The \textit{WUSCHEL} gene is required for shoot and floral meristem integrity in \textit{Arabidopsis}

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\section*{SUMMARY}

Self perpetuation of the shoot meristem is essential for the repetitive initiation of shoot structures during plant development. In \textit{Arabidopsis} shoot meristem maintenance is disrupted by recessive mutations in the \textit{WUSCHEL (WUS)} gene. The defect is evident at all developmental stages and is restricted to shoot and floral meristems, whereas the root meristem is not affected. \textit{wus} mutants fail to properly organize a shoot meristem in the embryo. Postembryonically, defective shoot meristems are initiated repetitively but terminate prematurely in aberrant flat structures. In contrast to wild-type shoot meristems, primordia initiation occurs ectopically across mutant apices, including the center, and often new shoot meristems instead of organs are initiated. The cells of \textit{wus} shoot apices are larger and more vacuolated than wild-type shoot meristem cells. \textit{wus} floral meristems terminate prematurely in a central stamen. Double mutant studies indicate that the number of organ primordia in the center of \textit{wus} flowers is limited, irrespective of organ identity and we propose that meristem cells are allocated into floral whorl domains in a sequential manner. \textit{WUS} activity also appears to be required for the formation of supernumerary organs in the center of \textit{agamous, superman} or \textit{clavata1} flowers, suggesting that the \textit{WUS} gene acts upstream of the corresponding genes. Our results suggest that the \textit{WUS} gene is specifically required for central meristem identity of shoot and floral meristems to maintain their structural and functional integrity.

Key words: \textit{Arabidopsis}, shoot meristem, floral meristem, meristem maintenance, \textit{WUSCHEL}

\section*{INTRODUCTION}

In contrast to animals, the adult plant body is formed postembryonically by the continuous activity of small cell clusters, the shoot and the root meristems. Cells produced by these meristems serve two purposes: the formation of organs and the self-perpetuation of the meristem (for review see Steeves and Sussex, 1989).

The shoot meristem is established during plant embryogenesis and together with cotyledons, hypocotyl, embryonic root and root meristem makes up the basic body plan (Jürgens et al., 1991). During postembryonic development of \textit{Arabidopsis}, the shoot meristem gives rise to the shoot axis (stem), leaves and axillary meristems in a repetitive indeterminate fashion while being maintained by self-renewal. During vegetative development it produces a rosette of leaves bearing indeterminate axillary shoot meristems. After floral induction the shoot meristem gives rise to determinate floral meristems.

Experimental studies suggest that the shoot meristem is organized in three zones: (1) the central zone at the very summit, (2) a zone underneath (rib meristem) which gives rise to the pith of the shoot axis and (3) the peripheral zone (flank meristem) where leaf and flower primordia are initiated (for review see Steeves and Sussex, 1989). In this model, the central zone contains undifferentiated stem cells that replenish the cells used up for primordia initiation and thus are the ultimate source of almost the entire shoot. These stem cells, often termed apical or embryonic initial cells (Wardlaw, 1957), can be active over extended periods of plant development. However, they are occasionally replaced by neighboring cells (Ruth et al., 1985), suggesting that positional information is instrumental in defining shoot meristem cell fates. Surgical experiments suggest that the central zone also plays a role in regulating functional shoot meristem integrity (Loiseau, 1959; for review see Steeves and Sussex, 1989).

Recently, the roles of several genes in shoot meristem development were investigated by mutant analyses. For example, embryonic and postembryonic initiation of shoot meristems in \textit{Arabidopsis} requires \textit{SHOOT MERISTEMLESS (STM)} activity (Barton and Poethig, 1993), while the \textit{ZWILLE (ZLL)} gene is essential for shoot meristem development specifically in the embryo (Jürgens et al., 1994). Shoot meristems can be initiated ectopically on leaf blades by maize \textit{KNOTTED-1} gene expression, suggesting that this gene plays a role in promoting meristicatic cell identity (Sinha et al., 1993). Shoot meristem size in \textit{Arabidopsis} is affected by \textit{CLAVATA1} (Leyser and ...
Furner, 1992) and CLAVATA3 activity, which appear to negatively regulate proliferation of the central meristem (Clark et al., 1993, 1995). Shoot meristem structure is disturbed by mutations in genes such as FOREVER YOUNG and SCHIZOID, but these genes also appear to affect other aspects of plant development (Medford et al., 1992).

