The **CLAVATA** and **SHOOT MERISTEMLESS** loci competitively regulate meristem activity in *Arabidopsis*

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**SUMMARY**

The **CLAVATA** (CLV1 and CLV3) and **SHOOT MERISTEMLESS** (STM) genes specifically regulate shoot meristem development in *Arabidopsis*. CLV and STM appear to have opposite functions: clv1 and clv3 mutants accumulate excess undifferentiated cells in the shoot and floral meristem, while stm mutants fail to form the undifferentiated cells of the shoot meristem during embryonic development. We have identified a weak allele of *stm* (stm-2) that reveals STM is not only required for the establishment of the shoot meristem, but is also required for the continued maintenance of undifferentiated cells in the shoot meristem and for proper proliferation of cells in the floral meristem. We have found evidence of genetic interactions between the CLV and STM loci. clv1 and clv3 mutations partially suppressed the *stm-1* and *stm-2* phenotypes, and were capable of suppression in a dominant fashion. clv *stm* double mutants and plants homozygous for *stm* but heterozygous for *clv* while still lacking an embryonic shoot meristem, exhibited greatly enhanced postembryonic shoot and floral meristem development. Although *stm* phenotypes are recessive, *stm* mutations dominantly suppressed *clv* homozygous and heterozygous phenotypes. These results indicate that the *stm* phenotype is sensitive to the levels of CLV activity, while the *clv* phenotype is sensitive to the level of STM activity. We propose that these genes play related but opposing roles in the regulation of cell division and/or cell differentiation in shoot and floral meristems.

Key words: organogenesis, cell division, cell differentiation, pattern formation, **CLAVATA**, **SHOOT MERISTEMLESS**, *Arabidopsis*, meristem

**INTRODUCTION**

In higher plants, organ formation occurs throughout life. Plants must therefore retain a pool of undifferentiated cells by balancing the proliferation of meristem cells with their subsequent incorporation into organ primordia. Plants solve this problem by developing and maintaining a collection of stem cells termed the shoot meristem. The shoot meristem originates during embryogenesis and is later responsible for generating the above-ground portion of the plant. The shoot meristem can be thought of as having two zones, a central zone containing meristematic cells in an undifferentiated state and a surrounding peripheral zone where cells enter a specific developmental pathway toward a differentiated state. This central zone/peripheral zone distinction has been recognized for decades and is based on differences in morphology and cell division rates (Steeves and Sussex, 1989).

The *Arabidopsis* genes CLAVATA1 (CLV1), CLAVATA3 (CLV3) and **SHOOT MERISTEMLESS** (STM) appear to play important roles in the regulation of shoot meristem development. The **CLV** loci (CLV1 and CLV3) appear to act to promote the transition towards differentiation of cells at the shoot and floral meristems, and/or to restrict the proliferation of cells at the center of these meristems (Clark et al., 1993, 1995). clv mutants accumulate massive pools of undifferentiated cells at the shoot meristem and at the normally determinate floral meristem. Root meristems are unaffected. An allelic series exists for CLV1, and there is both an intermediate and a strong allele of **CLV3** (Clark et al., 1993, 1995; Leyser and Furner, 1992), suggesting that the mutants are reduction of function alleles. In general, *clv1* mutants are identical in phenotype to *clv3* mutants and *clv1 clv3* double mutants are identical in phenotype to strong *clv1* or *clv3* single mutants, indicating that these genes function in the same pathway and also suggesting that the strong single mutants may represent nulls for the **CLV** pathway. Some *clv* alleles are recessive while others exhibit slight semidominance. **clv3** alleles dominantly enhance the semidominant **clv1** alleles, indicating that **CLV1** and **CLV3** work closely together to regulate shoot and floral meristem function.

**STM** is a gene required for the initiation of the embryonic shoot meristem in *Arabidopsis* (Barton and Poethig, 1993). *stm-1* mutants never develop an embryonic shoot meristem (the root meristem, however, is normal). Although the *stm-1* mutant shows that **STM** is necessary for the establishment of the shoot meristem, it does not reveal whether **STM** is also necessary for
the continued maintenance or function of the shoot meristem. *STM* has recently been shown to code for a homeodomain-containing protein, implying that it functions as a transcription factor (Long et al., 1996). Because *stm-1* is recessive and has been shown to have a stop codon upstream of the homeodomain (Long et al., 1996), it is likely to be a strong loss-of-function allele. It has been further shown that *STM* expression is limited to the center of the shoot meristem, while it is expressed throughout young floral meristems (Long et al., 1996).

Here we describe interactions among three genes regulating meristem development in Arabidopsis: *STM*, *CLV1* and *CLV3*. Our investigations indicate that *CLV* and *STM* play opposite and perhaps competitive roles in the regulation of meristem activity.

