POL and PLL1 phosphatases are CLAVATA1 signaling intermediates required for *Arabidopsis* shoot and floral stem cells

Sang-Kee Song1,*, Myeong Min Lee2 and Steven E. Clark1,†

The post-embryonic development of above-ground tissues in plants is dependent upon the maintenance and differentiation of stem cells at the shoot meristem. The *Arabidopsis* WUSCHEL (WUS) transcription factor establishes an organizing center within the shoot meristem that is essential for specification of stem-cell identity in overlying cells. The CLAVATA (CLV) signaling pathway, including the CLV1 receptor-kinase, promotes the differentiation of stem cells by limiting the WUS expression domain, yet the mechanism of CLV signaling is largely unknown. Previously, we have shown that mutations in two protein phosphatases, POLTERGEIST (POL) and PLL1, partially suppress *clv* mutant phenotypes. Here, we demonstrate that POL and PLL1 are integral components of the CLV1 signaling pathway. POL and PLL1 are essential for stem-cell specification, and can also block stem-cell differentiation when overexpressed. We provide extensive evidence that POL and PLL1 act downstream of CLV signaling to maintain WUS expression and that they regulate WUS at a transcriptional level. Our findings suggest that POL and PLL1 are central players in regulating the balance between stem-cell maintenance and differentiation, and are the closest known factors to WUS regulation in the shoot meristem.

**KEY WORDS:** Organogenesis, CLAVATA, Meristem, Differentiation, *Arabidopsis*

**INTRODUCTION**

The adult plant body is generated from the continuous and iterative organogenesis at two stem-cell containing structures: the shoot and root meristems. The plant shoot meristem is established at the apical end of the embryo, and is responsible for generating all of the organs and lateral meristems found above ground. A functional shoot meristem is maintained through a tightly controlled balance between the proliferation of a group of stem cells residing in the center, and the differentiation of their peripheral and basal progeny cells for the formation of organ primordia and other differentiated tissues. Flower meristems initiated during inflorescence development function similarly to shoot meristems, except for the transient nature of their stem-cell population.

The homeodomain-containing transcription factor WUSCHEL (WUS) is necessary and sufficient within the meristem to specify stem-cell identity. *wus* mutations lead to the loss of shoot meristem stem cells, and WUS overexpression gives rise to ectopic stem cells within the meristem (Brand et al., 2002; Gallois et al., 2002; Laux et al., 1996; Lenhard et al., 2002; Mayer et al., 1998; Schoof et al., 2000). WUS is expressed immediately basal to the stem cells in what is termed as an ‘organizing center’ (Mayer et al., 1998).

The CLAVATA (CLV) signal transduction components CLV1, CLV2 and CLV3 act to restrict the domain of WUS expression (Brand et al., 2000; Schoof et al., 2000). CLV1 encodes a receptor-like kinase containing leucine-rich repeat (LRR) motifs, CLV2 a LRR receptor-like protein and CLV3 a small secreted polypeptide (Clark et al., 1997; Fletcher et al., 1999; Jeong et al., 1999; Rojo et al., 2002; Ni and Clark, 2006). Recent findings have shown that transient inactivation of the CLV pathway leads to rapid alterations in the expression of meristem regulators, consistent with earlier studies investigating the effect of *clv* mutations on WUS expression (Reddy and Meyerowitz, 2005). Despite our improving understanding of the crucial biological role of this signaling pathway in regulating stem-cell specification and differentiation, no downstream signaling intermediates have been definitively identified.

The best candidate for a CLV signaling intermediate is the protein phosphatase kinase-associated protein kinase (KAPP) (Stone et al., 1994; Stone et al., 1998; Williams et al., 1997). KAPP binds, among many receptor-kinases, to CLV1, and both overexpression and co-suppression studies have suggested that KAPP plays a role in repressing CLV1 function (Stone et al., 1994; Stone et al., 1998; Williams et al., 1997). However, neither a definitive genetic study nor a clear mechanism for KAPP function has been reported.