While the Arabidopsis shoot meristem acts in an indeterminate fashion, the floral meristem initiates a limited number of organs in four concentric whorls: four sepals in the outermost whorl, four petals in the second, six stamens in the third and two fused carpels in the fourth whorl. Genetic and molecular analyses have revealed many genes involved in the regulation of floral meristem specification, number of floral organs and organ identity specification (for review see Ma, 1994).

We have identified the WUSCHEL (WUS) gene, mutations of which specifically disturb shoot and floral meristem development. Our results suggest that WUS promotes central identity in both indeterminate shoot and determinate floral meristems and plays an important role in maintaining their structural and functional integrity.

MATERIALS AND METHODS

Plant growth conditions and plant strains

Plants were grown at 18°C or 25°C as previously described (Mayer et al., 1993). The following strains were used: Landsberg erecta (Ler), W100 (an ap1; er py; hy2 g1; bp cer2; ms1 t3), pi-1, ag-1 and ap3-1 (obtained from Dr M. Koornneef, Agricultural University Wageningen), clv1-4 (obtained from Dr S. Clark, University of Michigan, Ann Arbor), ap1-1 (obtained from the Arabidopsis Stock center, Ohio), sup-6 (S. Ploenese and T. L., unpublished) and hy3 py as (G. J., unpublished data).

Isolation of mutants

Seeds were mutagenized in 0.2% and 0.4% EMS as previously described (Mayer et al., 1991). Segregating populations from 11000 families were screened for seedlings with a defective shoot meristem. Based on the frequency of albino and different trichome mutants, the number of lines screened represents an average of six mutant alleles for each gene. More than 100 shoot meristem defective mutants were initially identified at the seedling stage.

Mapping of the WUS gene

Using the W100 strain, the WUS gene was mapped to chromosome 2. For more precise mapping, F2 progeny from a cross of wus-1/+ to hy3 py as were analyzed: among 123 wus-1 heterozygotes, the only two that were homozygous wild type for HY3 were also homozygous wild type for PY, indicating that the chromosomal order is WUS, HY3 and PY.

Double mutant analysis

Organ numbers were counted in fully developed, open flowers. Rare cases of fused flowers were excluded. wus-1 ap1-1 and wus-1 clv1-4 double mutants were analyzed among the progeny of ap1-1 and clv1-4 homozygotes, respectively, that segregated the wus mutant. For the other cases, after preselection for the wus phenotype at the seedling stage, double mutants were identified as about 1 in 4 F2 plants. wus-1 pi-1 plants were identified by flowers with sepals in the second whorl and wus-1 ag-1 plants by flowers with petals in the third whorl.

Histological analysis

Tissues were fixed overnight at 4°C in 50% ethanol, 5% acetic acid and 3.7% formaldehyde. Dry seeds were soaked in ice-cold water for several hours, autoclaved for 5 minutes and the seed coat was subsequently removed before fixation. The tissue was dehydrated by one-hour treatments of 60%, 70%, 80%, 90% and three repeats of 100% ethanol. Infiltration was carried out at room temperature for 1 hour each with 25%, 50%, 75% and three times 100% Spurr’s resin (Spurr, 1969) in ethanol. The tissue-containing vials were left open in the chemical hood overnight to remove traces of ethanol and the resin was hardened for 12 hours at 65°C. Serial sections, 1 μm thick were cut with a Reichert-Jung microtome, transferred to polylysine-coated slides and stained with 0.01% toluidine-blue/0.1% borate for 10 (seeds) or 30 seconds (root, seedlings) at 75°C. When necessary, excess dye was rinsed off with ethanol. Photographs were taken with Kodak Gold 100 film and the contrast was adjusted with the Photoshop 3.0 computer program.

Fluorescence analysis and scanning electron microscopy

DAPI staining and fluorescence analysis were done as previously described (Hülskamp et al., 1994). For scanning electron microscopy, seedlings and flowers were fixed overnight at 4°C in 3% glutaraldehyde in PBST (130 mM NaCl, 70 mM Na2HPO4, 30 mM NaH2PO4, 0.01% Tween 20). The tissue was washed twice for 30 minutes in cold PBST, post-fixed for 2 hours in ice-cold 1% Os04/PBST, washed once for 30 minutes in water and dehydrated by one-hour treatments each with 30%, 50%, 70%, 90% and three times 100% Spurr’s resin (Spurr, 1969). The excess dye was rinsed off with ethanol. Photographs were taken with Agfapan APX 25 film and the contrast was adjusted using the Photoshop 3.0 computer program.

