POLTERGEIST functions to regulate meristem development downstream of the CLAVATA loci

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Accepted 31 January; published on WWW 21 March 2000

SUMMARY

Mutations at the CLAVATA loci (CLV1, CLV2 and CLV3) result in the accumulation of undifferentiated cells at the shoot and floral meristems. We have isolated three mutant alleles of a novel locus, POLTERGEIST (POL), as suppressors of clv1, clv2 and clv3 phenotypes. All pol mutants were nearly indistinguishable from wild-type plants; however, pol mutants provided recessive, partial suppression of meristem defects in strong clv1 and clv3 mutants, and nearly complete suppression of weak clv1 mutants. pol mutations partially suppressed clv2 floral and pedicel defects in a dominant fashion, and almost completely suppressed clv2 phenotypes in a recessive manner. These observations, along with dominant interactions observed between the pol and wuschel (wus) mutations, indicate that POL functions as a critical regulator of meristem development downstream of the CLV loci and redundantly with WUS. Consistent with this, pol mutations do not suppress clv3 phenotypes by altering CLV1 receptor activation.

Key words: Meristem, POLTERGEIST, CLAVATA, Phenotypic suppression, Arabidopsis thaliana

INTRODUCTION

The shoot meristem is the source of all above-ground adult organs in the plant. To function as a site of continuous organ formation, the shoot meristem must maintain a population of undifferentiated cells while directing descendant cells toward organ formation and eventual differentiation. A number of genes are specifically involved in maintaining the structure of the shoot and floral meristems in Arabidopsis. These include SHOOT MERISTEMLESS (STM), WUSCHEL (WUS) and the CLAVATA loci (CLV1, CLV2 and CLV3) (Leyser and Furner, 1992; Barton and Poethig, 1993; Clark et al., 1993, 1995; Laux et al., 1996; Kayes and Clark, 1998). Analysis of genetic interactions between mutations at these loci indicate that several of these genes function in the same pathway and that all appear to regulate a similar process (for review see Clark, 1997).

clv1, clv2 and clv3 mutants accumulate undifferentiated cells at both the shoot and floral meristems (Clark et al., 1993, 1995). In the clv flowers, the larger meristem leads to the initiation of additional organs in each whorl, and extra whorls of organs interior to the normally terminal whorl 4 carpels. In plants homozygous for the strongest clv1 and clv3 alleles, the floral meristem is indeterminate. clv1 and clv3 mutants exhibit identical phenotypes, are mutually epistatic, and exhibit dominant interactions, indicating that the CLV1 and CLV3 genes function in the same pathway (Clark et al., 1995). clv2 mutants exhibit only weak phenotypes at the shoot and floral meristem compared to clv1 and clv3 mutants (Kayes and Clark, 1998). clv2 mutants also affect the development of several organ types, including pedicels, stamens and gynoecia. Genetic analysis indicates that CLV2 functions in the same pathway as CLV1 and CLV3 to regulate meristem development, but in an independent pathway to regulate organ development (Kayes and Clark, 1998).

wus and stm mutants fail to maintain a population of undifferentiated cells at the shoot and floral meristems (Barton and Poethig, 1993; Laux et al., 1996). This results in prematurely terminated shoot and floral meristems, as well as reduced numbers of floral organs, especially the central stamens and carpels. The strongest stm mutants fail to initiate any shoot meristems, while plants homozygous for the weaker stm alleles initiate shoot meristems that are overtaken by organ primordia (Barton and Poethig, 1993; Clark et al., 1996; Endrizzi et al., 1996). wus mutants, in contrast, form meristem-like structures that differentiate across the apex (Laux et al., 1996). wus and stm mutants also display differences in genetic interactions with clv mutants. wus is epistatic to clv1 and clv2 mutations (Laux et al., 1996), while clv stm double mutant plants exhibit an additive phenotype (Clark et al., 1996). The dominant interactions between clv and stm mutations suggest that they act competitively on a common downstream target. However, the epistasis of the wus mutation indicates that either WUS functions downstream of the CLV loci, which act to negatively regulate WUS, or WUS functions to establish the meristem upon which the CLV loci act. Both STM and WUS code for homeodomain-containing proteins, and are expressed within a central region of the shoot and floral meristems (Long et al., 1996; Mayer et al., 1998).
To expand our understanding of the hierarchy of genes regulating meristem development, we undertook a screen for second-site mutations that enhanced or suppressed the phenotypes of intermediate clv1 and clv3 mutants. We identified three mutant alleles of a novel locus we term POLTERGEIST (POL) (Pogany et al., 1998). Here we describe in detail a morphological and genetic analysis of pol mutant plants. While pol single mutant plants were nearly identical to wild type, pol provided recessive, partial suppression of the phenotypes of strong clv1 and clv3 mutants, and nearly complete suppression of weak clv1 mutants. pol mutations partially suppressed clv2 phenotypes in a dominant manner, and almost completely suppressed clv2 phenotypes in a recessive manner. wus became semi-dominant in a pol background, and pol enhanced wus phenotypes, suggesting that POL functions redundantly with WUS. Finally, pol mutations did not suppress clv3 phenotypes by altering CLV1 receptor-kinase activation.