Another potential source of signaling components are modifier mutants. Previous studies have identified mutations in many genes that enhance or suppress the phenotype of *clv* mutants and are potential candidates for signaling intermediates, including *SHOOTMERISTEMLESS*, *ULTRAPETALA*, *REVOLUTA*, *PHABULOSA*, *PHAVOLUTA*, *CORONA*, *PERIANTHIA*, and *WIGGUM/ENHANCED RESPONSE TO ABA 1* (Clark et al., 1996; Fletcher, 2001; Green et al., 2005; Otsuga et al., 2001; Prigge et al., 2005; Running et al., 1998; Running and Meyerowitz, 1996). However, detailed analyses of all of these genes suggest that each acts independently of the CLV signaling pathway.

Two additional genes that modify the *clv* phenotype when mutated are POLTERGEIST (POL) and PLL1, which encode related protein phosphatases. Mutations in either gene provide partial, additive suppression of the stem-cell accumulation of *clv* mutants, raising the possibility that these two genes act redundantly to promote stem-cell identity (Song and Clark, 2005; Yu et al., 2003;
Yu et al., 2000). However, the pol pll1 double mutant is seeding lethal, complicating previous efforts to analyze these genes and their potential role in CLV signaling.

In this study, we report a detailed analysis of POL, PLL1, WUS and CLV genetics. We overcome seedling lethality through grafting, and show interactions between mutations in these genes and their overexpression. All of our findings are consistent with a model in which POL and PLL1 act downstream of the CLV proteins, CLV overexpression. All of our findings are consistent with a model in

MATERIALS AND METHODS

Plant materials and growth condition

The pol-1, pol-6 and pll1-1 mutants were obtained as described previously (Song and Clark, 2005; Yu et al., 2003; Yu et al., 2000). pol-6 pll1-1 plants were isolated from the F2 progeny obtained from the cross between pol and pll1-1, and, subsequently, pol-6 pll1-1 double mutants were identified among the progeny of pol-6 pll1-1 plants based on polymerase chain reaction (PCR) genotyping, as described previously (Song and Clark, 2005). Plants were grown as described previously (Song and Clark, 2005). When plants were grown in sterile conditions, seeds were germinated on the half strength of MS media (Sigma) containing 1% sucrose and 0.02% MES. Plants were grown as described previously (Song and Clark, 2005). When plants were grown in sterile conditions, seeds were germinated on the half strength of MS media (Sigma) containing 1% sucrose and 0.02% MES.

Reaction (PCR) genotyping, as described previously (Song and Clark, 2005). The seedlings of the triple mutants were grown in sterile conditions and grafted as described above, and their floral organ numbers were counted.

We used PLL1c1/PLL1c2 primers (PLL1c1, 5'-GAATTCAGACGACACAAAATTT-3') and PLL1c2/PLL1c3 primers (PLL1c2, 5'-GAATTCAGACGACACAAAATTT-3') for PCR amplification of PLL1 into a pUC19 vector (digested with SalI and SpeI) to test for the presence of both transgenes.

Expression pattern analyses of reporter genes

For ectopic WUS expression of WUS

For ectopic WUS expression, the pOpL two-component expression system was used (Moore et al., 1998). APETALA1 (AP1) (Hempel et al., 1998) and ERECTA (ER) promoters (Dievart et al., 2003) were used to drive the expression of LhG4 (kindly provided by Michael Prigge, University of Indiana, Indiana, USA). Several independent transgenic lines were screened and crossed with transgenic plants expressing pOp:WUS (Schoof et al., 2000) (kindly provided by Thomas Lux) to examine the strength of the promoter based on the phenotypes of F1 plants. F1 progeny obtained from the crosses between the selected AP1:LhG4 line (or ER:LhG4 line) and pol-6 pll1-1/+ were crossed to F1 progeny obtained from the crosses between pOp6:WUS and pol-6 pll1-1/. Among the resulting new progeny, pol pll1 plants were isolated, grafted and PCR genotyped using gene-specific primers (LhG4/LhG4r for AP1:LhG4 and ER:LhG4; WUS/N/WUS-C for pOp6:WUS) to test for the presence of both transgenes (LhG4, 5'-AATCTTTAACATACGATGCGAGAG-3'; LhG4r, 5'-CCATCTCGTTCTTGTTCGTGA-3'; WUS, 5'-CCGGATGCGAGAGAGAGAG-3'; WUS-C, 5'-GGATCTCTACCGAGAGAGAG-3'; AP1:LhG4 and pOp6:WUS transgenes were introduced into the wus-1 mutant background in a similar manner.

