, as evidenced by their ability to form abnormal rosettes

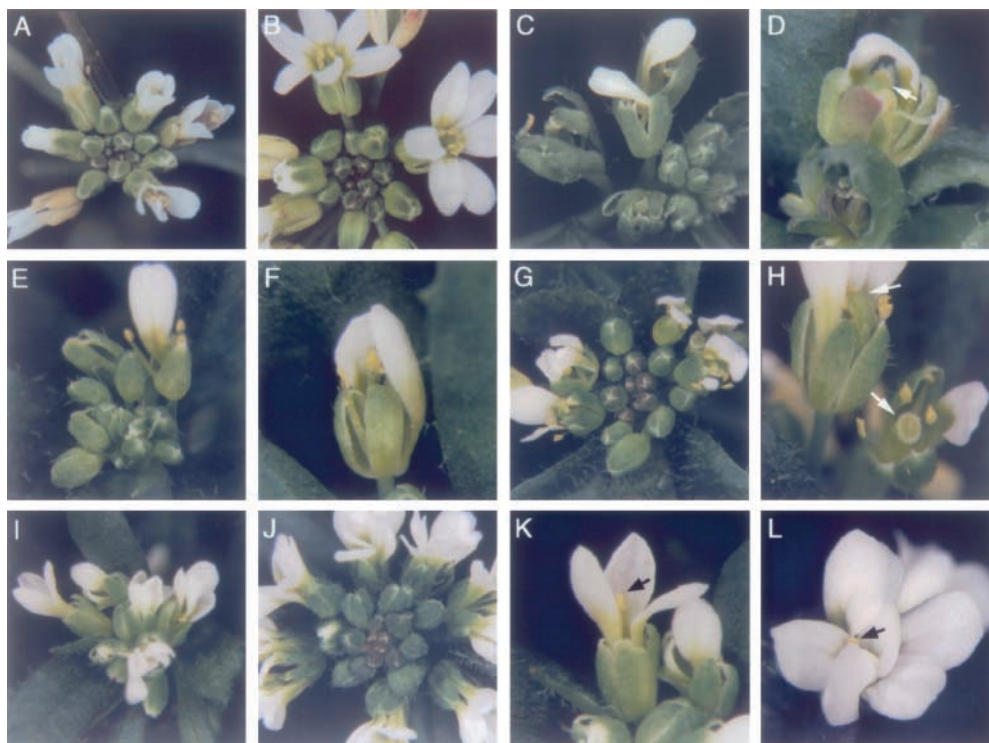


FIGURE 3.—Inflorescence meristem and flower phenotypes of *ult1 stm* and *ult1 wus* double mutants. (A) A wild-type *Ler* inflorescence meristem. (B) An *ult1-1* mutant inflorescence meristem, which generates flowers containing additional organs of all types, predominantly sepals and petals. (C) *ult1-1 stm-11* plants produce inflorescence meristems that generate a limited number of abnormal flowers. (D) Flowers produced by an *ult1-1 stm-11* inflorescence meristem contain fewer petals, stamens, and carpels than wild-type flowers do and resemble flowers generated by plants carrying weak *stm* alleles. Infrequently, flowers form carpeloid structures in the center of the flower (arrow). (E) Plants carrying the weak *stm-2* allele produce inflorescence meristems that generate a limited number of abnormal flowers. (F) Flowers produced by an *stm-2* inflorescence meristem lack the full complement of internal organs and fail to generate carpels. (G) *ult1-1 stm-2* plants produce inflorescence meristems that generate more flowers than *stm-2* inflorescence meristems do. (H) Flowers produced by an *ult1-1 stm-2* inflorescence meristem contain internal organs and can form unfused carpels or a normal, fused gynoecium (arrows). (I) A *wus-1* inflorescence meristem, which generates a small number of abnormal flowers in a disorganized phyllotactic pattern. (J) *ult1-1 wus-1* inflorescence meristems form many more flowers than do *wus-1* single-mutant meristems in a normal spiral phyllotaxy. (K) Flowers produced by *wus-1* plants lack the full complement of organs and generally terminate in a solitary stamen (arrow). (L) Flowers produced by *ult1-1 wus-1* plants can form more sepals and petals than either wild-type or *wus-1* flowers, but fail to form carpels and generally terminate in a solitary stamen (arrow).

ence meristem lack the full complement of internal organs and fail to generate carpels. (G) *ult1-1 stm-2* plants produce inflorescence meristems that generate more flowers than *stm-2* inflorescence meristems do. (H) Flowers produced by an *ult1-1 stm-2* inflorescence meristem contain internal organs and can form unfused carpels or a normal, fused gynoecium (arrows). (I) A *wus-1* inflorescence meristem, which generates a small number of abnormal flowers in a disorganized phyllotactic pattern. (J) *ult1-1 wus-1* inflorescence meristems form many more flowers than do *wus-1* single-mutant meristems in a normal spiral phyllotaxy. (K) Flowers produced by *wus-1* plants lack the full complement of organs and generally terminate in a solitary stamen (arrow). (L) Flowers produced by *ult1-1 wus-1* plants can form more sepals and petals than either wild-type or *wus-1* flowers, but fail to form carpels and generally terminate in a solitary stamen (arrow).