MATERIALS AND METHODS

Plant growth and genetic techniques

The isolation of the pol-1, pol-3 and pol-4 mutations was described previously (Pogany et al., 1998). Seeds were sown on a 1:1:1 mix of top soil:perlite:vermiculite and imbibed for 7 days at 4°C. Plants were grown at 22°C under approximately 800 foot-candles of constant cool white fluorescent light. Plants were given fertilizer once a week.

The isolation of pol was, pol stm, and pol ag double mutant plants were carried out as follows. pol clv3 double mutant plants were crossed to smi/STM and wus/WUS single mutant plants, and pol clv1 double mutant plants were crossed to ag/AG and was/WUS single mutant plants. Progeny were collected from individual F1 plants, and those F2 populations segregating out all three mutant phenotypes were used for further analysis (Fig. 1). Progeny exhibiting wild-type phenotypes were collected from individual F2 plants, and the segregation of the mutations in the F3 generation was assessed. F3 families segregating only wild-type, suppressed clv, and the other mutant-like phenotypes (ag, wus, or stm) were followed for subsequent analysis. The parent F2s for these families were homozygous for pol, and heterozygous for clv and the other mutation. Progeny were collected from wild-type individuals in these F3 families, and the F4 generation was assessed. F4 families that segregated only wild type and the mutation in question were from F3 individuals that were homozygous for pol, homzygous wild-type for CLV, and heterozygous for the mutation in question. The number of families in each phenotypic class is indicated in Table 1. For ag, additional steps were taken to ensure the identity of the pol ag double mutant, because plants heterozygous for ag exhibit phenotypes similar to pol clv double mutant plants (i.e., a low frequency of flowers with extra carpels). To further verify that the putative pol ag double mutant plants were homozygous for pol, two addition assays were performed. First, the 9019T7 and additional markers both north and south of POL on chromosome 2 were tested. The pol ag double mutants were all homozygous for the pol-1 (Columbia) polymorphism at these markers. Second, the pedicels of pol ag double mutant plants were compared to pedicels of ag single mutants, and the pol ag double mutants had significantly shorter pedicels (6.48±0.61 mm/ag-3 pedicel vs. 4.93±0.12 mm/pol-1 ag-3 pedicel).

Fig. 1. Generation of double mutant plants. Genotypes are represented as follows: P, wild-type POL allele; p, mutant POL allele; C, wild-type CLV1 or CLV3 allele; c, mutant CLV allele; M, wild-type AG, STM, or WUS allele; m, mutant AG, STM, or WUS allele.
Mapping
The clv3-1 line that was originally mutagenized to generate the pol-1 allele was a Columbia/Landsberg erecta mixture. Preliminary mapping in crosses with known genetic markers indicated that pol mapped to the bottom arm of chromosome 2 (data not shown). In the original clv3-1 line, the entire bottom arm of chromosome 2 was homozygous for Columbia polymorphisms. Thus, we used pol-1 that had been backcrossed two times to clv1-1 (Landsberg) for mapping. DNA was collected from each F2 plant from the final cross, and the genotype of the plant was assessed by analyzing F3 progeny. 477 progeny were assayed for CAPS markers on the bottom arm of chromosome 2. Tightest linkage was observed with marker 90J19T7, which showed only 5 recombination events over 954 chromosomes. Thus, pol is tightly linked to this marker.

Tissue and image processing
Scanning electron microscopy (SEM) was carried out as described by Bowman et al. (1989), except the Hitachi S3200N SEM allowed images to be collected digitally. Slides were scanned and digitized using a Polaroid SprintScan35. Brightness, contrast, and color balance were adjusted using Adobe Photoshop and figures were printed using a Kodak 8600 Digital Printer. pol and wild-type plants were fixed in formaldehyde, acetic acid and ethanol mixture (Clark et al., 1997), embedded in paraffin, and sectioned at 4 µm thickness.

Floral stages
In order to clearly categorize the stages of floral primordia, physical characteristics were defined for stages 1, 2, 3 and 4 flowers based on those of Smyth et al. (1990). Stage 1, primordia have formed a furrow between the shoot meristem and primordia; stage 2, the floral meristem physically contacts the inflorescence meristem; stage 3, floral meristems have formed sepal primordia; stage 4, the sepals physically contact the floral meristem prior to the formation of stamen primordia (Fig. 2C).

