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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

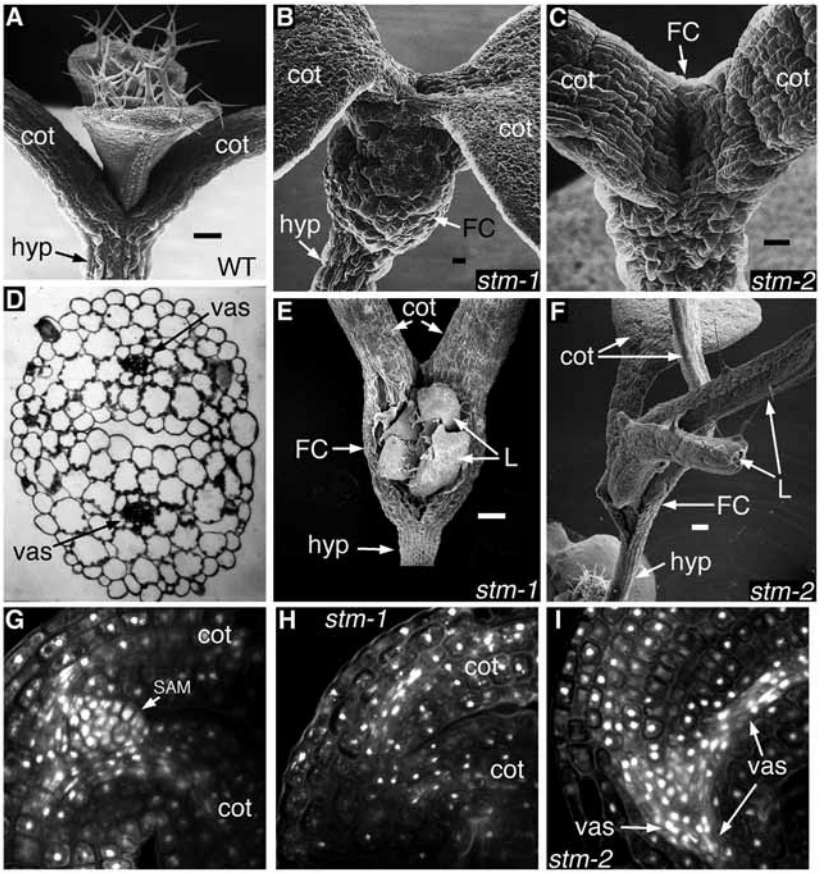

Fig. 1. Early phenotypes of *stm-1* and *stm-2* plants. (A-C) 5.5-day-old and (E,F) 16-day-old plants observed by SEM. Compared to (A) wild-type plants, (B) *stm-1* and (C) *stm-2* plants fail to initiate any organs initially, although (E) some *stm-1* and (F) all *stm-2* plants eventually develop organs 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 1I), a position similar to that occupied by the wild-type shoot meristem. These brightly staining cells were not observed in *stm-1* embryos. Thus differences between *stm-1* and *stm-2* development were observed as early as the mature embryo stage.

In *stm-1* seedlings, the region between the cotyledons and the hypocotyl was enlarged relative to wild-type (Fig. 1A,B). In *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 *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 *stm-1* plants (Fig. 2A). The rescued *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 *stm-2* plants was greater

Table 1. Effect of *clv* heterozygosity or homozygosity on shoot development in *stm-1* and *stm-2* plants

Genotype of parent	Number of plants with initial *stm* phenotype*	Percentage rescued†
Experiment I	Scored at 16 days	
stm-1/+	106	14
clv1-1 +/+ *stm-1*	70	50
clv1-4 +/+ *stm-1*	65	68
clv3-1/+ *stm-1*/+	65	45
clv3-2/+ *stm-1*/+	61	61
Experiment II	Scored at 16 days	
stm-1/+	19	32
stm-2/+	24	100
clv1-1 stm-1/+	35	74
clv1-4 stm-1/+	49	88
clv3-1 stm-1/+	61	85
clv3-2 stm-1/+	41	85
Experiment III	Scored at 5.5 days	
stm-1/+	27	0
stm-2/+	26	12
clv1-4 stm-1/+	33	0
clv3-2 stm-1/+	29	0
clv1-4 stm-2/+	8	50
clv3-2 stm-2/+	22	82
Wild-type Landsberg *erecta*	44‡	100

*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 *stm* phenotype (no leaves developing in the axils of the cotyledons).