of leaves followed by inflorescence stems that produce reduced numbers of flowers (CLARK *et al.* 1996). To determine whether the *ult1* mutations affected the production of these postembryonic structures, we generated *ult1 stm-2* double mutants and compared their growth with that of *stm-2* single-mutant plants. Because nearly all *stm-2* plants eventually form leaves, we compared the rate of postembryonic leaf production between the different genotypes. After 6 days of growth, 43% of *ult1-1 stm-2* and 37% of *ult1-2 stm-2* seedlings had produced one or more leaves, compared to 17% of *stm-2* seedlings (Figure 2H). After 10 days of growth, 100% of *ult1-1 stm-2* and *ult1-2 stm-2* seedlings had produced leaves, compared to 82% of *stm-2* seedlings (Figure 2H). Therefore, *stm-2* seedlings generate leaves at a slightly accelerated rate when *ULT1* activity is reduced or absent.

The extent of inflorescence and floral meristem development was also greater in *ult1 stm-2* plants compared to *stm-2* mutants. Approximately one-third of *stm-2* plants formed a solitary, terminal flower. The other two-thirds produced more than one flower, frequently four or five. Rarely, an *stm-2* plant formed >10 flowers before termi-

nating (Figure 3E). In contrast, 100% of *ult1-1 stm-2* and *ult1-2 stm-2* plants generated inflorescence meristems bearing more than one flower, and 30–40% of the double-mutant plants produced >10 flowers in a normal spiral phyllotaxy (Figure 3G). The number of floral organs generated per whorl, and per flower, was likewise increased in *ult1 stm-2* plants compared to *stm-2* plants (Figure 4A). Flowers produced by *stm-2* plants contained reduced numbers of sepals, petals, and stamens and rarely formed carpels (Figures 3F and 4A). We observed partial restoration of organogenesis in each whorl of *ult1 stm-2* flowers, including the formation of fused or unfused carpel structures in the center of the flower (Figures 3H and 4A). However, we did not detect dose dependence between *ult1* alleles and *stm* alleles in any combination, indicating that while these two genes appear to have opposite activities they do not function in a directly competitive manner.

We used scanning electron microscopy to determine the earliest stage at which *ult1-1 stm-2* flower development deviated from *stm-2* flower development. At stage 2, when floral meristems first become distinguishable as bulges on the flanks of the shoot apical meristem

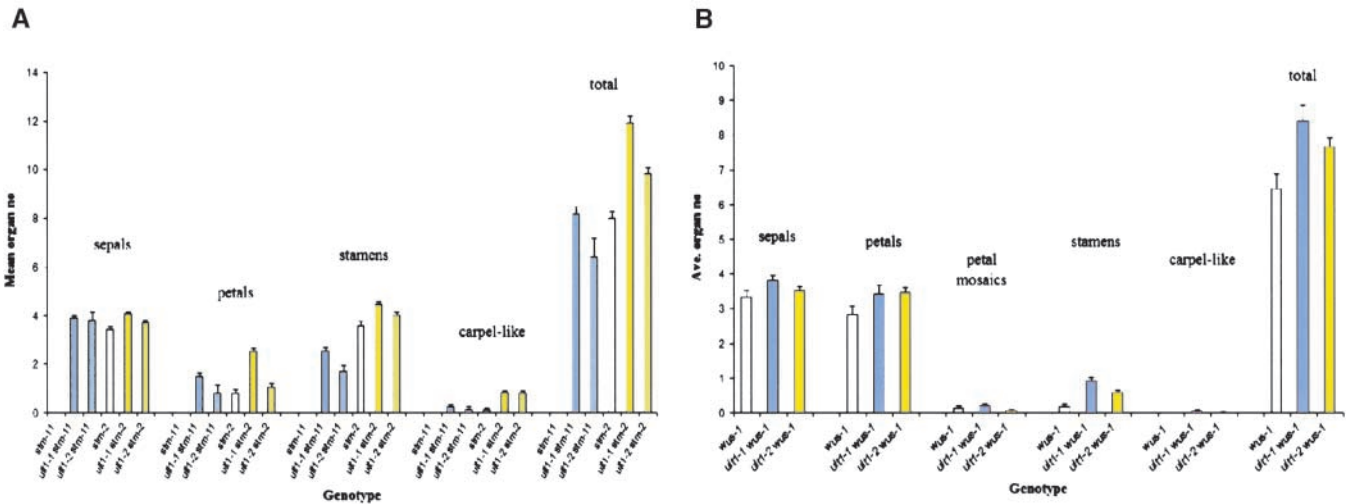


FIGURE 4.—Organ number in *ult1*, *stm*, and *wus* single- and double-mutant flowers. (A) *ult1* mutations restore organogenesis to *stm* floral meristems. The mean number of organs in the first 10 flowers of *stm* single-mutant and *ult1 stm* double-mutant plants is shown. If <10 flowers were produced by an individual plant, then all flowers on the primary inflorescence meristem were counted. At least 15 flowers were counted for each mean, and the standard error is indicated. (B) *ult1* mutations restore organogenesis to *wus-1* floral meristems, except in the carpel whorl in the center of the flower. The mean number of organs in the first 10 flowers of 10 *wus-1* single-mutant and *ult1 wus-1* double-mutant plants is shown. If <10 flowers were produced by an individual plant, then all flowers on the primary inflorescence meristem were counted. At least 38 flowers were counted for each mean, and the standard error is indicated.