Analysis of shoot meristem size
Scanning electron micrographs were taken from directly above the shoot meristem. The center of the furrow of the youngest stage 1 floral primordium was determined with a compass. A line connecting the furrow ends was drawn. The distance from the center of the furrow line perpendicularly across the shoot meristem was defined as the size of the shoot meristem (Fig. 2C).

RESULTS

Mutant isolation
Ethylmethane sulfonate mutagenesis was performed on the intermediate clv3-1 and clv1-1 mutant plants to identify enhancers and suppressors (Pogany et al., 1998). Three independent families segregated a recessive suppressor of the clv mutation (Fig. 2). When the suppressed clv1-1 plants were backcrossed to wild type, an approximate 12:3:1 ratio of...
(wild-type phenotype):(cvi1 phenotype):(suppressed cvi1 phenotype) plants were observed in the F2 generation (data not shown). Thus, we suspected that plants with the suppressing mutation(s) alone were indistinguishable from wild type. Seeds were collected from F2 individuals and progeny were scored for cvi1 phenotypes. Approximately two-thirds of the plants phenotypically cvi1 gave rise to progeny in which a 3:1 ratio of (cvi1 phenotype):(suppressed cvi1 phenotype) was observed, indicating that the suppression was caused by a single, recessive mutation. Among the phenotypically wild-type plants, a portion gave rise to progeny with a 3:1 ratio of (cvi1 phenotype):(suppressed cvi1 phenotype). We concluded that these plants were homozygous for the suppressor mutation. By collecting progeny from these F3 individuals, we were able to isolate lines that were both homozygous for the suppressing mutation and wild type for CLVI. These plants were indistinguishable from wild type (or nearly so—see below). Because the mutation could not be identified in an otherwise wild-type plant, we termed the original suppressor allele poltergeist (pol). Prior to further phenotypic and genetic analysis, the pol-1 cvl double mutant plant was backcrossed to wild type three times.

Allelism tests were performed by crossing a pol-1 single mutant plant with other suppressed cvl1-1 plants. For the mutations that were pol alleles, these crosses resulted exclusively in wild-type and suppressed cvl1 phenotypes in both the F2 and F3 generations.

For pol-1, which was isolated as a suppressor of cvl3-1, we observed linkage between the pol and cvl3-1 mutations (data not shown). Fine mapping on a large population revealed only 5 recombination events between pol and CAPS marker 90J19T7 on the bottom arm of chromosome 2 over 954 chromosomes (see Materials and Methods), indicating pol is tightly linked to this marker.

The three different pol alleles were analyzed for the relative severity of the mutant phenotype and the effect of pol on meristem development. Plants homozygous for pol alleles developed flowers with an identical number of floral organs as wild type (Table 2). To compare the relative strength of the pol alleles, the reduction in carpel number in the pol cvl1-1 double mutant plants was determined (Fig. 2H). The pol-1 allele provided the greatest suppression of cvl1-1, and was therefore used for all subsequent genetic and phenotypic analyses.

While visible inspection of pol single mutant plants revealed no shoot meristem abnormalities, scanning electron microscopy (SEM) analysis of pol shoot meristems revealed a possible slight reduction in meristem size (Fig. 2A,B). To determine whether the pol meristem was reduced compared to wild type Landsberg, the meristem sizes of 18 wild-type and pol-1 plants were determined by SEM (Fig. 2C; Table 3; see Materials and Methods). While the mean pol-1 meristem size was smaller than that of wild type, the average difference was only 5 μm. We also compared pol and wild-type meristem size by longitudinal serial sections of their apices (Fig. 3). Fewer sections were required for pol meristems and they were narrower than wild type. To confirm this reduction by a different measurement, we determined the mean number of stage 1, 2, 3 and 4 flowers present on pol-1 inflorescence shoot meristems. We hypothesized that a smaller meristem would initiate organs at a slower rate. We observed a slight reduction in the number of stage 1-4 flowers in pol mutants (7.7 / pol meristem vs. 9.0 / wild-type meristem; Table 3), which may indicate a reduced shoot meristem size, or a faster rate of pol flower development.