RESULTS

Isolation of wuschel mutants

The WUSCHEL (WUS) gene was identified by the isolation of two recessive allelic mutants (see Materials and Methods) which, in contrast to wild type, did not produce leaves 7 days after germination (Fig. 1A,B). While the wild-type shoot meristem gave rise to a rosette of leaves (Fig. 1C) and, after floral transition, to the inflorescence (Fig. 1E), wus mutants repetitively initiated defective shoot meristems, which gave rise to only a few leaves and then discontinued primordia initiation (Fig. 1D). Eventually, disorganized bunches of leaves were observed at the base and the tip of several stems in mutants (Fig. 1F). Mutant flowers were rare and differed from wild type in that they lacked most central organs and terminated most often in a single central stamen (Fig. 1G,H). The WUS gene was mapped close to the HY3 gene on chromosome 2 (see Materials and Methods). Since the two alleles, wus-1 and wus-2, appeared phenotypically indistinguishable, we performed most of our detailed studies on wus-1.

wus plants develop in a stop-and-go mode

Vegetative development

Under our growth conditions, the first leaves of wild-type seedlings are easily visible about 4 days after germination (Fig. 2A). Subsequently, the vegetative shoot meristem attains a convex structure (Fig. 2B). Successive leaf primordia initiation is restricted to the periphery of the shoot meristem and occurs in a spiral phyllotaxis to give rise to a basal rosette. By contrast, 7-day-old wus seedlings appeared to lack a shoot meristem or leaf primordia but displayed a flat and enlarged apex (Fig. 2C). Mutant seedlings eventually initiated between one and four leaf primordia about 14 days after germination (Fig. 2D). However,
subsequent development was discontinued and as before a flat enlarged apex was apparent (Fig. 2E; we refer to mutant apices that discontinued successive organ formation typical of wild-type shoot meristems as ‘terminated apices’). About 1 week later, numerous leaf primordia and secondary shoot meristems were initiated ectopically across the terminated apex, including its central region (Fig. 2F). Shoot meristems also emerged from the axils of leaves and cotyledons (Fig. 2G), indicating a loss of apical dominance in \textit{wus} shoots (see Discussion). \textit{wus} secondary shoot meristems, before termination, resembled wild-type shoot meristems (Fig. 2H). Some secondary shoot meristems initiated in \textit{wus} plants older than 1 month produced rosettes of leaves similar to the wild-type rosette, suggesting that the mutant defect was less pronounced at that stage. The \textit{wus} ‘stop-and-go’ development eventually resulted in plants with an disorganized bunch of up to one hundred leaves at their base (Fig. 1F).

\textbf{Inflorescence development}
After rosette formation, the wild-type shoot meristem undergoes a transition from vegetative to inflorescence development (Fig. 2I). About 3 weeks after germination the plant forms a stem, 2-3 cauline leaves with indeterminate shoot meristems in their axils and bractless, determinate floral meristems (Fig. 2J). The majority of \textit{wus} plants gave rise to stems 2-4 months after germination. \textit{wus} inflorescence meristems, however, showed a similar defect to \textit{wus} vegetative meristems: they terminated prematurely in a flat structure of variable size after 2-3 leaves had been formed (Fig. 2K). Similarly to the mutant vegetative development, repetitive initiation of defective inflorescence meristems across the terminated apices gave rise to disorganized bunches of cauline leaves along the plant (Fig. 1F). \textit{wus} inflorescence meristems occasionally gave rise to 1-5 flowers before termination occurred (Fig. 2L). The flowers were located at the very tip of the stem and the internodes were not elongated as in wild type.

The root, including the root meristem, and the majority of organs in \textit{wus} plants were indistinguishable from wild type (data not shown). There were a few exceptions, however: \textit{wus} cotyledons were slightly enlarged, a minority of mutant leaves were deformed, \textit{wus} stems infrequently were flattened, mutant floral organs occasionally were chimeric and \textit{wus} anthers released very little pollen (data not shown).