(stages according to SMYTH *et al.* 1990), we observed no difference between *stm-2* and *ult1-1 stm-2* floral meristems (see supplemental figure at <http://www.genetics.org/supplemental/>). However, a distinction was clearly detected when stage 3 floral meristems were compared. At this stage, wild-type floral meristems assume a dome-shaped structure, surrounded by four sepal primordia at the periphery (SMYTH *et al.* 1990). The stage 3 floral meristems of *stm-2* plants formed a very reduced apex between the developing sepal primordia (see supplemental figure at <http://www.genetics.org/supplemental/>). In contrast, the stage 3 floral meristems of *ult1-1 stm-2* plants formed a dome between the developing sepal primordia and more closely resembled the wild type (see supplemental figure at <http://www.genetics.org/supplemental/>). Thus, the effect of the *ult1-1* mutation on *stm-2* flower development could be detected at the time of sepal initiation, after the floral meristems had formed, consistent with the idea that *ULT1* acts competitively with *STM* during meristem maintenance but not meristem initiation.

***ult1* interactions with *wus*:** The *WUSCHEL* (*WUS*) gene is required for proper meristem function, as *wus* mutant plants are defective in shoot and floral meristem maintenance (LAUX *et al.* 1996). Plants homozygous for the *wus-1* null allele form a normal pair of cotyledons during embryogenesis, but produce only a few disorganized meristematic cells at their base (Figure 1E). Because *wus-1* plants are sterile, we crossed *ult1-1* and *ult1-2* plants to *wus-1/+* heterozygous plants and identified homozygous *ult1* plants in the F₂. Among the progeny

of *ult1-1* and *ult1-2* F₂ plants that segregated *wus-1*, we found that ~25% of embryos lacked a dome-shaped SAM between the cotyledons (Figure 1F). Thus neither the *ult1-1* nor the *ult1-2* mutation rescues the *wus-1* embryo shoot apical meristem defect. After germination the *wus-1* plants pause in their development and then produce multiple abbreviated rosettes of leaves from the axils of the cotyledons and across the flat shoot apex (LAUX *et al.* 1996). We compared postembryonic development between *wus-1* plants and *ult1 wus-1* plants and found that the rate of leaf production in the double mutants was indistinguishable from that of the single mutants. Thus the *ult1* mutations do not accelerate vegetative organ formation in *wus-1* mutant plants, as they do in *stm* mutant plants.

After the transition to flowering, *wus* mutant plants produce abnormal inflorescences and flowers due to reduced meristem activity (LAUX *et al.* 1996; SCHOOF *et al.* 2000). *wus-1* inflorescence meristems generate far fewer flowers than wild-type meristems do, and the flowers that do form arise in aerial rosettes with a disorganized phyllotaxic pattern (Figure 3I). Under our growth conditions, 45% of *wus-1* plants terminated development without flowering, and an additional 37% generated a solitary flower (Table 1). Only 18% of *wus-1* plants generated multiple flowers from their adventitious inflorescence meristems (Table 1). In contrast, 100% of *ult1-1 wus-1* plants and 82% of *ult1-2 wus-1* plants produced one or more flowers in a normal spiral phyllotaxy (Figure 3J, Table 1), indicating that mutations in *ULT1* restore some function to *wus-1* inflorescence meristems.

TABLE 1
Terminal structures produced by *wus-1* plants and
***ult1 wus-1* double-mutant plants**

Structure	<i>wus-1</i> (%)	<i>ult1-1 wus-1</i> (%)	<i>ult1-2 wus-1</i> (%)
Basal rosette	15	0	0
Aerial rosette	30	0	18
Solitary flower	37	28	18
Multiple flowers	18	72	64

Numbers represent the percentage of plants with the indicated structure as a terminal phenotype. Plants terminating in either a basal or an aerial rosette did not produce flowers. *wus-1*, $n = 33$; *ult1-1 wus-1*, $n = 36$; and *ult1-2 wus-1*, $n = 27$ plants scored.

wus-1 floral meristems generate reduced numbers of floral organs, producing on average three to four sepals, three to four petals, and zero to one stamen per flower (Figures 3K and 4B). *wus-1* flowers do not contain more than four sepals and petals and never form carpels (Figure 4B). *ult1-1 wus-1* and *ult1-2 wus-1* flowers have slightly more sepals and petals on average than *wus-1* flowers (Figure 4B). However, unlike *wus-1* flowers, *ult1-1 wus-1* flowers and *ult1-2 wus-1* flowers can contain up to six or seven sepals and petals (Figure 3L). The sepal and petal number increase observed in *ult1* flowers is therefore at least partially independent of *WUS*.