Genetic interactions with cvl mutations

To determine the position of POL within the hierarchy of genes regulating meristem development, double mutant plants were first generated between pol-1 and cvl1, cvl2 and cvl3 mutations (Fig. 4; Table 2). Plants homozygous for the strong cvl1-4 and cvl3-2 alleles give rise to dramatically enlarged shoot and floral meristems (Clark et al., 1993) (Fig. 4G,O). The pol-1 cvl1-4 and pol-1 cvl3-2 double mutant plants exhibited a cvl

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mean number of organs per flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (Ler)</td>
<td>4.00±0.2</td>
</tr>
<tr>
<td>pol-1</td>
<td>4.00±0.2</td>
</tr>
<tr>
<td>cvl1-6</td>
<td>4.07±0.03</td>
</tr>
<tr>
<td>pol-1 cvl1-6</td>
<td>3.98±0.02</td>
</tr>
<tr>
<td>cvl1-1</td>
<td>4.28±0.05</td>
</tr>
<tr>
<td>pol-1 cvl1-1</td>
<td>4.01±0.01</td>
</tr>
<tr>
<td>cvl1-4</td>
<td>4.92±0.07</td>
</tr>
<tr>
<td>pol-1 cvl1-4</td>
<td>4.04±0.02</td>
</tr>
<tr>
<td>cvl2-2</td>
<td>4.02±0.01</td>
</tr>
<tr>
<td>pol-1 cvl2-2</td>
<td>3.99±0.01</td>
</tr>
<tr>
<td>cvl2-3</td>
<td>4.22±0.05</td>
</tr>
<tr>
<td>pol-1 cvl2-3</td>
<td>4.00±0.0</td>
</tr>
<tr>
<td>cvl2-4</td>
<td>4.23±0.05</td>
</tr>
<tr>
<td>pol-1 cvl2-4</td>
<td>4.05±0.02</td>
</tr>
<tr>
<td>cvl3-1</td>
<td>4.56±0.06</td>
</tr>
<tr>
<td>pol-1 cvl3-1</td>
<td>4.00±0.0</td>
</tr>
<tr>
<td>cvl3-2</td>
<td>4.96±0.05</td>
</tr>
<tr>
<td>pol-1 cvl3-2</td>
<td>4.02±0.01</td>
</tr>
</tbody>
</table>

Values represent the mean number ± standard error of the mean of indicated floral organs. 100 flowers were counted for each mean calculated. Only the first ten flowers of any given plant were analyzed. Whorl 3 organs that developed as filamentous organs were included in the counts of stamens.

Table 3. Floral initiation and shoot meristem size are reduced in pol-1 mutants

<table>
<thead>
<tr>
<th>Floral stage§</th>
<th>Mean number of floral primordia per meristem* ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landsberg</td>
<td>pol-1</td>
</tr>
<tr>
<td>1</td>
<td>1.78±0.25</td>
</tr>
<tr>
<td>2</td>
<td>2.89±0.14</td>
</tr>
<tr>
<td>3</td>
<td>1.94±0.20</td>
</tr>
<tr>
<td>4</td>
<td>2.39±0.12</td>
</tr>
<tr>
<td>total</td>
<td>9.00±0.33</td>
</tr>
</tbody>
</table>

*Samples were collected 28 days after germination. 18 samples were counted for each mean calculated.
‡Values represent the mean number of floral primordia ± standard error of the mean.
§See Material and Methods for definition of floral stages.
¶Values represent the mean shoot meristem size (μm) ± standard error of the mean.
phenotype, but it was strongly suppressed as measured by shoot meristem size, the number of floral organs, which is indicative of floral meristem size (Clark et al., 1993), and the extent of indeterminacy at the floral meristem (Fig. 4G,H,O,P; Table 2; data not shown). This recessive suppression resulted in plants similar to plants homozygous for weak clv alleles (Fig. 4; Tables 2, 4).

When double mutant plants were generated between pol-1 and weaker clv1 alleles (clv1-6 and clv1-7), a nearly complete suppression was observed (Tables 2, 4). The mean number of floral organs in these double mutants varied only slightly from wild type (Table 2; data not shown). Similarly, the shoot meristems of the pol-1 clv1-6 and pol-1 clv1-7 double mutants were reduced in size to that seen in wild-type plants (Fig. 4A,B; Table 4). To determine if pol could provide semi-dominant suppression of clv1-1 mutant phenotypes, the progeny of pol-1/Pol clv1-1/clv1-1 plants were scored for the number of carpels in each of the first ten flowers (Fig. 5B). Each plant was then tested for the pol genotype (pol/pol, pol/Pol, or Pol/Pol) by examining progeny. Mean carpel number for pol/Pol heterozygotes was less than Pol/Pol homozygotes, indicating that pol suppression of clv1-1 was semi-dominant.

clv2 mutant plants are somewhat different than clv1 or clv3 mutant plants in that: (1) all clv2 mutant plants exhibit weak phenotypes despite being putative nulls (Jeong et al, 1999); (2) clv2 phenotypes, but not clv1 or clv3 phenotypes, are altered under short-day photoperiod conditions; and (3) CLV2 plays a role independent from CLV1 and CLV3 to regulate the development of several organ types (Kayes and Clark, 1998). One of the organ types regulated by CLV2 is the floral pedicel. In clv2 mutant plants, pedicels are on average approximately 50% longer than wild type (Kayes and Clark, 1998). clv2 mutants under short-day photoperiod develop wild-type flowers with elongated pedicels, indicating that the regulation of pedicel length by CLV2 is independent of its regulation of the floral meristem.