\textbf{Fig. 1.} Postembryonic development of \textit{wuschel} plants. (A,C,E,G) wild type; (B,D,F,H) \textit{wus-1}. (A,B) 7-day-old seedlings. Young leaves (l) are visible in the wild type, but not in the mutant. (C,D) 2-week-old plants stained with DAPI (see Material and Methods). The brightness of the signal reflects the number of nuclei in a region; proliferating regions consist of small cells and result in a bright signal. (C) Successively younger rosette leaves (l) are visible in wild type, with the youngest leaf primordia being located in the center (arrow). (D) \textit{wus-1} plant with cotyledonary petioles (p) and two leaves (l). No succession of leaf primordia is visible, only a flat apex (arrow). (E,F) Adult phenotypes. (E) 6-week-old wild-type plant with cauline leaves (c), siliques and flowers. (F) An approx. 3-month-old \textit{wus-1} plant with bunches of leaves at different positions along the plant. (G,H) Flowers with the four sepals removed. (G) Wild-type flower with petals, stamens and the central gynoecium. (H) In the \textit{wus-1} flower only one central stamen is present. Bars: (A,B,G,H) 1 mm; (C,D) 500 \mu m; (E,F) 5 cm.
In summary, our results showed that mutations in the WUS gene result in premature termination of both vegetative and inflorescence meristems. Therefore, maintenance and structural integrity of the shoot meristem, but not the root meristem nor organ formation appear to be defective in wus mutants.

**Shoot meristem organization is altered in wus mutants**

In order to determine the cellular basis of the defects in wus shoot meristems, we analyzed mutant apices at the histological level.

**Embryo development**

The shoot meristem can be recognized in wild-type embryos as early as the torpedo stage and becomes most distinct in the mature embryo as a group of small, intensely staining cells with large nuclei and without large central vacuoles (Fig. 3A). By contrast, no wild-type shoot meristem was recognized at any stage of wus embryo development, which was most evident in mature embryos (Fig. 3B). Rather, the corresponding position was occupied by a few cells that were slightly larger, more vacuolated and lacked prominent nuclei compared to the wild-type shoot meristem, suggestive of an aberrant apex organization. Thus, wus shoot meristem development appears to be defective at the earliest recognizable stages during embryogenesis.

**Postembryonic development**

In wild-type seedlings, the shoot meristem and the first two leaf primordia are visible within 2 days after germination (Fig. 3C). Subsequently, the convex shoot meristem has two distinct outer cell layers (Fig. 3D), suggestive of a tunica-corpus organ-
ization. Periclinal cell divisions in the subepidermal layer are restricted to the meristem periphery where primordia are initiated, while cells in the central region stain less intensely and are slightly larger than those at the flanks (Vaughan, 1955). By contrast, no convex shoot meristem or leaf primordia were recognized in wus seedlings up to 10 days after germination (Fig. 3E). The mutant apex was flat and had two outer cell layers. The cells in the wus apex were slightly larger and stained less intensely than cells of a wild-type shoot meristem, but were smaller and less vacuolated relative to differentiated cortex or epidermal cells. Periclinal cell divisions in the subepidermal layer appeared to occur ectopically across the apex, including the central region (Fig. 3E, arrow). After two leaf primordia were eventually initiated, there was often no recognizable meristem found between the primordia (Fig. 3F).

Mutant apices at later stages showed no similarity to wild-type shoot meristems: they were enlarged and flat, and the cells resembled those of the seedling apex (Fig. 3G). Occasionally, bulges of intensely staining small cells emerged from the mutant apices (Fig. 3H).

Taken together, our results indicate that at all development stages examined, the mutant apex organization was aberrant and no histological differences between central and peripheral regions within the apex were evident.

**wus** floral meristems terminate prematurely

Wild-type floral meristems are determinate and produce four whorls of organs; four sepals, four petals, six stamens and two fused carpels, respectively (Fig. 4A). By contrast, wus flowers lacked most of the central organs, stamens and carpels, and generally terminated in a single central stamen (Fig. 4D; Table 1). Occasionally, flowers without stamens and others with up to three central stamens were found. We never observed carpels, carpeloid tissue or ‘unused’ meristem in wus-1 flowers (data not shown). By contrast, the number of organs in the outer two whorls were on average only slightly reduced compared to wild type (Table 1). Similarly to the organs derived from the shoot meristem, organ development once initiated by the floral meristem was generally not affected by the wus mutation. Thus, like shoot meristems, wus floral meristems terminated prematurely, but gave rise to organs in a manner indistinguishable from wild type prior to termination.