In contrast to the other *wus-1* reproductive meristem phenotypes, the premature floral meristem termination phenotype is not rescued by the *ult1-1* or *ult1-2* mutations. *wus-1* flowers contain an average of less than one stamen per flower and completely lack carpels (Figures 3K and 4B). Similarly, *ult1-1 wus-1* flowers contain an average of less than one stamen per flower and lack carpels (Figures 3L and 4B; of 88 counted, a single carpel-like structure was formed in 1 *ult1-1 wus-1* flower). Examination of floral meristem development using scanning electron microscopy revealed that the floral primordia of single- and double-mutant plants are indistinguishable at both stage 2 and stage 3 (see supplemental figure at <http://www.genetics.org/supplemental/>). Like *wus-1* stage 3 floral meristems, *ult1-1 wus-1* stage 3 floral meristems lack a detectable dome of meristematic cells interior to the developing sepal primordia (see supplemental figure at <http://www.genetics.org/supplemental/>). Thus, *wus* is epistatic to *ult1* in the center of the floral meristem, revealing that the *WUS* and *ULT1* genes play antagonistic roles in the same genetic pathway that controls floral meristem determinacy.

wus and *ult1* mutant plants have opposite phenotypes in the center of the flower. *wus-1* floral meristems are smaller than those of the wild type, generate reduced numbers of stamens, and terminate prematurely prior to carpel formation (LAUX *et al.* 1996; SCHOOF *et al.* 2000). *ult1-1* and *ult1-2* floral meristems, in contrast, are larger than those of the wild type and can generate

extra whorls of floral organs within the normal fourth whorl of carpels (FLETCHER 2001; Figure 5B; compare with wild type in Figure 5A). Since no fourth whorl carpels or internal fifth whorl organs are formed by *ult1-1 wus-1* double mutants, the partial loss of determinacy observed in *ult1-1* flowers depends upon *WUS* activity. To confirm this result, we used *in situ* hybridization to visualize the *WUS* expression pattern in developing wild-type and *ult1-1* mutant flowers.

In wild-type floral meristems, *WUS* transcripts are first detected in stage 2 primordia budding from the flanks of the inflorescence meristem. In wild-type stage 2 floral meristems, *WUS* expression is restricted to the interior cells in the central zone (Figure 5E). This area of the meristem has been proposed to act as an organizing center, specifying the overlying neighbor cells to maintain their pluripotent state (MAYER *et al.* 1998). *WUS* expression in *ult1-1* floral meristems at this stage of development (Figure 5F) is indistinguishable from that in the wild type. *WUS* transcription is repressed in wild-type floral meristems after stage 6 (MAYER *et al.* 1998), when two carpel primordia are initiated in the center of the flower and meristematic activity ceases (Figure 5G). The formation of additional whorls of floral organs in *ult1-1* and *ult1-2* flowers is correlated with the presence of a dome of cells that separate the two developing carpel primordia detectable in stage 6 and stage 7 flowers (Figure 5D). In contrast, the carpel primordia produced by wild-type flowers abut each other (Figure 5C). In *ult1-1* stage 7 flowers displaying such a dome of tissue between the carpel primordia, *WUS* transcription is still detectable in cells underlying this dome (Figure 5H). This *WUS* expression domain corresponds to cells for which floral organ identity specification has been delayed, on the basis of our observation that *AG* induction in the center of the floral bud occurs later in *ult1-1* than in wild-type plants (FLETCHER 2001). These data show that *ULT1* negatively regulates *WUS* to establish floral meristem determinacy and that the partial loss of determinacy observed in *ult1* flowers depends on *WUS* activity.

DISCUSSION

Shoot and floral meristem maintenance in Arabidopsis depends upon the activity of networks of meristem-restricting and meristem-promoting factors. Our previous experiments have shown that *ULT1* is an important meristem-restricting factor that limits the accumulation of cells in both inflorescence and floral meristems. *STM* and *WUS* represent two meristem-promoting factors that act in separate genetic pathways, with the *STM* pathway maintaining meristem cells in an uncommitted, proliferative state and the *WUS/CLV* pathway maintaining stem cell fate at the meristem apex. To determine the genetic interaction between the meristem-restricting *ULT1* factor and the *STM* and *WUS* pathways,