We sought to determine whether pol was capable of suppressing clv2 meristem and/or organ defects. Double mutant plants were generated between pol-1 and clv2-1, clv2-2, clv2-3 and clv2-4. Similar to the double mutant plants of pol with weak clv1 alleles, the pol clv2 double mutant plants were nearly identical to wild type in terms of the size of the shoot meristem and the number of floral organs (Fig.

### Table 4. pol-1 clv double mutant phenotypes

<table>
<thead>
<tr>
<th>clv1-6</th>
<th>clv1-7</th>
<th>clv2-1</th>
<th>clv2-2</th>
<th>clv2-3</th>
<th>clv2-4</th>
<th>clv3-1</th>
<th>clv3-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength of clv phenotype*</td>
<td>Weak</td>
<td>Weak intermediate</td>
<td>Intermediate</td>
<td>Strong intermediate</td>
<td>Strong</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strength of pol clv phenotype*</td>
<td>Wild type‡</td>
<td>Wild type‡</td>
<td>Weak</td>
<td>Weak</td>
<td>Weak intermediate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The phenotypes of the pol-1 clv and clv mutant plants are indicated. Phenotype classification is based on the extent of shoot meristem proliferation, the number of floral organs, and the extent of floral indeterminacy. See Table 2.

‡Mean number of floral organs was nearly identical to wild type.
4E,F,1J,M,N; Tables 2, 4). The mean number of floral organs varied only slightly from those of wild type (Table 2). Interestingly, pol also suppressed the elongation of the pedicel caused by clv2 mutations (Fig. 5A). This prompted us to examine the length of pedicels in pol single mutant plants compared to wild type, which revealed that pol mutant plants developed significantly shorter pedicels than wild type (Fig. 5A).

While generating the pol clv2 double mutant plants, we observed that a significant portion of the progeny of pol/POL clv2/clv2 plants exhibited partial suppression. To determine if this was the result of semi-dominant suppression of clv2 by pol, each individual plant was scored for floral organ number and pedicel length, and subsequently scored for pol genotype by testing the progeny. pol heterozygotes were significantly reduced in both floral organ number and pedicel length compared to plants wild-type for POL (Fig. 5). Thus, pol was semi-dominant in its suppression of clv2, and suppressed both meristem and organ defects.

**pol** suppression of indeterminacy is specific to **clv** mutants

As mentioned above, pol suppressed the indeterminate floral meristems that develop in strong clv1 and clv3 mutant plants. To determine if this suppression was specific for the CLV loci, we tested the ability of pol to suppress the indeterminate agamous (ag) floral meristem. ag mutations result in the replacement of stamens by petals, and a new flower in the place of carpels (Bowman et al., 1989). Thus, the ag flower initiates an indeterminate series of (sepal, petal, petals)n. We generated pol ag double mutant plants, by crossing pol-1 clv1-4 to ag-3/AG. By testing large numbers of F2 progeny, we identified a plant pol-1 pol-1 clv1-4/CLV1 ag-3/AG (Fig. 1; see Materials and Methods). By collecting seeds from a number of progeny of this plant, we isolated a plant pol-1 pol-1 clv1-4/CLV1 clv3-1 ag-3/AG, and compared the progeny of this plant to the progeny of an ag-3/AG plant. We observed no difference in the development of the ag-3 and pol-1 ag-3 flowers (Fig. 6), indicating that pol had no effect on the indeterminacy found in ag flowers.