†Percentage of plants with an early 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 *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.

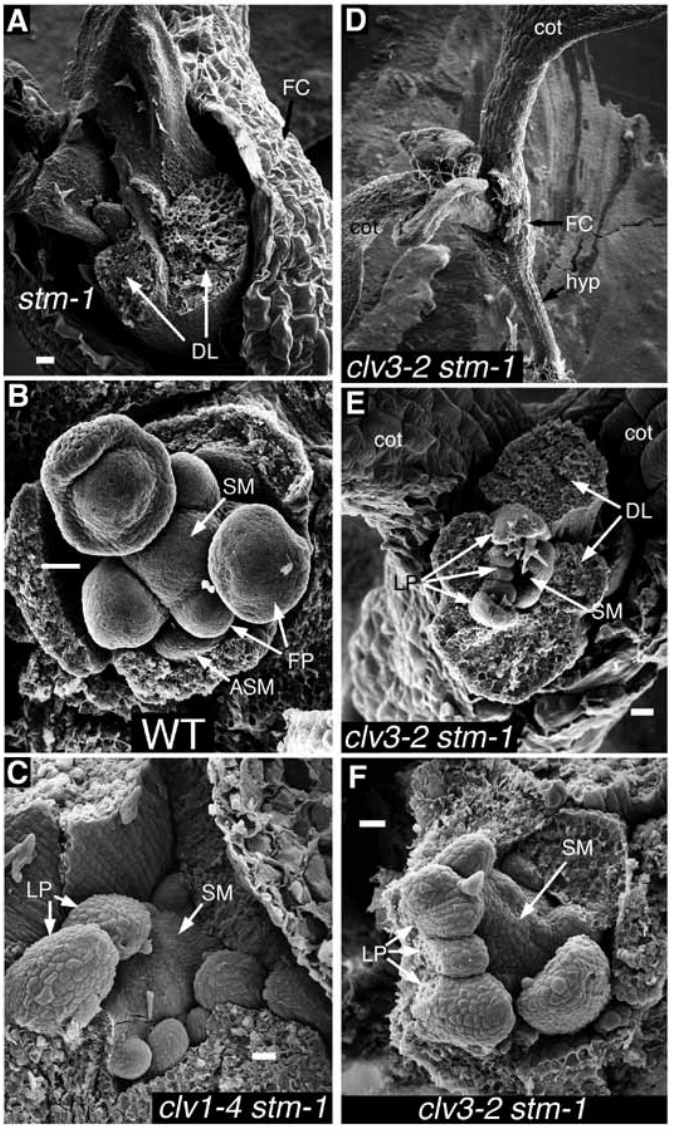

Fig. 2. *clv stm-1* seedlings develop meristems. (A,C-F) 16-day-old and (B) 14-day-old seedlings observed by SEM. (A) Dissection of an *stm-1* seedling that has developed leaves fails to reveal a structure resembling a shoot meristem. In contrast, *clv stm-1* seedlings do develop identifiable meristems. The *clv3-2 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 *clv1-4 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 μm (A,E) and 20 μm (B,C,F).

than that seen in *stm-1* plants. First, leaves developed from the FC region of *stm-2* plants as early as 5.5 days after sowing, earlier than that observed in *stm-1* plants (Table 1). By 16 days of development, while less than a third of *stm-1* plants exhibited the rescued phenotype, all *stm-2* plants had visible leaves (Fig. 1F, Table 1). *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 *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 *stm-2* plants exhibited more extensive shoot development than *stm-1* plants, the shoots produced by *stm-2* plants were not indeterminate structures as are found in wild-type plants.

stm-2 affects floral meristem structure

Although flowers were never observed on *stm-1* plants, *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 *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 *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). *stm-2* plants, therefore, have floral meristems that fail to produce a full complement of floral organs.

clv mutations increase the frequency and speed of postembryonic leaf development in *stm* mutants