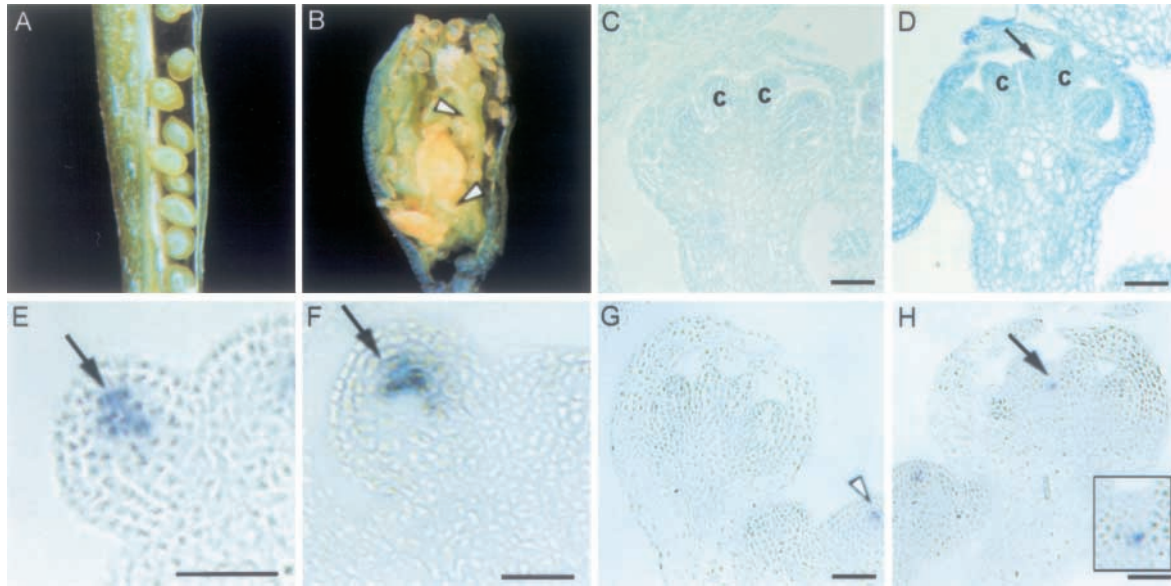


FIGURE 5.—*WUS* expression in wild-type and *ult1-1* floral meristems. (A) A wild-type silique dissected lengthwise to reveal the ovules within one valve. (B) An *ult1-1* silique dissected lengthwise to reveal the presence of supernumerary whorls of stamens and carpels (arrowheads). (C) Wild-type stage 7 flower with two carpel primordia (c) developing in the center of the flower. (D) *ult1-1* stage 7 flower with a dome of tissue (arrow) between the two developing carpel primordia (c). (E–H) Wild-type and *ult1-1* floral meristems hybridized with an antisense *WUS* riboprobe. (E) A wild-type stage 2 floral meristem. *WUS* expression is detected in a small group of cells beneath the outermost two cell layers (arrow). (F) An *ult1-1* stage 2 floral meristem. The pattern and level of *WUS* expression (arrow) are similar to those observed in wild-type stage 2 floral meristems. (G) A wild-type stage 7 flower. After the carpel primordia have emerged, *WUS* transcription is not detected in the center of the flower. The arrowhead points to the signal detected in the inflorescence meristem, testifying to the integrity of the tissue section. (H) An *ult1-1* stage 7 flower. *WUS* expression is observed in the cells within the dome of tissue between the two carpel primordia (arrow). The inset shows the region depicted by the arrow at higher magnification. Bars, 30 μ m.

we generated double mutants between strong and weak *ult1* alleles and *stm* and *wus* alleles. We determined that while the *ult1* alleles cause no detectable phenotypes during embryonic or vegetative development, they can partially suppress the vegetative and inflorescence meristem defects that result from reduced meristem activity in both *stm* and *wus* mutant plants. *ult1* mutations also restored organogenic potential to *stm* floral meristems, leading to increased organ production in all whorls. However, we found that *wus* mutations are epistatic to *ult1* mutations in the center of the flower and that *WUS* transcripts persist longer than normal in developing *ult1* flowers. Thus, *ULT1* controls floral determinacy by negatively regulating *WUS* expression during floral meristem development.

***ULT1* function early in Arabidopsis development:**

Plants carrying loss-of-function *ult1* alleles are indistinguishable from wild-type plants during embryonic and vegetative development. This may be because either *ULT1* does not function prior to the inflorescence phase or its activity earlier in development is masked by the activity of another gene or genes. We find that loss of *ULT1* activity restores postembryonic SAM structure and organogenesis function to *stm* mutant plants and also partially suppresses the *wus* vegetative terminal meristem phenotype. Thus our analysis of *ult1 stm* and *ult1 wus* double mutants reveals that *ULT1* is functional dur-

ing vegetative development, prior to the stage at which an *ult1* single-mutant phenotype is detectable. However, lack of *ULT1* activity does not restore embryonic SAM structure in *stm-11* and *wus-1* mutants.

***ULT1* regulation of shoot and floral meristem activity:**

Genetic and molecular studies have defined the homeobox genes *STM* and *WUS* as essential regulators of shoot and floral meristem formation and maintenance. *WUS* and *STM* are induced independently of one another in embryonic SAMs (LONG and BARTON 1998; MAYER *et al.* 1998), and the evidence to date indicates that these two genes promote meristem activity in independent but complementary ways and function in distinct regulatory pathways (LENHARD *et al.* 2002). The *STM* pathway suppresses cell differentiation throughout the meristem, while the *WUS* pathway specifies a subset of cells at the meristem apex as stem cells. These pathways ultimately converge to maintain meristem cells in an undifferentiated state (GALLOIS *et al.* 2002; LENHARD *et al.* 2002).