**MATERIALS AND METHODS**

**Mutant lines**

*stm-1*, which was kindly provided by Kathy Barton, and the *stm-2* allele (this study) were both ethyl methanesulfonate-induced in the Landsberg erecta wild type. The origins of *clv* alleles were previously described (Clark et al., 1993, 1995). The strengths of the *clv* mutant alleles from strongest to weakest are: *clv1-4 > clv3-2 > clv3-1 > clv1-1*. Based on the chromosome 1 location of *STM* reported by Barton and Poethig (1993), *STM* lies approximately 20 cM proximal to the centromere from *CLV1*. This is in agreement with limited measurements of 12 cM provided by an analysis of F3 families scored during attempts to generate *clv1 stm* double mutants (data not shown). *CLV3* maps on chromosome 2 (Clark et al., 1995). All plants used in this study were homozygous for *erecta*. Plants were grown as previously described (Clark et al., 1995).

**Tissue and image processing**

Scanning electron microscopy (SEM) was performed as described (Bowman et al., 1989). Confocal laser scanning microscopy (CLSM) of embryos was performed as described (Running et al., 1995). JB-4 infiltration resin was used for plastic sectioning as recommended by the supplier (Polysciences, Inc. Warrington, PA). Negatives and slides were scanned and digitized using either a Nikon Coolscan or a Kodak RFS 2035 slide scanner. Brightness, contrast and color balance were adjusted using Adobe Photoshop 3.0 and figures were printed using a Kodak 8300 Digital Printer.

**RESULTS**

**stm mutations disrupt shoot meristem initiation and maintenance**

*stm-1* mutant embryos have previously been shown to lack an embryonic shoot meristem (Barton and Poethig, 1993). It was also reported that in *stm-1* seedlings, the hypocotyls swelled and, occasionally, leaves emerged from this region. These results indicated that *STM* is required for embryonic shoot apical meristem development, but not for the formation of leaves. To address whether *STM* is also required for postembryonic meristem function, we compared the phenotypes of the original *stm-1* allele and a weak allele that we recently identified, *stm-2*.

As shown in Fig. 1, both *stm-1* and *stm-2* seedlings apparently lacked embryonic shoot meristems as judged by the absence of organ formation between the cotyledons in 5.5-day-old plants (Fig. 1A-C). In our previous work (Running et al., 1995; Clark et al., 1995), we found that meristematic cells of the embryonic shoot meristem correspond with small, densely staining cells visualized by confocal microscopy (Fig. 1G). Confocal imaging of *stm-1* mature embryos revealed no densely staining cells (Fig. 1H; see also Barton and Poethig, 1993). In *stm-2* embryos, no organized shoot meristem was evident, although a variable number of densely staining cells

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Fig. 1. Early phenotypes of *stm-1* and *stm-2* plants. (A–C) 5.5-day-old and (E,F) 16-day-old plants observed by SEM. Compared to (A) wild-type plants, (B) *stm-1* and (C) *stm-2* plants fail to initiate any organs initially, although (E) some *stm-1* and (F) all *stm-2* plants eventually develop organs from the fused cotyledon (FC) region. (D) A 2 μM plastic cross section of the FC region of a 5.5-day-old *stm-1* seedling, stained with toluidine blue. (G–I) Mature embryos observed with confocal microscopy. Among the seeds of an *stm-1/+* plant, the majority of embryos displayed a normal shoot meristem (SAM in G) and presumably had the genotypes *stm-1/+ or +/-*. 5 of the 21 embryos examined, however, lacked any evidence of a shoot meristem and presumably represented the *stm-1* homozygous seeds (H). Similarly, for seeds from an *stm-2/+* parent, 9 of 29 embryos examined lacked an identifiable shoot meristem (I), although a number of brightly staining cells were visible above the junction of the cotyledon vascular elements (vas). Bars, 100 μm (A), 50 μm (B,C) and 200 μm (E,F). G–I shown at same magnification. cot, cotyledon; hyp, hypocotyl; vas, vascular bundles; L, leaf.
was observed above the intersection of the cotyledon vascular elements (Fig 11), a position similar to that occupied by the wild-type shoot meristem. These brightly staining cells were not observed in \textit{stm-1} embryos. Thus differences between \textit{stm-1} and \textit{stm-2} development were observed as early as the mature embryo stage.

In \textit{stm-1} seedlings, the region between the cotyledons and the hypocotyl was enlarged relative to wild-type (Fig. 1A,B). In \textit{stm-2} mutants, this enlargement was not as pronounced (Fig. 1C). This swollen region corresponds to an area of fused cotyledon petioles and not the hypocotyl. Cell morphology of this fused cotyledon (FC) region was similar to that of the cotyledon petiole, but different from that of the hypocotyl (data not shown). An analysis of vascular bundles in the FC region indicated they are contiguous with those of the cotyledon petioles and merge at the junction between the FC region and hypocotyl (data not shown). In addition, cross-sections of the FC region indicate that the petioles only fuse at the edges (Fig. 1D).