RESULTS

Grafted pol pll1 tissue phenocopies wus mutants

A major obstacle in analyzing POL/PLL1 function in stem-cell specification was the embryo/seeding lethality of the pol pll1 double mutants (Song and Clark, 2005). We determined that pol pll1 lethality was largely the result of major defects in basal embryo patterning, and that we could grow pol pll1 double-mutant tissue by grafting the apical portion of a pol-6 pll1-1 seedling onto the hypocotyl/root of a wild-type seedling (Fig. 1A-E). Both pol-6 and pll1-1 are T-DNA insertion alleles that are putative nulls (Song and Clark, 2005).

The pol pll1 tissues (Fig. 1F,G) growing in such grafted plants closely phenocopied wus mutants (Fig. 1I), with re-iterative termination of shoot apices during vegetative development.
Eventually transitioning to flowering as wus mutants do, the pol pll1 grafted tissue (Fig. 1K,L) gave rise to inflorescence phenotypes similar to wus mutants (Fig. 1N), including flowers with reduced numbers of floral organs, presumably as a result of the loss of flower-meristem stem cells (compare Fig. 1P,Q with Fig. 1S). The meristem-termination phenotypes were also observed, albeit less frequently, in pol pll1 mutants (Fig. 2). The meristem-termination phenotypes were also observed, albeit less frequently, in pol pll1 mutants (Fig. 2). The meristem-termination phenotypes were also observed, albeit less frequently, in pol pll1 mutants (Fig. 2).

To examine the post-embryonic phenotypes, clv3-2 pol pll1 mutants were grafted onto the wild-type hypocotyls. clv3-2 pol pll1 tissue developed in an identical fashion to clv3-2 pol pll1/+/+ plants (79:31) and clv3-2 pol pll1/pll1 plants with incomplete complementation by PLL1 (Fig. 1U-Y). This indicates that these phenotypes are not related to the grafting technique used to generate pol pll1 tissue, but are a consequence of reduced POL/PLL1 activity.

**pol pll1 is epistatic to clv3**

The severe loss of meristem activity in pol pll1 double-mutant tissue allowed us to address whether POL/PLL1 act upstream or downstream of the CLV signaling pathway by generating clv pol-6 pll1-1 triple mutants. A similar genetic approach was previously used to establish that WUS acts downstream of CLV signaling, a finding bourn out by subsequent detailed studies (Laux et al., 1996; Mayer et al., 1998; Schoof et al., 2000). The seedling lethality of pol pll1 mutants was unaffected by the introduction of the putative null clv3-2 mutation. The ratio between the viable plants and seedling-lethal plants in the progeny of clv3-2 pol pll1/+ plants (79:31) and the progeny of clv3-2 pol pll1/+ plants (54:19) did not vary significantly from 3:1 based on χ² analysis. clv2-1 pol pll1, clv1-7 pol pll1 and clv1-1 pol pll1 triple mutants also exhibited seedling-lethal phenotypes.

To examine the post-embryonic phenotypes, clv3-2 pol pll1 mutants were grafted onto the wild-type hypocotyls. clv3-2 pol pll1 tissue developed in an identical fashion to pol pll1 double-mutant tissue during vegetative and inflorescence development (Fig. 1H,K). The mean number of organs developing in clv3-2 pol pll1/+/+ plants (207:69) was statistically indistinguishable from that of pol pll1/+/+ plants (207:69). These results indicate that pol pll1 is fully epistatic to clv3-2, indicating that POL/PLL1 act downstream of the CLV signaling pathway.