The *CLV* loci act to restrict meristem activity, having the opposite effect to *STM* and *WUS* on shoot and floral meristems. Genetic analysis showed that *wus* mutations were epistatic to *clv* mutations in both shoot and floral meristems, placing *WUS* and the *CLV* genes in the same genetic pathway (SCHOOF *et al.* 2000). In contrast, *clv* mutations partially suppressed the *stm* mutant pheno-

types and vice versa, and the suppression occurred in a dominant fashion (CLARK *et al.* 1996). The occurrence of dominant interactions between *clv* and *stm* mutations was interpreted to mean that these genes play opposing and possibly competitive roles in shoot and floral meristem regulation.

Like the *CLV* loci, *ULT1* acts opposite to *STM* and *WUS* in that it functions to restrict the excess accumulation of cells in shoot and floral meristems. We have shown that *ult1* mutations restore organized vegetative shoot apical meristems to *stm* mutant plants, allowing postembryonic organ formation to proceed, albeit in an abbreviated manner. In addition, we observe restoration of floral meristem and floral organ formation in both *ult1 stm-11* and *ult1 stm-2* plants, including the development of carpel tissue in the latter genotype. In sum, the *ult1* alleles reverse many of the effects of weak and strong *stm* alleles, but do not suppress them completely. Similarly, the *stm* mutations partially suppress the *ult1* phenotypes, showing that a wild-type level of *STM* activity is necessary for excess meristem cell accumulation in *ult1* plants. The *ult1 stm* double-mutant phenotypes can therefore be considered additive, from which we conclude that *STM* and *ULT1* act oppositely through separate genetic pathways to regulate shoot and floral meristem activity. However, the lack of dose dependency between *stm* and *ult1* alleles suggests that the two genes do not function competitively to regulate the same process.

The interaction between *ULT1* and *WUS* is more complex. Similar to *stm-2* plants, *wus-1* plants produced some lateral organs from disorganized meristems that initiate randomly across the entire differentiated shoot apex (LAUX *et al.* 1996). Yet unlike *stm-2* plants, the rate at which *wus-1* plants produced leaves was not accelerated in the absence of *ULT1*. However, *ult1 wus-1* double mutants bolted at a higher frequency and formed many more floral meristems than did *wus-1* single mutants. These results indicate that *ult1* mutations partially suppress *wus*, restoring a greater amount of shoot and floral meristem activity. However, *ult1 wus-1* double-mutant inflorescences still terminated prematurely and produced flowers with fewer organs than *ult1* single mutants did, indicating that the *wus-1* mutation also partially suppresses the *ult1* mutations. We also observe significant sepal and petal restoration in *ult1-1 wus-1* and *ult1-2 wus-1* flowers, and, in fact, supernumerary sepals and petals could be produced by *ult1* floral meristems even in the absence of *WUS*. Thus *ULT1* acts in a separate pathway from *WUS* to control shoot apical meristem activity, and the sepal and petal number increase in *ult1* flowers is largely *WUS* independent. However, the *wus-1* mutation is epistatic to the *ult1* mutations in the inner two whorls of the floral meristem, indicating that *WUS* is absolutely required for the formation of supernumerary whorls of organs by *ult1* floral meristems.

***ULT1* regulation of floral meristem determinacy:** Nor-

mal *Arabidopsis* flower development requires that floral stem cell activity terminate upon formation of the central carpel primordia, which consumes the floral meristem. Floral meristem termination occurs via a temporal autoregulatory loop involving *WUS* and *AG* (LENHARD *et al.* 2001; LOHMANN *et al.* 2001). *AG* encodes a MADS domain transcription factor that is required to terminate floral meristem activity and also to specify stamen and carpel identity (YANOFSKY *et al.* 1990). Early in flower development, *WUS* and the floral meristem identity factor *LEAFY* (*LFY*) activate *AG* transcription by binding to adjacent sites in the second intron (LOHMANN *et al.* 2001). *AG* expression is restricted to the interior two whorls of the flower bud, where the stamen and carpel primordia form (DREWS *et al.* 1991). At stage 6 of flower development, *AG* switches off the organizing center activity by repressing *WUS* expression, resulting in the differentiation of the remaining stem cells into carpel tissues. However, genetic evidence indicates that *AG* alone is not sufficient to repress *WUS* in the center of the flower, because ectopic activation of *AG* in the inflorescence meristem does not cause meristem arrest (MIZUKAMI and MA 1997). Therefore *AG* requires an additional factor or factors to achieve downregulation of *WUS* transcription (LENHARD *et al.* 2001).