Most of the \textit{stm-1} plants died without producing leaves, but 14% in one experiment and 32% in a second experiment eventually gave rise to a ‘rescued’ phenotype (Table 1; Fig. 1E). A rescued phenotype was scored as any plant in which leaves were able to develop from the axils of the cotyledons. As previously reported (Barton and Poethig, 1993), these leaves were not organized in a manner reminiscent of the rosettes seen in wild-type. No tissues resembling meristems were found in dissected 16 day-old rescued \textit{stm-1} plants (Fig. 2A). The rescued \textit{stm-1} plants would often continue to grow for months. In these plants, single leaves appeared to develop from axils or petioles of the leaves that developed from the cotyledons and this process would reiterate several times (Fig. 3A,B). Only rarely would a set of leaves form in the axil of a leaf in a pattern suggesting a meristem (i.e., multiple leaves originating from one point) and only once was a stem, suggestive of an inflorescence, observed out of the hundreds of plants analyzed (data not shown).

The shoot development observed in \textit{stm-2} plants was greater

<table>
<thead>
<tr>
<th>Genotype of parent</th>
<th>Number of plants with initial \textit{stm} phenotype*</th>
<th>Percentage rescued†</th>
</tr>
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<tbody>
<tr>
<td>\textit{stm-1/+}</td>
<td>Scored at 16 days</td>
<td></td>
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<tr>
<td>\textit{clv1-1 +/+ stm-1}</td>
<td>106</td>
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<td>61</td>
</tr>
<tr>
<td>\textit{stm-2/+}</td>
<td>Scored at 16 days</td>
<td></td>
</tr>
<tr>
<td>\textit{stm-1/-+}</td>
<td>19</td>
<td>32</td>
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<tr>
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<td>100</td>
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<td>0</td>
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<td>12</td>
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<td>50</td>
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<td>\textit{clv3-2 sm-2/-}</td>
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<td>82</td>
</tr>
<tr>
<td>\textit{clv1-4 sm-2/-}</td>
<td>44†</td>
<td>100</td>
</tr>
</tbody>
</table>

*Populations were initially scored four days after transferring plants from 4°C to 22-24°C. This column shows the number of seedlings with an \textit{stm} phenotype (no leaves developing in the axils of the cotyledons).
†Percentage of plants with an early \textit{stm} phenotype which later exhibited a ‘rescued’ phenotype (leaf development visible, see Figs 1E,F, 3A-C).
‡The wild-type plants did not have an \textit{stm} phenotype, but are included for comparison. The populations included in each experiment (I, II, and III) were grown side by side under identical conditions.

\textit{clv} \textit{stm-1} seedlings develop meristems. (A,C,F) 16-day-old and (B) 14-day-old seedlings observed by SEM. (A) Dissection of an \textit{stm-1} seedling that has developed leaves fails to reveal a structure resembling a shoot meristem. In contrast, \textit{clv} \textit{stm-1} seedlings do develop identifiable meristems. The \textit{clv3-2} \textit{stm-1} seedling in D is shown partially (E) and completely (F) dissected to reveal a single shoot meristem (SM) initiating several leaf primordia (LP). The meristem of a similarly dissected \textit{clv1-4} \textit{stm-1} seedling is shown in C. A wild-type meristem is shown in B. cot, cotyledon; FC, fused cotyledon; FP, floral primordia; hyp, hypocotyl; ASM, axillary shoot meristem; DL, dissected leaf. Bars, 50 \(\mu\)m (A,E) and 20 \(\mu\)m (B,C,F).
than that seen in \textit{stm-1} plants. First, leaves developed from the FC region of \textit{stm-2} plants as early as 5.5 days after sowing, earlier than that observed in \textit{stm-1} plants (Table 1). By 16 days of development, while less than a third of \textit{stm-1} plants exhibited the rescued phenotype, all \textit{stm-2} plants had visible leaves (Fig. 1F, Table 1). \textit{stm-2} initial vegetative shoot development ranged from two leaves with no further growth to arrays more similar to the rosettes that are found on wild-type plants (Fig. 3C). However, these meristems did not produce inflorescence stems and apparently terminated in leaf structures. New meristems then formed in the axils of the rosette leaves (Fig. 4A). While most of these meristems also terminated prior to flowering, some went on to produce inflorescence stems bearing several cauline leaves and a variable number of flowers, usually less than five (Fig. 3C,H inset). These inflorescence meristems then terminated in floral structures (described below). Regardless of whether \textit{stm-2} shoots terminated during vegetative or inflorescence development, new growth occurred from the axils of leaves, reiterating the process and giving rise to plants with a bushy appearance (Fig. 3C). Thus, while \textit{stm-2} plants exhibited more extensive shoot development than \textit{stm-1} plants, the shoots produced by \textit{stm-2} plants were not indeterminate structures as are found in wild-type plants.