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

In the second experiment, *clv stm-1* double mutant plants were examined. In this case, plants with an initial *stm* phenotype among the progeny of *clv*/*clv stm-1*/+ plants were compared to the progeny of *stm-1*/+ and *stm-2*/+ plants (Table

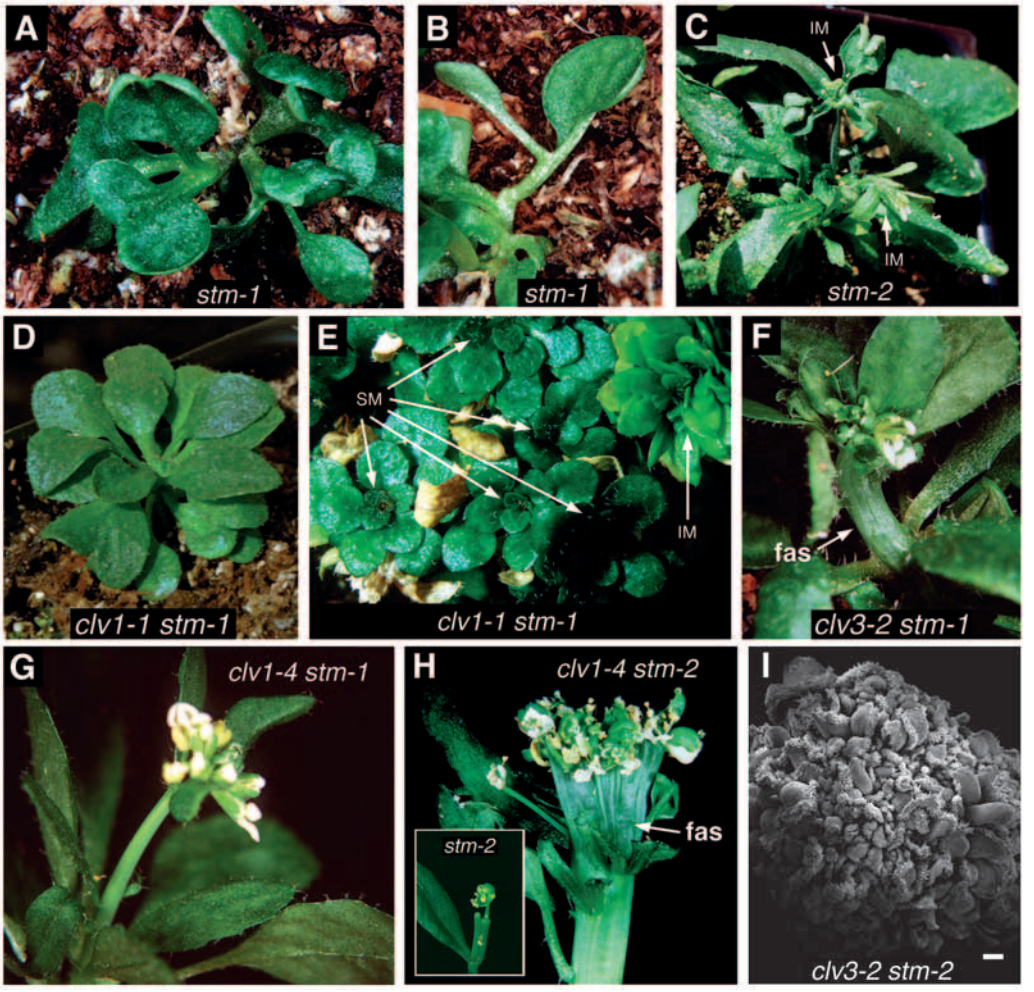

Fig. 3. Phenotypes of mature *stm-1*, *stm-2* and *clv stm* plants. (A,B) Mature *stm-1* and (C and H inset) *stm-2* plants are presented. (D) The initial rosette of leaves of a *clv 1-1 stm-1* plant and (E) new rosettes of leaves formed in the older leaf axils of a single *clv1-1 stm-1* plant are shown. Inflorescence shoots (IM) of (F,G) *clv stm-1* and (H,I) *clv stm-2* are presented. Note the fasciated stems (fas). SM, shoot meristem. Bar in I, 200 μm.