**Double mutant combinations of wus with flower mutations**

What is the basis for the defects in wus-1 floral meristems? Since wus-1 inflorescence meristems gave rise to flowers very infrequently, a systematic morphological analysis of early flower development was not feasible. However, once wus-1 flowers were observed, their phenotypes could be scored quantitatively by counting organ numbers. Furthermore, a number of well-defined flower mutants were available (for review see Ma, 1994), providing the opportunity to genetically study the role of the WUS gene during floral development. We thus analyzed several double mutant combinations of wus-1 with

<table>
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<tr>
<th>Genotype</th>
<th>n</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
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<td>Wild type</td>
<td>10</td>
<td>4.0±0.0</td>
<td>4.0±0.0</td>
<td>6.0±0.0</td>
<td>2.0±0.0</td>
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<tr>
<td>ag-1</td>
<td>10</td>
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<td>5.7±0.5</td>
<td>4.4±1.1</td>
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<tr>
<td>clv1-4</td>
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<td>4.3±0.6</td>
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<td>wus-1</td>
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<tr>
<td>wus-1 clv1-4</td>
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<td>4.1±0.7</td>
<td>3.8±0.8</td>
<td>1.2±0.7</td>
<td>0.0±0.0</td>
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Average values with standard deviations are presented. n, numbers of flowers analyzed.

**Table 1. Floral organ numbers of wus-1 ag-1 and wus-1 clv1-4 double mutants**

Fig. 3. Median sections through wild-type and wus shoot apices. (A,C,D) Wild type; (B,E-H) wus-1 mutant. (A,B) Mature embryos. (A) The shoot meristem (arrow) is distinct in wild type. (B) No shoot meristem is visible in mutant embryos. (C) 2-day-old wild-type seedling with the primordia of the first leaves and the shoot meristem (arrow). (D) 15-day-old wild-type seedling. Two leaf primordia (p) and the convex vegetative shoot meristem (arrow) are visible. (E) 7-day-old mutant seedling. The apex is flat and no primordia are present. Periclinal cell divisions appear to have occurred in the center (arrow). (F) 2-week-old mutant seedling. The first two leaf primordia appear without interspace; compare with C. (G,H) An approx. 2-month-old mutant. (G) The terminated mutant inflorescence apex is flat and resembles the vegetative apex structure; compare with E. (H) Bulge of intensely staining cells emerging from a flat inflorescence apex. Bars: 20 μm.
flower mutations that alter identities or numbers of floral organs.

In *agamous-1 (ag-1)* flowers the outer two whorls are wild type, but third-whorl stamens are replaced by petals, and fourth-whorl carpels by new flowers, resulting in an indeterminate floral meristem (Fig. 4B; Bowman et al., 1989). *wus-1 ag-1* flowers terminated prematurely in a central, third-whorl petal (Fig. 4E). Third-whorl organ numbers in *wus-1 ag-1* flowers were more variable but on average only slightly increased compared to *wus-1* flowers (Table 1). Therefore, termination of *wus* floral meristems appears to occur irrespective of whether stamen or petals are specified in the third whorl.

In *pistillata-1 (pi-1)* flowers (Fig. 4C), first-whorl and fourth-whorl organs are wild type, but second-whorl petals are transformed into sepals and most third-whorl stamens are missing, with the cells that would normally give rise to stamens presumably being incorporated into the gynoecium (Bowman et al., 1989; Hill and Lord, 1988). *wus-1 pi-1* flowers formed sepals in the second whorl and terminated either without a central organ or in a single central carpel, indicating that *WUS* is not required for carpel development. In rare cases two partially fused carpels were observed (Fig. 4F). In two cases two carpels appeared completely fused but were deformed and attempts to demonstrate fertility were unsuccessful (data not shown). Carpels were not observed in mutant flower combinations between *wus-1* and *apetala3-1*, which as a single mutant differed from *pi-1* flowers under our growth conditions only by the presence of third-whorl stamens or carpeloid stamens.