\textbf{\textit{stm-2} affects floral meristem structure} \\
Although flowers were never observed on \textit{stm-1} plants, \textit{stm-2} plants produced inflorescence stems bearing several flowers. These flowers exhibited defects in floral meristem structure; inner organs were either absent or present in reduced numbers (Table 2). Specifically, a nearly normal number of sepals developed in the outermost whorl 1, a reduced number of petals and stamens developed in whorls 2 and 3, respectively, and very few carpels were present in whorl 4. Although most of the floral organs formed in \textit{stm-2} flowers were normal, some were fused together and some were mosaics with characteristics of two or more floral organ types (Table 2). The flowers at the tip of a terminated inflorescence meristem were often partially fused with each other, reminiscent of inflorescences of plants mutant for \textit{terminal flower} (Alvarez et al., 1992; Shannon and Meeks-Wagner, 1991). These partially fused flowers contained organs with carpel characteristics more often than did the solitary flowers (Table 2). \textit{stm-2} plants, therefore, have floral meristems that fail to produce a full complement of floral organs.

\textbf{clv mutations increase the frequency and speed of postembryonic leaf development in \textit{stm} mutants} \\
We compared the frequency of the rescued phenotype among the \textit{stm} homozygous progeny in three separate experiments. In the first, we found that the \textit{stm} homozygous plants exhibited a rescued phenotype more often in progenies of \textit{clv1 +/+} \textit{stm-1} or \textit{clv3/+} \textit{stm-1/}+ plants than in the progeny of \textit{stm-1/}+ plants (Table 1). Because most of the plants in these populations are heterozygous for \textit{clv}, this suggests that \textit{clv1} or \textit{clv3} heterozygosity increases the frequency of leaf development in \textit{stm-1} plants.

In the second experiment, \textit{clv} \textit{stm-1} double mutant plants were examined. In this case, plants with an initial \textit{stm} phenotype among the progeny of \textit{clv/clv} \textit{stm-1/+} plants were compared to the progeny of \textit{stm-1/+} and \textit{stm-2/+} plants (Table S. E. Clark and others

\textbf{Fig. 3.} Phenotypes of mature \textit{stm-1}, \textit{stm-2} and \textit{clv} \textit{stm} plants. (A,B) Mature \textit{stm-1} and (C and H inset) \textit{stm-2} plants are presented. (D) The initial rosette of leaves of a \textit{clv 1-1} \textit{stm-1} plant and (E) new rosettes of leaves formed in the older leaf axils of a single \textit{clv1-1} \textit{stm-1} plant are shown. Inflorescence shoots (IM) of (F,G) \textit{clv} \textit{stm-1} and (H,I) \textit{clv} \textit{stm-2} are presented. Note the fasciated stems (fas). SM, shoot meristem. Bar in I, 200 \textmu m.
A total of 43 solitary flowers occupying the first 5 positions on the inflorescence stem from 15 stm-2 plants were analyzed. The fused flowers which terminate the inflorescence stem were not included. The stereotypic number of wild-type organs numbers are shown to the right.

Table 2. Floral organs produced by stm-2 plants

<table>
<thead>
<tr>
<th>Organ type</th>
<th>Mean number per flower</th>
<th>Wild-type Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepal**</td>
<td>3.88</td>
<td>4</td>
</tr>
<tr>
<td>Petal</td>
<td>0.47</td>
<td>4</td>
</tr>
<tr>
<td>Petal mosaic†</td>
<td>0.16</td>
<td>0.26</td>
</tr>
<tr>
<td>Stamen</td>
<td>2.30</td>
<td>6</td>
</tr>
<tr>
<td>Fused stamen‡</td>
<td>0.70</td>
<td>0.19</td>
</tr>
<tr>
<td>Filament†</td>
<td>0.26</td>
<td>2</td>
</tr>
<tr>
<td>Carpel**</td>
<td>0.19</td>
<td>2</td>
</tr>
<tr>
<td>All organs</td>
<td>7.98</td>
<td>16</td>
</tr>
</tbody>
</table>

*Includes single sepals and fused sepals, each sepal is counted separately. Also includes one petal/sepal mosaic organ. All organs occupy the first whorl.
†Organs found include petal/sepal, petal/stamen, petal/sepal/stamen, and petal/filament mosaics. All organs occupied whorls other than the first whorl.
‡Includes stamens whose filament was fused with that of an adjacent stamen.