**wus-pol** dominant interactions

To better place POL within the hierarchy of genes regulating meristem development, we also investigated interactions between pol and wus mutations. wus mutants fail to maintain organogenesis at both the shoot and flower meristems (Laux et al., 1996). This results in the repeated initiation of adventitious meristems that quickly terminate, and flowers that largely lack central organs (stamens and carpels). pol-1 wus-1 double mutant plants were isolated in a manner similar to the isolation of the pol ag double mutant plants (Fig. 1; Table 1; see Materials and Methods). In comparison to wus single mutant plants, the pol wus plants exhibited less overall growth, reduced plant stature, abnormal leaf development, and a reduced number of flowers (Fig. 7A). A comparison of the number of floral organs initiated by wus and pol wus flowers revealed that pol enhanced the reduction in floral organ number observed in wus mutants (Table 5). In addition, we observed among the progeny of pol-1 wus-1 pol-1 WUS plants a significant portion of plants that exhibited weak wus phenotypes. These phenotypes consisted primarily of terminated vegetative shoot meristems, but rarely the termination of inflorescence shoot meristems as well (Fig. 7BE). To determine if the terminated meristem phenotype was the
result of *wus* heterozygosity, we tested progeny of all fertile plants. Every plant that exhibited these phenotypes was heterozygous for *wus*, while phenotypically normal plants were largely wild-type for *WUS*, although some were *wus*/WUS (Table 6). Thus, *wus* semi-dominance in the pol mutant background was not fully penetrant.

**pol enhances *stm* phenotypes**

Because of the competitive nature of the interactions between the CLV loci and STM (Clark et al., 1996), we predicted that pol should enhance *stm* mutant phenotypes. Utilizing techniques similar to that for isolating pol ag plants, double mutant plants of *pol-1* with the strong and weak *stm* alleles, *stm-1* and *stm-2*, respectively, were generated (Fig. 1; Table 1; see Materials and Methods). We observed that pol enhanced the phenotypes of both *stm* alleles. *stm-1* germinates without a shoot meristem, but is capable of developing post-embryonic leaf initiation on a portion of the mutant plants (Barton and Poethig, 1993). The formation of post-embryonic leaves was significantly reduced in *pol-1 stm-1* double mutant plants compared to *stm-1* single mutant plants (Table 7). The weak *stm-2* mutant plants initiate meristems that are eventually overtaken by organ formation, resulting in multiple rounds of meristem initiation and termination (Clark et al., 1996). *pol-1 stm-2* double mutant plants developed noticeably less post-embryonic growth compared to *stm-2* single mutant plants (Fig. 8), indicating that pol enhances the *stm-2* phenotype as well.

**Table 5. pol-1 reduces floral organ number in wus-1 mutant flowers**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Average number of organs per flower*†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sepals§</td>
</tr>
<tr>
<td><em>pol-1 wus-1</em></td>
<td>3.02±0.1</td>
</tr>
<tr>
<td><em>wus-1</em></td>
<td>3.5±0.1</td>
</tr>
</tbody>
</table>

*Values represent the mean number ± standard error of the mean of indicated organs.
†61 flowers were counted for each mean and standard error of the mean calculated.
§P<0.005.
¶P<0.001.
**Whorl 3 organs that developed as filamentous organs were included in the count of stamens.

**Fig. 5.** pol dominantly suppresses both the carpel defects of *clv1* and *clv2* and the pedicel defects of *clv2*. The progeny of *clv1-1/clv1-1 pol-1/POL and *clv2/clv2 pol-1/POL* were scored for carpel number. The *clv2* plants were also scored for pedicel length. These plants were then tested for POL genotype by progeny testing. (A) The mean length of pedicels (mm) for each genotype. (B) The mean number of carpels per flower. White bars represent POL/POL, gray bars represent *POL/pol-1*, and black bars represent *pol-1/pol-1*. At least 80 flowers and pedicels were counted for each mean calculated. Only the first ten flowers and pedicels of any given plant were analyzed. Standard errors of the mean are indicated by vertical black lines.

**Fig. 6.** pol specifically suppresses the indeterminacy of *clv* flowers. Flowers of *pol-1 ag-3* plants (A,C) are identical to those of *ag-3* plants (B,D) both in terms of overall morphology and in terms of floral meristem structure as analyzed by scanning electron microscopy. Scale bars, 20 μm.
pol does not affect CLV1 receptor-kinase activation

The observations that pol dominantly suppresses the phenotypes of many clv alleles, and that it suppresses the plants homozygous for the null clv3-2 allele, suggests that POL functions downstream of the CLV loci (see Discussion). The CLV loci all appear to act as signaling molecules at the cell surface, with CLV3 activating the membrane-localized CLV1 and CLV2 (Trotochaud et al., 1999). CLV1 activity can be assayed by examining CLV1 complex formation in vivo. CLV1 is found in two complexes: an inactive 185 kDa complex (fractions 17-18) that may be a CLV1-CLV2 heterodimer, and an active 450 kDa complex (fractions 12-13) that includes the protein phosphatase KAPP and a Rho GTPase-related protein (Trotochaud et al., 1999). In clv3 mutant plants, CLV1 is found exclusively in the inactive 185 kDa complex, indicating that the formation of the active complex requires CLV3.