**Altering stem-cell marker gene expression in pol pll1 mutants**

To determine the nature of meristem defects in pol pll1 mutants, we crossed pol pll1 to well-characterized transgenes in which the CLV3 and WUS cis regulatory elements drive β-glucuronidase (GUS) expression (P<sub>CLV3</sub>GUS and P<sub>WUS</sub>GUS) and monitored the expression of these key meristem regulatory factors (Gross-Hardt et al., 2002; Lenhard et al., 2002). For P<sub>WUS</sub>GUS, we observed a clear spot of GUS activity at the shoot meristem in wild-type seedlings, but no signal at all was observed in pol pll1 seedlings (data not shown). Around the transition to flowering, when WUS becomes expressed in the many wild-type flower meristems, we observed occasional punctate spots of WUS expression in pol pll1 plants (Fig. 3A-F). These spots corresponded to expression in internal cell layers.
of what morphologically appeared to be nascent meristems (Fig. 3C,F). When compared to P\textsubscript{WUS}:GUS expression in wild-type plants, the spots in pol pll1 mutants appeared to correspond to transient apices forming in leaf axils. These results suggest that POL/PLL1 are required for the maintenance, but not the initiation, of apices forming in leaf axils. These results suggest that POL/PLL1 are important for maintenance, but not initiation, of expression. Because data indicate that CLV signaling is also important for maintenance, but not initiation, of WUS expression, these results are consistent with the hypothesis that POL/PLL1 functions within the CLV pathway. If CLV signaling achieves repression of WUS through the inhibition of POL/PLL1 activity, one would expect constitutive inhibition of WUS in pol pll1 mutants after initiation.

\(P_{\text{Ctv3}}:GUS\) activity was detected in the initiating shoot apical meristem of pol pll1 embryos; however, the activity was weaker, compared with wild type, and was restricted to the epidermal layer (see Fig. S2 in the supplementary material). Post-embryonically, \(P_{\text{Ctv3}}:GUS\) behaved similarly to \(P_{\text{WUS}}:GUS\) in wild-type plants and in pol pll1 mutants, with punctate spots of \(P_{\text{Ctv3}}:GUS\) activity in pol pll1 mutants in apparent transient shoots (Fig. 3G-O). Similar to wild-type meristems, the \(P_{\text{Ctv3}}:GUS\) signal was largely within apical cells layers within these apparently transient meristems (Fig. 3L,O). Both reporter-gene expression patterns and morphology suggest that meristems are initiated but immediately lost in pol pll1 plants, consistent with hyper-repression of WUS after meristem initiation.

**Ectopic WUS expression bypasses the requirement of POL/PLL1 for stem cells**

To definitively test whether the loss of meristem activity in pol pll1 mutants was the consequence of the loss of WUS expression maintenance, we designed a transgene arrangement to determine if ectopic WUS expression could bypass the requirement for POL/PLL1. We set expression of WUS under the control of the flower-specific APETALA1 (AP1) cis regulatory elements in a transactivation system, in which AP1-driven WUS expression would only occur in the progeny of plants carrying both the \(P_{\text{AP1}}:LHG4\) driver and the \(P_{\text{OPC}}:WUS\) responder (Hempel et al., 1998; Moore et al., 1998; Schoof et al., 2000). In wild-type plants with \(P_{\text{AP1}}:LHG4\) driven WUS, \(P_{\text{OPC}}:WUS\) was not under autoregulatory control for the receptor-kinase ERECTA (ER) (Diévart et al., 2003; Yokoyama et al., 1998). Using the same transactivation transgene arrangement followed by grafting to generate \(P_{\text{ER}}:WUS\) pol pll1 tissue, we observed restoration of meristem proliferation that was less extensive than \(P_{\text{AP1}}:WUS\) and gave rise to more normal floral organs (Fig. 4LJ). Thus, the loss of pol pll1 meristem activity is directly attributable to the loss of WUS expression maintenance, indicating that POL/PLL1 act through WUS to promote stem-cell identity.

**Ectopic PLL1 expression blocks differentiation in clv mutants**

If POL/PLL1 are indeed targeted for negative regulation by CLV signaling, one would predict that overexpressing POL/PLL1 would enhance clv mutants, providing de-repression of excess

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Fig. 2. **pol pll1 mutations are epistatic to clv3.** Mean number of organs from flowers of grafted pol pll1 in the er\(^*\), ER\(^*\) and clv3-2 backgrounds, wus-1 and clv3-2 mutants, and wild-type Ler plants. Error bars indicate standard error of the mean (s.e.m.). At least 50 flowers were counted for each mean.