**Thin cylindrical organs resembling stamen filaments and with no obvious sepaloid, petaloid, or carpeloid character. Includes filaments, fused filaments and filament/sepal mosaic organs.

1). While 32% of the stm-1 plants showed a rescued phenotype, 74 to 88% of the clv stm-1 double mutants exhibited a rescued phenotype. Examples of these 16-day-old rescued seedlings are shown in Fig. 2C-F. Thus, clv homozygosity increased the frequency of the rescued phenotype of stm-1 plants.

In the third experiment, clv stm-2 double mutants were compared to stm-2 single mutants. Because all stm-2 plants eventually exhibited a rescued phenotype (Table 1, Experiment II), the comparison between clv stm-2 and stm-2 was made in younger, 5.5 day-old, plants (Table 1). While only 12% of stm-2 plants exhibited rescue after 5.5 days, 50% of clv1-4 stm-2 and 82% of clv3-2 stm-2 exhibited rescue after 5.5 days. Therefore, clv1-4 and clv3-2 increased the speed at which stm-2 seedlings produced postembryonic leaves.

clv mutations restore identifiable shoot meristems to stm mutants

Dissection of 16 day-old clv stm-1 plants revealed the presence of shoot meristems (Fig. 2C-F). These clv stm-1 meristems were often larger than comparable wild-type shoot meristems (Fig. 2B). The clv stm-1 meristems often formed rosettes with more than 10 leaves (Fig. 3D), which is similar to the number of leaves produced by clv1-1 plants (Clark et al., 1995). The initial shoot meristem sometimes continued to initiate new organs and develop an inflorescence. clv stm-1 inflorescences formed several cauline leaves and often more than 10 flowers (Fig. 3F,G). The inflorescence stem was usually fasciated (Fig. 3F), similar to clv inflorescence stems (Clark et al., 1993). As in stm-2, the clv stm-1 shoot meristems eventually terminated and new meristems formed in the axils of leaves (Fig. 3E). These secondary meristems were also capable of forming rosettes of leaves and inflorescence stems. The number of organs formed on secondary inflorescence stems varied, but most consisted of one to three flowers and several carpelloid bracts (data not shown).

clv1-4 stm-2 and clv3-2 stm-2 double mutants were also examined. These two double mutants (the phenotypes of which were similar) displayed more shoot meristem activity than stm-2 plants. Double mutant plants, although still lacking an embryonic shoot meristem, developed shoot meristems that formed rosettes and inflorescences. The inflorescence stem was generally fasciated or massively enlarged, often giving rise to more than twenty flowers (Fig. 3H). These meristems eventually terminated in hundreds of carpelloid bracts before ceasing growth (Fig. 3I). These carpelloid bracts had sectors of sepal and carpel tissue (data not shown).

Although clv stm-2 double mutants displayed extensive shoot meristem development these plants still appeared to lack an embryonic shoot meristem based on the observation that the seedlings did not initiate leaves as rapidly as does wild-type. To conclusively determine if clv stm-2 embryos lacked shoot meristems, the embryos from seeds of a clv3-2 stm-2/+ parent were examined by confocal microscopy. As expected, most of the progeny had enlarged embryonic shoot meristems characteristic of clv3-2 embryos (Clark et al., 1995; Fig. 5A). These progeny were presumably clv3-2 SM and clv3-2 stm-2+/+. In eight out of the 42 embryos examined, however, no embryonic meristem was present (Fig. 5B,C). Similar to what was observed in stm-2 embryos, some small densely staining cells were often present above the junction of the vascular elements in clv3-2 stm-2 meristemless embryos. In summary, clv mutations enhance the speed, frequency and extent of postembryonic meristem development in stm-1 and stm-2 plants, but do not appear to restore the formation of embryonic meristems.

Rescued meristems form between the cotyledons

To determine how meristemless embryos generate active shoot meristems postembryonically, we examined 8-day-old clv3-2 stm-1 double mutant seedlings by confocal microscopy. In some plants, we observed no evidence of small, densely staining cells that might indicate active cell division and meristem activity (Fig. 5D). In other plants, however, a clear region of densely staining cells was

Fig. 4. Meristems of stm-2 and clv/+ stm-1/stm-1 plants. (A) stm-2, (B) clv1-4/+ stm-1 and (C) clv3-2/+ stm-1 plant tissue was observed by SEM. Older leaves were dissected to reveal apparently normal meristems (arrow), except for C, where the meristem appears to be highly reduced. Bars, 10 μm.
observed, indicating that meristem activity had begun (Fig. 5E). Similarly to wild-type or clv3-2 shoot apical meristems, clv3-2 stm-1 double mutant meristematic cells were positioned between the vascular bundles of the cotyledons, and included both epidermal and subepidermal cells (cf. Fig. 5E with Fig. 5A,F).