Table 2. Floral organs produced by *stm-2* plants

Organ type	Mean number per flower	Wild-type Standard
Sepal*	3.88	4
Petal	0.47	4
Petal mosaic†	0.16	
Stamen	2.30	6
Fused stamen‡	0.70	
Filament¤	0.26	
Carpel**	0.19	2
All organs	7.98	16

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.

*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.

**Includes carpels, stamen/carpel mosaics, and filament/carpel mosaic organs. None of these organs fused to form a normal gynoecium.

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 STM* 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

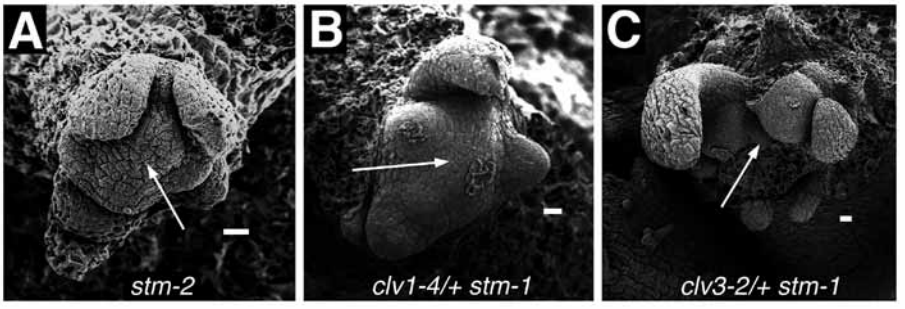

Fig. 4. Meristems of *stm-2* and *clv*/+ *stm-1/stm-1* plants. (A) *stm-2*, (B) *clv1-4*/+ *stm-1* and (C) *clv3-2*/+ *stm*-1 plant tissue was observed by SEM. Older leaves were dissected to reveal apparently normal meristems (arrow), except for C, where the meristem appears to be highly reduced. Bars, 10 μm.

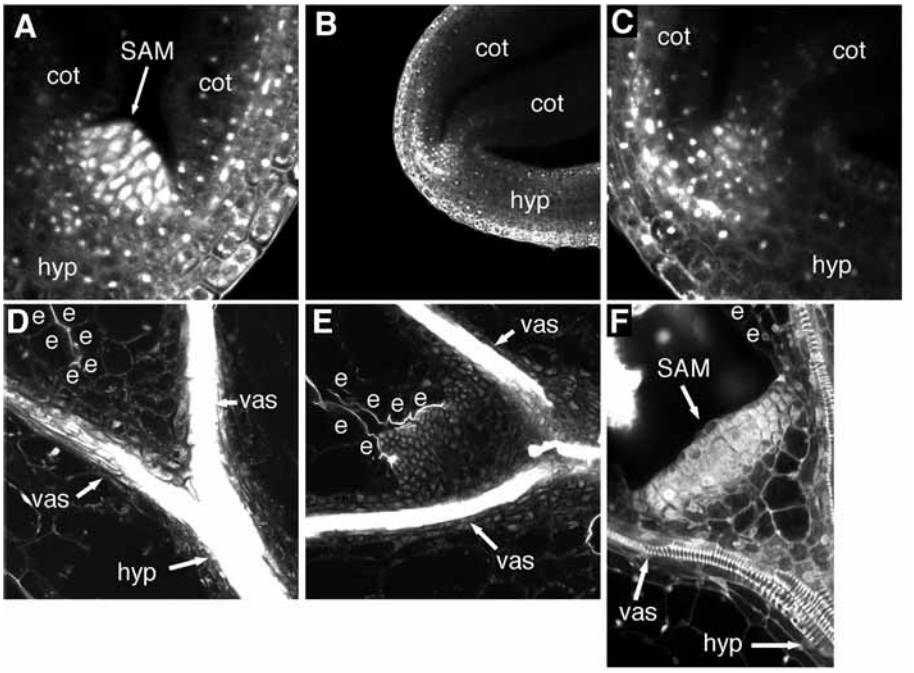

Fig. 5. Confocal imaging of meristematic cells in *clv stm* plants. Seeds from (A-C) a *clv3-2 stm-2*/+ parent, (D,E) 8-day-old *clv3-2 stm-1* seedlings and (F) a 6-day-old *clv3-2* seedling, observed by confocal microscopy. Among seeds of *clv3-2 stm-2*/+ plants, the majority of embryos displayed an 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.