If POL functions downstream of CLV1, then pol mutations should have no direct effect on CLV1 complex formation. A rigorous way to test this idea would be to examine CLV1 complex formation in pol-1 clv3-2 double mutant plants. If pol suppression is downstream of CLV1, the pol-1 clv3-2 plants should accumulate no active CLV1. On the other hand, if pol suppression acts by increasing activation of CLV1, then CLV1 in the active 450-kD complex may be observed in the pol-1 clv3-2 double mutant plants. When CLV1 complex formation was assayed in clv3-2 and pol-1 clv3-2 plants, no difference was observed (Fig. 9). In other words, all of the CLV1 remained in the inactive 185 kDa complex despite the pol mutation.

DISCUSSION

Research into meristem development in Arabidopsis has only uncovered a handful of specific regulators. However, even in plants with the most severe mutant alleles of these regulators and in various double mutants, organ formation still occurs. This suggests that additional genes are present that allow for organ formation even in the absence of these genes. The additional genes may have remained unidentified for several reasons. First, the additional genes may regulate multiple development processes leading to pleiotropic mutant

Table 6. wus-1 is semi-dominant in a pol-1 background

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. plants</th>
<th>WUS/WUS</th>
<th>wus-1/WUS</th>
<th>wus-1/pol-1 WUS</th>
<th>wus-1/pol-1 WUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>32</td>
<td>26</td>
<td>10.7</td>
<td>6</td>
<td>21.3</td>
</tr>
<tr>
<td>was-1-like rosette with wild-type inflorescence</td>
<td>24</td>
<td>0</td>
<td>8</td>
<td>24</td>
<td>16</td>
</tr>
</tbody>
</table>

Progeny of a pol-1 was-1/pol-1 WUS plant were scored as having wild-type phenotype, was-1-like rosette with wild-type inflorescence phenotype, or was-1-like rosette and inflorescence phenotype. The number of plants segregating wus-1 was compared with the number that would be expected to segregate wus-1, if wus-1/WUS had no effect on the phenotype of these plants.

Table 7. pol-1 reduces the rate of postembryonic development in stm-1 mutants

| Days after germination | Rate of postembryonic development*‡ | | | | | |
|------------------------|------------------------------------|---|---|---|---|
|                        | stm-1                             | pol-1                                      |
| 15                     | 0                                 | 0                                         |
| 24                     | 0.29                              | 0.14                                      |
| 27                     | 0.39                              | 0.21                                      |
| 30                     | 0.48                              | 0.27                                      |

*Values represent the number of plants that developed leaves in the axes of the cotyledons per total number of surviving mutant plants.
‡More than 129 viable mutant plants were counted for each rate calculated.

Fig. 7. wus-1 is semi-dominant in a pol-1 homozygous background. wus-1 and pol-1 was-1 plants and leaves at 70 days (A) and pol-1 was-1/pol-1 WUS plants where the primary shoot meristem terminated leading to the development of multiple lateral shoot meristems indicated by white arrows (B,C).

Fig. 8. pol enhances stm phenotypes. Representative stm-2 (A) and pol-1 stm-2 (B) plants at 70 days.
meristems. The indeterminacy is not altered in mutant plants, while it is suppressed in mutations. Both suppression of flower meristem determinacy is specific to the activity partially is consistent with both hypotheses. However, several additional pol suppresses the phenotypes of all CLV antibodies. dotted onto nitrocellulose al. (1999). Fractions were described by Trotochaud et superose 6 column as by gel filtration on a (B) plants were fractionated to altered CL V1 receptor accumulation of undifferentiated cells observed in indeterminate from the fact the stm phenotypes, as pol clv mutations (Laux et al., 1996), becomes semi-dominant in a pol mutant background. This demonstrates that pol mutant plants require a full level of WUS activity to develop in a largely normal manner, indicating that POL and WUS function closely together in the regulation of meristem development. (4) pol enhances the wus-1 phenotype, suggesting either that POL functions redundantly with WUS, or that wus-1 is not a null allele. wus-1 contains a nonsense mutation downstream of the coding sequence for the homeodomain, but displays phenotypes similar to alleles with nonsense mutations near the very beginning of the coding sequence, suggesting that wus-1 is a null allele (Mayer et al., 1998). pol wus double mutants also exhibit synergistic phenotypes, suggesting again that these genes are redundant. Thus, the specificity for suppressing clv and the extensive dominant interactions are all consistent with POL acting within the CLV pathway. However, genetic analysis can never constitute proof that genes function in the same pathway – this must await a biochemical analysis of the respective gene products.

The interpretations of POL function should hold whether the pol alleles are partial or complete loss-of-function mutations. pol alleles are very unlikely to be gain-of-function alleles because of the relatively high number of independent pol alleles (three) that were isolated from a relatively small mutagenized population (Pogány et al., 1998).