**clv mutations restore floral meristem activity to stm mutants**

Because clv mutations increase cellular proliferation in the floral meristem (Clark et al., 1993, 1995), we analyzed floral development of clv stm-1 plants. Whereas stm-1 plants never produced flowers, clv1-4 stm-1 and clv3-2 stm-1 plants (Fig. 3F,G) and clv1-1 stm-1 and clv3-1 stm-1 plants (not shown), produced flowers largely similar to those on stm-2 plants. While stm-2 flowers only occasionally initiate carpels, more carpelloid organs were produced in clv1-4 stm-2 and clv3-2 stm-2 flowers, which were fertile on rare occasions.

**clv mutations dominantly restore shoot and floral meristem development to stm mutants**

In the progeny of clv1 +/- stm-1 and clv3+/+ stm-1/+ plants (Table 1), a large proportion of the rescued plants developed rosettes, inflorescence stems and flowers. Rescued plants capable of initiating flowers were testcrossed to clv plants (clv1 or clv3 as appropriate) to determine whether they were clv+/+ or clv+/clv. SEM analysis of plants identified as being clv+/+ revealed the presence of meristems (Fig. 4B,C). Of 19 plants tested, 10 were homozygous for clv and 9 were heterozygous for clv. The inflorescence stems of clv+/+ stm-1/+/stm-1/+ plants fascinated less often and bore fewer flowers than those of clv+/clv stm-1/+/stm-1/+ plants, suggesting that the shoot and floral meristem activity of the clv heterozygous plants was less than that of the clv homozygous plants. Thus clv mutations are partially dominant with respect to their suppression of the stm phenotype.

**stm acts dominantly to partially suppress the clv3 homozygous phenotype**

Among the progeny of clv3-1/clv3-1 stm-1/+ plants, some of the plants were observed with a partially suppressed clv3-1 floral phenotype. The suppression was recognized based on the shape of the gynoecium. Strong clv mutants develop very distorted gynoecia as a result of the continued growth of the floral meristem inside the gynoecium. The degree of distortion in various clv alleles correlates with the severity of other clv phenotypes, including the number of floral organs and shoot meristem size (Clark et al., 1993). Of the progeny of clv3-1/clv3-1 stm-1/+ plants, those plants lacking the initial stm seedling phenotype were scored for their gynoecial phenotype (Table 3). 13 plants were scored as having a normal clv3-1 phenotype, while 25 plants were scored as having a weaker, suppressed phenotype. Seeds were collected from the plants and scored for the segregation of stm-1. For all 25 plants showing a suppressed clv phenotype, stm-1 segregated in the self-fertilized progeny, indicating that those weaker phenotype plants were all stm-1/+. However, none of the 13 plants exhibiting a normal clv3-1 phenotype were heterozygous for stm-1. This perfect correlation between stm-1 heterozygosity

![Image](image.png)

**Fig. 5.** Confocal imaging of meristematic cells in clv stm plants. Seeds from (A-C) a clv3-2 stm-2/+ parent, (D,E) 8-day-old clv3-2 stm-1/+ seedlings and (F) a 6-day-old clv3-2 seedling, observed by confocal microscopy. Among seeds of clv3-2 stm-2/+ plants, the majority of embryos displayed an enlarged shoot meristem, characteristic of clv3-2 embryos (A; see also Clark et al., 1995) and were presumably clv3-2 stm-1/+ or clv3-2 +/+ . However, eight out of 44 embryos lacked identifiable shoot meristems (B and higher magnification in C). Among 8-day-old clv3-2 stm-1/+ seedlings, various states of cellular proliferation were observed ranging from none detected (D), to considerable proliferation (E, compare to clv3-2 single mutant in F), cot, cotyledon; hyp, hypocotyl; SAM, shoot apical meristem; vas, vascular bundles; e, epidermal cells. A and C are shown at the same magnification, as are D and E.

**Table 3. Dominant suppression of clv3-1 homozygous phenotype by stm-1**

<table>
<thead>
<tr>
<th>Carpel phenotype</th>
<th>Number of plants</th>
<th>Number of plants segregating stm-1 observed</th>
<th>Number of plants segregating stm-1 expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal clv3-1</td>
<td>13</td>
<td>0</td>
<td>8.7</td>
</tr>
<tr>
<td>Weak clv3-1</td>
<td>25</td>
<td>25</td>
<td>16.7</td>
</tr>
</tbody>
</table>

The self progeny of clv3-1/clv3-1 stm-1/+ plants were scored as either having the normal clv3-1 carpel phenotype or a weak phenotype. Plants were then scored for stm-1 segregation. For each phenotypic class, the number of plants segregating stm-1 is compared with the number that would be expected to segregate stm-1, if stm-1 had no effect on the carpel phenotype of these plants.
and the reduced clv3-1 gynoecium defect suggests that stm-1 heterozygosity suppresses the clv3-1 phenotype. Thus, while the stm-1 mutation is normally recessive (Barton and Poethig, 1993), in a clv3-1 homozygous background stm-1 becomes semidominant.