Fig. 3. **pol pll1 tissue initiates, but does not maintain, WUS and CLV3 expression.** (A-O) \(P_{\text{WUS}}:GUS\) (A-F) and \(P_{\text{CLV3}}:GUS\) (G-O) signal in pol pll1 seedlings germinated on MS media (G-I), inflorescence tissue from grafted pol pll1 plants (A-C,J-L), and phenotypically wild-type siblings (D-F,M-O). Each sample is shown at increasing magnifications to provide context for the location of the signal within the plant.
POL/PLL1. Given the extensive gene families for \( CLV1, CLV2 \) and \( CLV3 \) (Botella et al., 1997; Sharma et al., 2003; Shiu and Bleecker, 2001), and evidence that \( clv1 \) and \( clv2 \) null alleles exhibit rather weak phenotypes (Diévart et al., 2003; Kayes and Clark, 1998), it is unclear whether any \( clv \) single mutant represents a complete loss of signaling. We have previously shown that \( PLL1 \) overexpression in an otherwise wild-type background gives rise to weak \( Clv^- \) phenotypes (Song and Clark, 2005). Here, we drove \( PLL1 \) expression under the control of both \( ER \) (\( P_{ER} \)) and the cauliflower mosaic virus (CaMV) \( 35S \) (\( P_{35S} \)) cis regulatory elements in the \( clv2-1 \) and \( clv3-2 \) mutant backgrounds, and observed dramatic enhancement of the \( Clv^- \) phenotype (Fig. 5). In many cases, the transgenic plants simply produced meristem tissue at the shoot apex, with a complete absence of organ formation over a long developmental period. Some plants senesced and died without producing organs, whereas others developed ‘escape’ tissue, presumably as a result of transgene repression, that went on to produce a small number of organs after a sustained period without organogenesis (see Fig. S1 in the supplementary material). To determine whether the overproliferating tissue was stem cell-like in nature, we introduced \( P_{CLV3}:GUS \) and \( P_{WUS}:GUS \) (Gross-Hardt et al., 2002; Lenhard et al., 2002) into the \( P_{35S}:PLL1 \ clv3-2 \) background (Fig. 5). In both cases, we observed GUS activity throughout the apex, with the strongest \( P_{WUS}:GUS \) signal in internal cell layers and the strongest \( P_{CLV3}:GUS \) signal in the overlying cells. The presence and pattern of both meristem markers, as well as the morphology of these structures, indicate that these accumulated cells are equivalent to the central zone of a wild-type meristem, including stem cells in the top three cell layers. This data also indicate that \( clv \) mutants have a partial loss of differentiation that is further antagonized by the overexpression of \( PLL1 \). This is consistent with both a detailed analysis of \( clv \) mutants, as well as a recent study showing that inducible downregulation of \( CLV3 \) leads to the rapid expansion of the \( CLV3 \) expression domain (Reddy and Meyerowitz, 2005). Indeed, it would be of interest to repeat this sort of analysis in a \( PLL1 \) overexpression background, as \( P_{35S}:PLL1 \ clv3 \) results in a more complete loss of differentiation than a \( clv3 \) single mutant.

Ectopic stem cells induced by \( PLL1 \) overexpression require \( WUS \) and suppress \( CLV3 \) overexpression effects

To determine whether the effect of \( PLL1 \) overexpression was entirely dependent upon \( WUS \), we assessed the effect of the \( P_{35S}:PLL1 \) transgene in the \( wus-1 \) mutant background. \( P_{35S}:PLL1 \ wus \) plants were readily distinguishable from \( wus \) single mutants during vegetative development because \( PLL \) overexpression results in altered leaf morphology, namely smaller, rounder leaves (Song and Clark, 2005). Beyond this difference in leaf development, \( P_{35S}:PLL1 \) had no effect that we could detect on the terminated vegetative or inflorescence meristems observed in \( wus \) mutants (see Fig. S1 in the supplementary material). Similarly, within the flowers, \( P_{35S}:PLL1 \) had no statistically measurable effect on the number of organs formed in \( wus \) flowers. Most importantly, no increase in the number of stamens or carpels per flower as a result of \( PLL1 \) overexpression was observed (Fig. 6). \( wus \) is thus fully epistatic to \( P_{35S}:PLL1 \) within the meristem, consistent with the hypothesis that \( PLL1 \) acts within the meristem by regulating \( WUS \).