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**Fig. 4.** *wus-1* flowers in different genetic backgrounds. (A-C,G-I) *WUS* control. (D-FJ-L) *wus-1*. (A,D) Wild-type background. (A) *WUS*. Petals, stamens and gynoecium are visible. Sepals were removed. (D) *wus-1* flowers lack most central organs and terminate in a central stamen. (B,E) *ag-1* background. (B) In *ag-1*, petals replace stamens and new flowers replace the gynoecium. Several sepals and petals facing the front were removed. (E) *wus-1 ag-1* flowers terminate in a central petal. Sepals were removed. (C,F) *pi-1* background. *pi-1* flowers (C) have sepals in the second whorl and lack third-whorl organs. First-whorl sepals were removed. (F) *wus-1 pi-1* flower with two partially fused carpels. Four sepals each in the first and the second whorl were removed. (G,J) *ap1-1* background. (G) *ap1-1* flower with bract-like first-whorl organs and an axillary flower. No petals are present. (J) *wus-1 ap1-1* flower. Two leaf structures encompassing a single stamen. (H,K) *sup-6* background. (H) *sup-6* flowers with supernumerary stamens and reduced carpels. Sepals and petals were removed. (K) Typical flower of *wus-1* mutants among the progeny of heterozygous *sup-6/+* plants. The flower terminates prematurely in a single stamen as in *wus-1* shown in D. (L) *clv1-4* background. (I) *clv1-4* flower with supernumerary organs. Five petals and five of a total of six carpels are visible. (L) *wus-1 clv1-4* flower which terminates prematurely in a single stamen as in *wus-1* shown in D. (A-D,F-I,K,L) SEMs; (E,J) Live plant. Bars: 500 μm.
phenotype appears not to be severely affected by the mutation. Third-whorl-domain size is measured in absolute terms and cells are being completely consumed; no fourth-whorl domain is established. (b) Proportional partitioning mechanism. Cells are allocated into third and fourth-whorl domains, proportionally reduced in size relative to wild type.

(Bowman et al., 1989; T. L., unpublished data). Thus, carpels were formed in wus floral meristems if no stamens were initiated in the pi-1 background.

The apetalal-1 (ap1-1) mutation results in a partial transformation of flowers into inflorescence shoots and disruption of first-whorl and second-whorl organ development, while third-whorl and fourth-whorl organs are wild type (Fig. 4G; Bowman et al., 1993; Mandel et al., 1992). Most wus-1 ap1-1 plants, 27 out of 33, lacked any floral structures, suggesting that WUS is required for floral meristem initiation in the ap1-1 background. The remaining six double mutants displayed a total of 14 extremely reduced flowers consisting of one or two sepaloid leaves, sometimes with carpeloid tissue at their margins and often accompanied by thin filaments (data not shown), and two flowers, consisting of a single stamen, surrounded by nectaries, filaments and two sepaloid leaves (Fig. 4J). Thus, wus-1 ap1-1 double mutants display floral phenotypes that appear to be more severe than the phenotype expected from a simple combination of floral defects observed in ap1-1 and wus-1 single mutants (see Discussion).

Mutations in the SUPERMAN (SUP) gene result in an increased number of stamens to a maximum of 26 relative to 6 in wild-type flowers whereas carpels are reduced in number or are malformed (Fig. 4H; Bowman et al., 1992). By contrast, first and second whorls of sup flowers are normal. In a population that segregated the wus-1 and the sup-6 mutations, no mutant phenotype other than wus-1 or sup-6 was found (data not shown). In 63 wus-1 mutant plants identified at the seedling stage, all flowers examined displayed a wus-1 phenotype and terminated prematurely (Fig. 4K; Table 2). Thus, the wus-1 phenotype appears not to be severely affected by the sup-6 mutation.

cvl1 flowers have an increased number of central organs, stamens and carpels, relative to wild type (Fig. 4I; Clark et al., 1993; Leyser and Furner, 1992), thus displaying an opposite phenotype to wus flowers. We did not observe any obvious difference between wus-1 cvl1-4 and wus-1 mutants. The double mutant flowers terminated prematurely, most often in a single stamen (Fig. 4L). Although third-whorl stamen numbers in wus-1 cvl1-4 flowers were more variable and on average slightly increased compared to wus-1 flowers (Table 1), the wus-1 floral phenotype appears to be essentially unaltered by the cvl1-4 mutation.

In summary, premature termination of floral meristems due to the wus-1 mutation appeared unaffected in all double mutant combinations examined.

**DISCUSSION**

The shoot meristem is established during embryogenesis and is maintained by self-renewal throughout plant development.