**stm dominantly suppresses clv1 semidominance**

Plants heterozygous for the clv1-1 allele display a slight, but reproducible semidominant phenotype. While for wild-type plants 99% of gynoecia are composed of two carpels, approximately 25% of gynoecia from clv1-1/+ plants are composed of three or four carpels (Clark et al., 1995). We sought to determine if this semidominant phenotype could be dominantly suppressed by stm-1 or stm-2.

In the first experiment, clv1-1 homozygous plants were crossed to stm-1 heterozygous plants. For the F1 progeny, the number of carpels constituting the first ten gynoecia was recorded (Fig. 6A). Seeds were collected from the plants and tested for stm-1 segregation (and also to confirm clv1-1 segregation). Fig. 6A shows the distribution of extra carpels for plants clv1-1/+ and for plants clv1-1 /+ stm-1. An unpaired t-test revealed that the distribution is significantly different for the two populations (P=0.01).

In a separate experiment, clv1-1 gynoecia were fertilized with stm-2 or wild-type pollen. The clv1-1/+ and clv1-1 +/- stm-2 plants were grown side-by-side (pots were randomly intermixed) and the number of extra carpels on the first 10 flowers of each plant were recorded (Fig. 6B). Again, the unpaired t-test comparing these two populations indicated that their distributions were significantly different (P=0.001). These results indicate that stm-1 and stm-2 dominantly suppress the clv1-1 semidominant phenotype.

**DISCUSSION**

**STM is required for maintenance of the shoot meristem**

Previous work by Barton et al. (1993) demonstrated that STM is required for the formation of the embryonic shoot meristem. In this report, we have presented the phenotype of a weak stm allele, stm-2, which has provided insights into the role of STM during postembryonic development. stm-2 plants lack embryonic shoot meristems, but quickly form new shoot meristems between the cotyledons. The shoots in stm-2 plants are not indeterminate structures, instead they terminate after initiating several primordia. This result demonstrates a continuing need for STM activity to maintain meristem function. The organs initiated by the stm-2 meristems are largely normal in morphology. Thus, the primary defect in these plants is the inability to maintain organogenesis, implying that these mutants are unable to maintain undifferentiated cells.

**STM is required for floral meristem patterning**

A second insight provided by the weak stm-2 allele is the role of STM in floral meristem development. As stm-1 plants do not develop flowers, whether STM played a role in flower development was previously unknown. Our observations demonstrated that stm-2 plants are unable to develop flowers with a full complement of organs. Previous studies on clv flowers revealed that additional floral organs developed as a consequence of an enlarged floral meristem at the earliest stages of organ initiation (Clark et al., 1993). By analogy, it is possible that the reduced number of floral organs in stm-2 flowers is the result of a smaller floral meristem.

**clv mutations reverse many of the effects of stm mutations**

The formation of the embryonic shoot meristem was not
restored in the clv stm double mutants. This suggests that STM has a CLV-independent role in embryogenesis, perhaps by activating genes required solely for formation of the embryonic shoot meristem. However, clv mutations greatly enhanced the postembryonic meristem activity observed in plants homozygous for stm mutations. Reducing CLV activity (with either clv1 or clv3 mutations) in clv mutants not only sped the generation of new organs from the originally meristemless seedling, but also restored the postembryonic development of shoot and floral meristems.

One feature of the clv stm-1 shoot meristems is that they can become either larger or smaller than wild-type shoot meristems (i.e. meristems can either fasciate or terminate prematurely). One interpretation of this result is that there are redundant pathways capable of regulating meristem activity and that in the absence of normal CLV and STM activity, the balance between meristem cell proliferation and differentiation is not properly controlled, allowing large variation in the extent of meristem development.

**clv and stm mutations dominantly suppress each others’ phenotypes**

The most sensitive tests of the potential genetic interactions of CLV and STM came from an analysis of the effects of clv heterozygosity on the stm-1 homozygous phenotype and the effects of stm heterozygosity on the clv homozygous or heterozygous phenotype. In the former example, clv1 or clv3 heterozygosity suppressed the stm-1 phenotype by restoring meristem activity. While the restoration of meristem activity was less extensive than that seen in clv stm-1 double homozygotes, clv heterozygosity restored the ability of stm-1 plants to form rosettes of leaves, inflorescence stems and flowers. These results suggest that the stm phenotype is sensitive to the level of the wild-type CLV1 and CLV3 gene products.