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 fasciated 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

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

Carpel phenotype	Number of plants	Number of plants segregating *stm-1*	
		Observed	Expected
Normal *clv3-1*	13	0	8.7
Weak *clv3-1*	25	25	16.7

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 F_1 progeny, the number of carpels constituting the first ten gynoecia was recorded. For each plant, the number of extra carpels (above the normal two carpels) was calculated (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

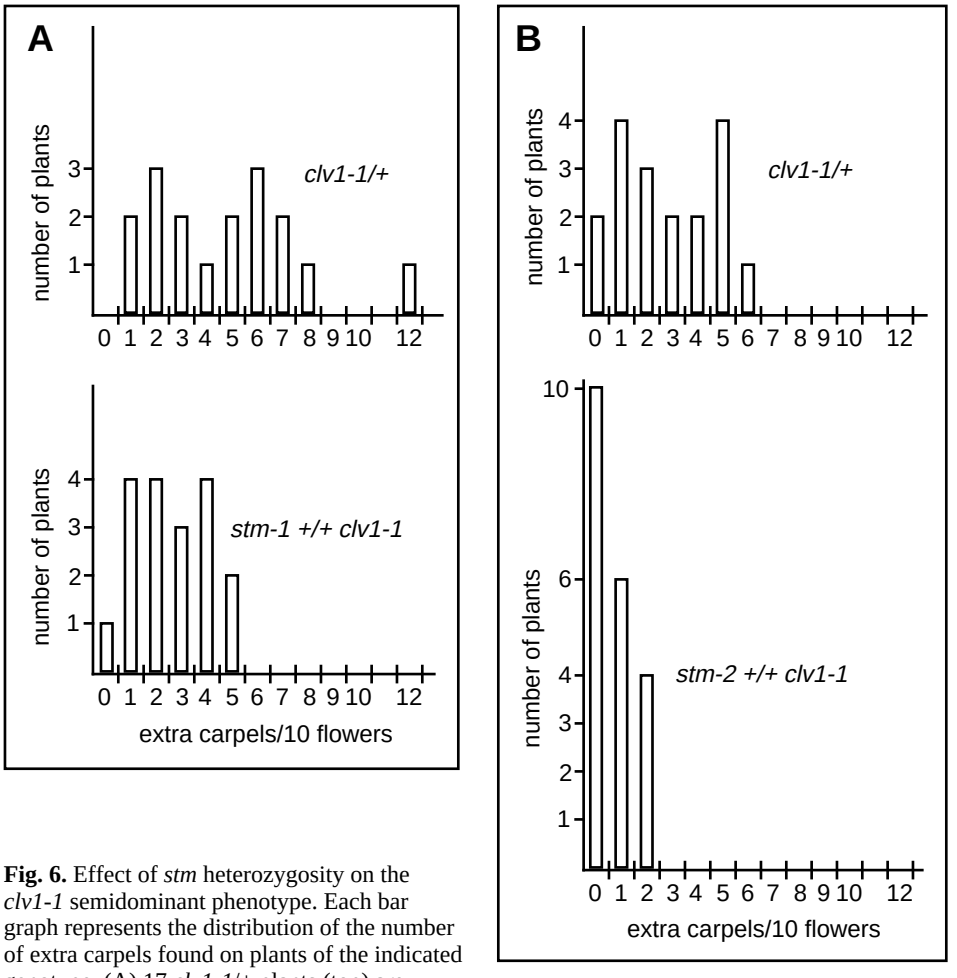

Fig. 6. Effect of *stm* heterozygosity on the *clv1-1* semidominant phenotype. Each bar graph represents the distribution of the number of extra carpels found on plants of the indicated genotype. (A) 17 *clv1-1*/+ plants (top) are compared with 18 *clv1-1*/+ *stm-1*/+ plants (bottom). (B) 18 *clv1-1*/+ plants (top) are compared with 20 *clv1-1*/+ *stm-2*/+ plants. Experiment A was carried out separately from experiment B.

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 *stm* 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.

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