**Fig. 6.** Central identity of shoot and floral meristems. (Early) Meristem primordia with central (C) and peripheral (P) anlagen in wild type. In wus plants, central anlagen (?) are defective. (Shoot) Organs are initiated successively on the periphery (P) of wild-type shoot meristem, which is renewed by the central zone (C; arrows). No renewal of periphery but termination in enlarged structures occurs in wus apices (?). (Flower) The determinate wild-type floral meristem is consumed during the initiation of a defined number of primordia in four concentric whorls. Early central anlagen are drawn to give rise to inner two whorls (stamens and carpels). However, the actual contribution is unclear. The wus floral meristem is consumed prematurely; the region derived from the early central anlage is reduced.

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**Table 2. Frequency of stamen numbers in wus-1 and putative wus-1 sup-6 flowers**

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<th>Genotype</th>
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<td>130</td>
<td>5</td>
<td>85</td>
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</table>

†Progeny of selfed wus-1+/ sup-6/+ plants, preselected for wus-1 phenotype at the seedling stage.

The total numbers of plants and flowers examined and the frequency as percentage of flowers with 0, 1, 2 or 3 stamen are presented.
While giving rise to the shoot (for review see Steeves and Sussex, 1989). What are the regulatory mechanisms that underlie shoot meristem maintenance and integrity? Recessive mutations in the Arabidopsis WUS gene result in premature termination of shoot and floral meristems, whereas the root meristem is not visibly affected. The similar defects in embryonic and postembryonic shoot meristems as well as floral meristems of wus plants suggest that there are common aspects of development in these two meristem types. We propose that mutations in the WUS gene primarily affect central regions in shoot and floral meristems, as this interpretation appears to account for all aspects of the wus-I phenotype observed. Here we discuss (1) the evidence for our interpretation and (2) the possible function of the WUS gene during wild-type development.

The WUS gene is required for shoot meristem integrity and maintenance

Two functions have been attributed to the central zone of the shoot meristem, self-renewal and regulation of meristem integrity (for review see Steeves and Sussex, 1989). Self-renewal of the shoot meristem is believed to be a function of stem cells that reside in the central zone and give off daughter cells to replenish the adjacent zones, flank and rib meristems. Regulation of shoot meristem integrity by the central zone has been inferred from surgical experiments (for review see Steeves and Sussex, 1989). For example, mechanical destruction of the shoot meristem summit resulted in adventitious meristem. (ii) Primordia initiation and periclinal cell division of mutant shoot meristems. Second, the organization of shoot meristem identity required for indeterminate development is different. Since only limited information can be obtained from histological analyses of mutant embryos, examination of postembryonic phenotypes may be helpful to infer the specific functions of a given gene in shoot meristem development. The fact that wus seedling apices eventually give rise to a few leaf primordia before termination occurs indicates that lateral anlagen of the shoot meristem are formed in wus apices, either in the embryo or thereafter, whereas central shoot meristem identity required for indeterminate development appears absent.

The WUS gene affects central floral meristem formation

What is the basis for the wus defect in floral meristems? In all mutant combinations examined the wus defect was essentially restricted to the center of the floral meristem, while the periphery was unaffected, suggesting that WUS is essential for development of the floral meristem center. Our double mutant analyses indicate that premature termination of wus-I floral meristems occurs irrespective of organ identity and that WUS is not required for carpel development. In wus-I pi-I flowers, the floral meristems apparently reallocated cells that in wus-I would give rise to stamens into carpel primordia. Therefore, precocious consumption of the central region during definition of the third-whorl domain appears to be the basis of the wus floral phenotype.

Other developmental aspects affected in wus mutants appear to be consequences of the primary defect in the shoot meristem. For example, the precocious initiation of meristems in the axils of leaves and cotyledons suggests a lack of apical dominance by the shoot apex, consistent with the lack of a functional apex in wus plants.