In the latter experiments, although stm mutations are recessive, stm-1 heterozygosity reduced the severity of the clv3-1 gynoecium phenotype. This indicates that the clv3-1 mutant phenotype is sensitive to the level of the wild-type STM gene product. Furthermore, the semidominant phenotype of clv1-1 was reduced in plants that were also heterozygous for either stm-1 or stm-2. Therefore, clv1-1 semidominance also requires full STM activity. Thus the genetic interactions observed between the CLV and STM loci suggest that these genes may function to regulate the same process(es).

**The roles of CLV and STM**

CLV and STM appear to play opposite roles in shoot and floral meristem development: STM promoting meristem formation and maintenance; and the CLV loci repressing meristem proliferation. These roles are supported by the mutant phenotypes, where stm mutants fail to initiate and maintain shoot and floral meristems, and clv mutants fail to restrict the growth of these meristems. These roles are also supported by the present work. We have shown that a wild-type level of CLV activity is required for the lack of postembryonic meristem development observed in stm mutants and a wild-type level of STM activity is required for the meristem over-proliferation phenotype of clv mutants. The dominant interactions observed between the clv and stm mutations suggest that these genes function closely in their competing roles in meristem development. That clv mutants have a phenotype in an stm mutant background, and stm mutants have a phenotype in a clv background, shows that neither type of gene requires the other to be fully active.

We propose that the roles of these genes are to regulate the boundary between the peripheral and central zone. One model that has been proposed for CLAVATA activity is that the CLV genes act in the peripheral zone of meristems, or in the transmission of a signal from the peripheral zone, to limit the domain of a ‘meristem promoting activity’ (MPA) to the central zone (Clark et al., 1995). In the absence of CLV activity, MPA is present in much of what would normally be the peripheral zone. This is postulated to repress organ formation and differentiation in the peripheral zone, and displace differentiating organs to far below the apex, as is characteristic of clv1 and clv3 mutants. STM could be a component or positive regulator of the MPA, active in the central zone to prevent differentiation or to enhance cell division. The recent finding that STM encodes a homeodomain protein (Long et al., 1996) suggests that this action would involve transcriptional regulation. When the activity of STM is reduced, as in stm-2, the central zone is smaller than normal. This would cause the early disappearance of the central zone in determinate meristems such as those of flowers, thus explaining the stm-2 floral phenotype; and would cause the eventual disappearance of the central zone in shoot meristems, accounting for their determinate growth in stm-2 mutants. The increased activity of shoot meristems in stm-2 mutants that also lack CLV activity and the reappearance of the central organs in clv stm-2 double mutant flowers fit this model.

STM cannot be the only component of the MPA, because the apparent loss of STM activity in the stm-1 mutant does not eliminate development of shoots or flowers in a clv mutant background. Likewise, the CLV genes must have activities other than regulating the STM domain, because clv homozygotes have different phenotypes than clv stm-1 double homozygotes. Nonetheless, the dominant effects of stm and clv on each other’s phenotypes imply a balanced quantitative relationship between the activities of the two sets of genes; this fits a model where CLV regulates the STM domain, so as to maintain the boundary between the central and the peripheral zones.

An alternative model is that CLV acts in the central zone to repress cell division, while STM acts to enhance it. One possibility is that CLV acts by negatively regulating STM transcriptional activity in the central zone. However, as discussed above, this is unlikely to be the only function of CLV, since stm mutations are not epistatic to clv mutations. Another possibility is that CLV and STM act to competitively regulate one or more components of the MPA, STM acting as a positive regulator and CLV acting as a negative regulator. One possible mechanism for this is that the CLV pathway could activate a transcription factor which competes with the action of STM. This would explain the balanced quantitative relationship between these gene products.

The authors would like to thank members of the Meyerowitz and Schiefelbein labs for critical reading of the manuscript, and Thomas Laux for challenging discussions on the nature of meristems. This material is based upon work supported by NSF grant MCB-9204839 and an award from the Strategic Research Fund of Zeneca Seeds to E. M. M., by NSF grant IBN 9419457 to S. E. C., and by postdoctoral fellowships to S. E. C. (NSF, DIR-9104342), S. E. J. (NIH, GM15964) and J. Z. L. (NIH, GM15132). The scanning electron microscope used at the University of Michigan was acquired under
grant #BSR-83-14092 from the NSF. S. E. C was and S. E. J. is currently a Zeneca Fellow.

REFERENCES


(Accepted 5 February 1996)