ULT1 also plays a role in specifying floral meristem determinacy. Mature *ult1* flowers can contain more than four whorls of organs, such as fifth and sixth whorls of carpels or a fifth whorl of stamens and a sixth whorl of carpels (FLETCHER 2001; Figure 5B). In this way the *ult1* flowers are reminiscent of *ag* flowers, which produce an indeterminate number of floral whorls as a result of active maintenance of a stem cell reservoir and organizing center at the apex of the floral meristem. When the stamen and carpel specification functions of *AG* are separated from the floral meristem determinacy function via site-directed mutagenesis, the resemblance is even more striking: a synthetic partial loss-of-function *ag* mutation, *AG-Met205*, causes production of extra whorls of stamens and carpels in the *ag-3* background (SIEBURTH *et al.* 1995), closely resembling the *ult1* mutant phenotype. Transgenic plants carrying an antisense *AG* construct in which *AG* expression is reduced to approximately half the normal level also display the nested stamen and carpel phenotype (MIZUKAMI and MA 1995). However, *ult1* mutant flowers, unlike *ag* null mutant flowers, are never completely converted to an indeterminate fate, and, as expected, *ag* mutations are epistatic to *ult1* mutations with respect to floral meristem determinacy (data not shown). Since floral stem cell termination eventually occurs in *ult1* mutants, it appears that *AG* can partially compensate for the absence of *ULT1*, but *ULT1* cannot compensate for the absence of *AG*.

Our results demonstrate that *ULT1* is a new component of the *AG*-*WUS* temporal feedback loop that controls floral meristem termination. We have shown that

ult1 floral meristems contain a dome of cells between the developing carpel primordia, which normally abut one another. Proliferating cells that separate the two carpel primordia are also observed in *pAG::WUS* transgenic plants in which *WUS* activity is prolonged in the center of the flower (LENHARD *et al.* 2001). Similarly, *ag* mutants maintain a dome of proliferating cells in the center of the flower, even after multiple whorls of organs have formed, which sustains *WUS* expression beneath the two outermost cell layers (LENHARD *et al.* 2001; LOHMANN *et al.* 2001). In addition, carpel number is not restored in *ult1 wus* double mutants, revealing that the *ULT1* indeterminacy phenotype is dependent on the activity of *WUS*. *wus* mutations are in fact epistatic to *ult1* mutations with respect to floral meristem determinacy, indicating that *ULT1* acts in the same pathway as *WUS* to control floral stem cell termination. Finally, we have previously observed that *AG* expression is delayed in the center of *ult1* mutant floral meristems (FLETCHER 2001), which correlates with delayed floral meristem termination. This result suggests that *ULT1* repression of stem cell activity may work through *AG*.

We can invoke two possible models for *ULT1* function in floral meristem termination that are consistent with our data. In the first model, *ULT1* is necessary to induce *AG* at the correct time during flower development to ensure that *WUS* repression occurs at the time of carpel initiation. Therefore we propose that in wild-type floral meristems, *ULT1* acts (directly or indirectly) with *LFY* and *WUS* to activate *AG* at the proper time and place in the most central region of the flower. *AG* activation leads to *WUS* repression and consequent stem cell termination. In *ult1* floral meristems, *AG* activation in the central region is delayed, causing a delay in *WUS* repression and thus permitting amplification of additional stem cells that become incorporated into extra whorls of organs. This model is attractive in that it accounts for the delayed activation of *AG* in the center of the flower, although we note that, because *AG* is properly induced in the third whorl even when *ULT1* is absent, some other factor(s) is likely to activate *AG* in this region. An alternative scenario is that *ULT1* may be required in addition to and independently of *AG* to repress *WUS* in the center of the flower. In this case, we predict that, in the absence of *ULT1*, *AG* is eventually able to repress *WUS* on its own, but only after a delay, during which the level of *AG* itself or a secondary activator may rise sufficiently to terminate *WUS* transcription. While we favor the first model because of its simplicity (it invokes only a single regulatory pathway rather than two separate pathways), its confirmation or rejection will await further experiments to determine whether *AG* is directly or indirectly activated by *ULT1*.

We thank Jeff Long, Kathy Barton, and Thomas Laux for providing *stm* and *wus* alleles, and Michael Lenhard for the *WUS* antisense construct. We are grateful to Karen Osmont, Leor Williams, Dan Choffnes, Robert Blanvillain, and George Chuck for helpful comments on the manuscript. This work was supported by the National Science Foundation (IBN-0110667).