Evidence for the role of WUS
The indication that POL and WUS function redundantly clarifies the role of WUS in meristem development. The previously demonstrated epistasis of wus mutations over clv1 mutations (Laux et al., 1996) left open two possible roles for WUS in meristem development. WUS could function either upstream of the CLV loci to pattern the meristem upon which these genes act, or downstream as a target for CLV signaling. Previous genetic and molecular genetic analyses have failed to distinguish between these possibilities. The uncertainty arises from the fact the wus mutants do not develop functional meristems. Thus, it was unclear if the CLV loci are ever active in wus mutants. The evidence that POL functions redundantly with WUS indicates that WUS functions downstream of the CLV loci. This is because even though pol suppresses the phenotype of the putative null clv3-2 allele and is epistatic to weak clv mutations, these results are unlikely to be the result of POL functioning upstream, as pol mutants generate functional meristems that clearly require the CLV loci. (3) The normally recessive wus mutation, which is known to be epistatic to clv mutations (Laux et al., 1996), becomes semi-dominant in a pol mutant background. This demonstrates that pol mutant plants require a full level of WUS activity to develop in a largely normal manner, indicating that POL and WUS function closely together in the regulation of meristem development. (4) pol enhances the wus-1 phenotype, suggesting either that POL functions redundantly with WUS, or that wus-1 is not a null allele. wus-1 contains a nonsense mutation downstream of the coding sequence for the homeodomain, but displays phenotypes similar to alleles with nonsense mutations near the very beginning of the coding sequence, suggesting that wus-1 is a null allele (Mayer et al., 1998). pol wus double mutants also exhibit synergistic phenotypes, suggesting again that these genes are redundant. Thus, the specificity for suppressing clv and the extensive dominant interactions are all consistent with POL acting within the CLV pathway. However, genetic analysis can never constitute proof that genes function in the same pathway – this must await a biochemical analysis of the respective gene products.

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phenotypes in pol stm plants. This evidence supports the idea that POL is redundant with WUS, but not STM.

**POL regulates pedicel length**

One of the remarkable aspects of the pol phenotype is that it suppresses both the meristem defects and pedicel defects of clv2 mutant plants. We have previously demonstrated that CLV2 regulates pedicel length independent of its regulation of the floral meristem, and independent of CLV1 and CLV3 (Kayes and Clark, 1997). An attractive model is that POL functions downstream of CLV2 in both the regulation of meristem development and the regulation of pedicel length. If this is the case, then POL is again redundant with a separate factor, because CLV2 stills functions to repress pedicel length in the pol mutant background (i.e., pol clv2 plants developed longer pedicels than pol plants). An alternative hypothesis is that POL functions in an independent pathway to regulate pedicel development, and that the pol clv2 double mutant phenotype can be viewed as additive.

**POL function**

The likely function of POL is to promote the undifferentiated state of cells at the center of the shoot and floral meristems. This would be consistent with the reduction in the number of undifferentiated cells in pol clv double mutant plants, as well as the reduction in adventitious meristem activity in pol wus and pol stm double mutant plants. If POL functions downstream of the CLV loci as indicated by the present study, then it may be a target for CLV1 signal transduction. CLV1 encodes a receptor-like kinase that is hypothesized to relay positional information within the meristem (Clark et al., 1997). CLV1 is activated by CLV3 (TROTOCHAU et al., 1999), which codes for a putative proteinaceous ligand (Fletcher et al., 1999). The active CLV1 is associated with a Rho GTPase-related protein (TROTOCHAU et al., 1999) which may then relay a signal that targets both POL and WUS. The genetic models of POL function make further predictions about the molecular nature of the POL gene product. The proposed redundant nature of POL and WUS function would suggest that because WUS encodes a putative transcription factor, POL may code either for a transcription factor or a protein that regulates one. POL should be expressed both in the shoot meristem in an overlapping domain with WUS, and in the developing floral pedicel. Because POL function in the shoot meristem is not readily detectable, we cannot rule out the possibility that POL functions in many developmental processes, but that phenotypes in these processes are also not readily apparent. Resolution of these ideas await the identification of the POL gene.

Authors would like to thank Jeffrey Pogany and Rebecca Katzman for isolating the original pol alleles, members of the Clark lab and Dr Keiko Torii for their helpful comments and critical reviews of the manuscript. Dr David Meinke for providing embt34-1 and embt101-1 seeds, and David Kay for photographic assistance. This material is based upon work supported by a grant from the Department of Energy (DE-FG02-96ER20227) to S. E. C. L. P. Y. is supported by the National Institute of Health, Cellular Biotechnology Training Program (Grant No. GM08353).

**REFERENCES**