The WUS gene affects shoot meristem development in the embryo

The shoot meristem comprises a few dozens cells and multicellular anlagen for the first two leaves in the mature Arabidopsis embryo (Furner and Pumfrey, 1992; Irish and Sussex, 1992). By contrast, no conspicuous shoot meristem was found at the corresponding position in wus embros; instead a small number of abnormal cells resembling those of the flat apex of wus seedling were observed. Thus, the shoot meristem appears to be initiated in wus embryos, but to display a defective organization at the mature embryo stage. Mutations in several genes other than WUS, such as STM (Barton and Poethig, 1993; K. Endrizzi and T.L., unpublished data) and ZLL (Jürgens et al., 1994; B. Moussian and T.L., unpublished data) can result in similar embryonic phenotypes, although their postembryonic development is different. Since only limited information can be obtained from histological analyses of mutant embryos, examination of postembryonic phenotypes may be helpful to infer the specific functions of a given gene in shoot meristem development. The fact that wus seedling apices eventually give rise to a few leaf primordia before termination occurs indicates that lateral anlagen of the shoot meristem are formed in wus apices, either in the embryo or thereafter, whereas central shoot meristem identity required for indeterminate development appears absent.
explained by the combination of the respective single mutant floral phenotypes in addition to defective inflorescence development caused by the wus-1 mutation.

In summary, our results indicate that in wild type, WUS is required for the formation of the floral meristem center, irrespective of how the cells will develop under the control of flower organ identity genes. Allocation of cells into third and fourth-whorl domains appears not to occur by proportional partitioning but rather in a ‘first-come-first served’ manner: only those cells, that have not been incorporated into the third-whorl domain after it has been defined to its normal size are available for carpel development (Fig. 5). It is tempting to speculate that the same mechanism operates during the definition of all four whorls of the Arabidopsis flower and that the floral meristem is consumed in an acropetal sequence. Our interpretation is not to be confused, however, with a sequential specification of whorl identity in which positional information of one whorl is provided by the developing adjacent whorl and which appears to contradict many observations (Meyerowitz et al., 1989).

Variability of the wus phenotype

While the wus defects were always obvious in embryos, seedlings and flowers, wus plants older than one month initiated secondary shoot meristems that gave rise to leaf rosettes resembling the wild-type rosette. What is the basis of the variable requirements of WUS activity during plant development? One explanation is that there are other genes encoding partially overlapping functions with WUS, similar to the partial redundancy observed between LEAFY and AP1 functions in floral meristem specification (Bowman et al., 1993; Weigel et al., 1992).

The wus-1 floral phenotype appears to be epistatic to ag-1 and clv1-4 regarding floral meristem termination, but due to the variability of organ numbers we cannot exclude the possibility that the double mutants represent additive functions. The variability was higher in wus-1 ag-1 and wus-1 clv1-4 flowers than in wus-1 flowers. While the significance of this observation is unclear, one possible explanation for it is that the accumulation of defective functions, each of which leads to specific developmental defects, may result in a general decrease of regulatory stringency.

Does the WUS gene promote central meristem identity?

It is not known whether the two wus mutants identified represent null mutations. However, their very similar phenotype makes it likely that the WUS gene activity is reduced or even eliminated. The similar defects in wus shoot and floral meristems suggest that both meristems share common organizational and functional aspects for which WUS function is required, i.e. central identity. This interpretation is consistent with the view that floral meristems are specialized shoot meristems of determinate growth (for review see Steeves and Sussex, 1989). The different consequences of the wus mutations in shoot and floral meristems can be explained by different roles of the central region in the two meristems (Fig. 6). In the indeterminately growing shoot meristem, the central zone appears to be required for maintenance of structural and functional integrity and these aspects are defective in wus mutants.

What is the role of central identity in determinate floral meristems? We propose that central identity is instrumental for growth of the floral meristem to its final size, allowing for the initiation of the appropriate number of primordia, and this aspect is affected in wus flowers. This view is consistent with findings in plant species other than Arabidopsis indicating that the central zone of floral meristems proliferates rapidly before being consumed in central organ primordia initiation (for review see Steeves and Sussex, 1989). WUS thus appears to play a unique role in shoot and floral meristem development which is clearly distinct from that of other genes, such as STM (Barton and Poethig, 1993; K. Endrizzi and T.L., unpublished) and ZLL (Jürgens et al., 1994).

In agreement with the wus-1 clv1-4 double mutant phenotype, WUS resembles a meristem promoting factor that is negatively regulated and restricted to the meristem center by CLV genes (Clark et al., 1995). But the phenotypes observed also allow for a different conclusion, which is that WUS and CLV1 act at different regulatory levels, with WUS promoting central meristem identity and CLV1 negatively regulating cell proliferation within this region. The isolation of the WUS gene, currently in progress, should enable us to test our hypotheses as well as current models for shoot meristem development.

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