LITERATURE CITED

- BARTON, M. K., and R. S. POETHIG, 1993 Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the *shoot meristemless* mutant. *Development* **119**: 823–831.
- BOWMAN, J. L., D. R. SMYTH and E. M. MEYEROWITZ, 1989 Genes directing flower development in *Arabidopsis*. *Plant Cell* **1**: 37–52.
- BRAND, U., J. C. FLETCHER, M. HOBE, E. M. MEYEROWITZ and R. SIMON, 2000 Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by *CLV3* activity. *Science* **289**: 617–619.
- BYRNE, M. E., R. BARLEY, M. CURTIS, J. M. ARROYO, M. DUNHAM *et al.*, 2000 *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* **408**: 967–971.
- BYRNE, M. E., J. SIMOROWSKI and R. A. MARTIENSEN, 2002 *ASYMMETRIC LEAVES1* reveals *knox* gene redundancy in *Arabidopsis*. *Development* **129**: 1957–1965.
- CLARK, S. E., S. E. JACOBSEN, J. Z. LEVIN and E. M. MEYEROWITZ, 1996 The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in *Arabidopsis*. *Development* **122**: 1567–1575.
- CLARK, S. E., R. W. WILLIAMS and E. M. MEYEROWITZ, 1997 The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. *Cell* **89**: 575–585.
- DREWS, G. N., J. L. BOWMAN and E. M. MEYEROWITZ, 1991 Negative regulation of the *Arabidopsis* homeotic gene *AGAMOUS* by the *APETALA2* product. *Cell* **65**: 991–1001.
- FLETCHER, J. C., 2001 The *ULTRAPETALA* gene controls shoot and floral meristem size in *Arabidopsis*. *Development* **128**: 1323–1333.
- FLETCHER, J. C., U. BRAND, M. P. RUNNING, R. SIMON and E. M. MEYEROWITZ, 1999 Signaling of cell fate decisions by *CLAVATA3* in *Arabidopsis* shoot meristems. *Science* **283**: 1911–1914.
- GALLOIS, J.-L., C. WOODWARD, G. V. REDDY and R. SABLONSKI, 2002 Combined *SHOOT MERISTEMLESS* and *WUSCHEL* trigger ectopic organogenesis in *Arabidopsis*. *Development* **129**: 3207–3217.
- JACKSON, D., 1991 *In situ* hybridization in plants, pp. 163–174 in *Molecular Plant Pathology: A Practical Approach*, edited by D. J. BOWLES, S. J. GURR and R. MCPHERSON. Oxford University Press, Oxford.
- JEONG, S., A. E. TROTOCHAUD and S. E. CLARK, 1999 The *Arabidopsis* *CLAVATA2* gene encodes a receptor-like protein required for the stability of the *CLAVATA1* receptor-like kinase. *Plant Cell* **11**: 1925–1933.
- LAUX, T., K. F. X. MAYER, J. BERGER and G. JURGENS, 1996 The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* **122**: 87–96.
- LENHARD, M., and T. LAUX, 2003 Stem cell homeostasis in the *Arabidopsis* shoot meristem is regulated by intercellular movement of *CLAVATA3* and its sequestration by *CLAVATA1*. *Development* **130**: 3163–3173.
- LENHARD, M., A. BOHNERT, G. JURGENS and T. LAUX, 2001 Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. *Cell* **105**: 805–814.
- LENHARD, M., G. JURGENS and T. LAUX, 2002 The *WUSCHEL* and *SHOOTMERISTEMLESS* genes fulfill complementary roles in *Arabidopsis* shoot meristem regulation. *Development* **129**: 3195–3206.
- LOHMANN, J. U., R. L. HONG, M. HOBE, M. A. BUSCH, F. PARCY *et al.*, 2001 A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* **105**: 793–803.
- LONG, J. A., and M. K. BARTON, 1998 The development of apical embryonic pattern in *Arabidopsis*. *Development* **125**: 3027–3035.
- LONG, J. A., E. I. MOAN, J. I. MEDFORD and M. K. BARTON, 1996 A member of the *KNOTTED* class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. *Nature* **379**: 66–69.
- MAYER, K. F. X., H. SCHOOF, A. HAECKER, M. LENHARD, G. JURGENS *et al.*, 1998 Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* **95**: 805–815.
- MIZUKAMI, Y., and H. MA, 1995 Separation of *AG* function in floral meristem determinacy from that in reproductive organ identity by expressing antisense *AG* RNA. *Plant Mol. Biol.* **28**: 767–784.
- MIZUKAMI, Y., and H. MA, 1997 Determination of *Arabidopsis* floral meristem identity by *AGAMOUS*. *Plant Cell* **9**: 393–408.
- ROJO, E., V. K. SHARMA, V. KOVALEVA, N. V. RAIKHEL and J. C. FLETCHER, 2002 *CLV3* is localized to the extracellular space, where it activates the *Arabidopsis* *CLAVATA* stem cell signaling pathway. *Plant Cell* **14**: 969–977.

- RUNNING, M. P., S. E. CLARK and E. M. MEYEROWITZ, 1995 Confocal microscopy of the shoot apex. *Methods Cell Biol.* **49**: 217–229.
- SCHOOF, H., M. LENHARD, A. HAECKER, K. F. X. MAYER, G. JURGENS *et al.*, 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.
- SIEBURTH, L. E., M. P. RUNNING and E. M. MEYEROWITZ, 1995 Genetic separation of third and fourth whorl functions of *AGAMOUS*. *Plant Cell* **7**: 1249–1258.
- SMYTH, D. R., J. L. BOWMAN and E. M. MEYEROWITZ, 1990 Early flower development in *Arabidopsis*. *Plant Cell* **2**: 755–767.
- STEEVES, T. A., and I. M. SUSSEX, 1989 *Patterns in Plant Development*. Cambridge University Press, New York.
- TROTOCHAUD, A. E., T. HAO, G. WU, Z. YANG and S. E. CLARK, 1999 The *CLAVATA1* receptor-like kinase requires *CLAVATA3* for its assembly into a signaling complex that includes KAPP and a Rho-related protein. *Plant Cell* **11**: 393–405.
- YANOFSKY, M. F., H. MA, J. L. BOWMAN, G. N. DREWS, K. A. FELDMANN *et al.*, 1990 The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. *Nature* **346**: 35–39.

Communicating editor: D. WEIGEL