Overexpression of \( CLV3 \) leads to shoot- and flower-meristem termination, resulting in plants phenotypically similar to \( wus \) mutants, albeit less severe (Brand et al., 2000). If \( CLV \) signaling acts to repress \( POL/PLL1 \) activity, then overexpression of \( PLL1 \) in the \( P_{35S}:CLV3 \) background would be predicted to suppress, at least partially, the meristem termination phenotype. Progeny from a cross between plants carrying the \( P_{35S}:CLV3 \) transgene and plants carrying the \( P_{35S}:PLL1 \) transgene were assayed for the shoot-meristem termination phenotype and genotyped for the presence of the two transgenes. Whereas every plant carrying \( P_{35S}:CLV3 \) alone developed terminated shoot meristems, 80% of the plants carrying both transgenes developed normal shoot meristems (Table 1). Transgene expression analysis indicated that this was not the result of suppression of the \( P_{35S}:CLV3 \) transgene (see Fig. S3 in the supplementary material).

DISCUSSION

Taken together, our analyses strongly suggest that \( POL/PLL1 \) are intermediates downstream of the CLV factors in the regulation of WUS expression and stem-cell differentiation. \( POL/PLL1 \) are required for stem-cell maintenance through their regulation of WUS expression. \( POL/PLL1 \) overexpression blocks differentiation and drives stem-cell accumulation, especially in a \( clv \) mutant background. This would suggest that CLV signaling inhibits \( POL/PLL1 \) post-
transcriptionally and that a combination of excess PLL1 transcription and loss of CLV-mediated inhibition is sufficient to block stem-cell differentiation.

POL/PLL1 regulation of WUS appears to be on the level of transcription, as evidenced by the loss of WUS transcription in pol pll1 mutants, as well as by the ability to bypass POL/PLL1 by expressing WUS under a different set of cis regulatory elements. The initiation of WUS expression in transient shoots in pol pll1 double mutants, as well as the slightly weaker phenotype of pol pll1 double-mutant tissue in comparison with wus null alleles, reflects that POL/PLL1 regulation of WUS expression acts only after meristem initiation. Thus, POL/PLL1 are primarily regulators of the maintenance, not the initiation, of WUS expression. This is fully consistent with data on the function of the CLV pathway in regulating WUS expression.

It is formally possible that POL/PLL1 act independently on WUS rather than acting as intermediates of CLV signaling, but this alternative hypothesis is not supported by the data. First, pol pll1 mutants do express WUS, albeit only in transient shoots. If POL/PLL1 and CLV were acting separately, one would expect the removal of CLV signaling to expand WUS expression, and hence alter phenotypes, in pol pll1 mutants. Therefore, this alternative hypothesis would predict that clv should suppress, to some extent, pol pll1 double-mutant phenotypes. This is especially true in the flower meristem, where we see transient WUS and CLV3 expression in pol pll1, and these mutants exhibit flower-meristem defects that are slightly weaker than wus. Even here, there is no effect of removing CLV signaling, as evidenced by the full epistasis of pol pll1 to clv mutants. In addition, there is an incredible level of dosage sensitivity between CLV, POL/PLL1 and WUS in both loss- and gain-of-function situations, suggesting a common pathway.

CLV repression of POL/PLL1 is likely to be post-transcriptional, based on both the broad expression of POL and PLL1 throughout the plant and within the meristem (Yu et al., 2003; Song and Clark, 2005), and on the interaction of clv mutants with PLL1 overexpression. This would be quite typical for receptor signaling intermediates.

Little is known about downstream targets of other receptor kinases. POL/PLL1 share similarity in general pathway function with the BIN2 kinase in BRI1 receptor kinase-mediated brassinosteroid signaling (Li and Nam, 2002). POL/PLL1 and BIN2

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**Table 1. PLL1 overexpression suppresses CLV3 overexpression meristem termination**

<table>
<thead>
<tr>
<th>Genotype of F1 plants</th>
<th>n</th>
<th>Wus− phenotypes</th>
<th>Wild-type phenotypes</th>
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<tbody>
<tr>
<td>P3SS:CLV3</td>
<td>26</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>P3SS:PLL1 P3SS:CLV3</td>
<td>16</td>
<td>3</td>
<td>13</td>
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*Number of plants; †plants exhibiting shoot termination typical of wus mutants; ‡plants with superficially wild-type shoot development.
appear to be negatively regulated by the corresponding receptor kinase, and said repression allows for signaling to occur. In the case of BIN2 repression, brassinosteroid signaling occurs as the result of the loss of BIN2-mediated phosphorylation and subsequent degradation of a set of transcription factors (Wang et al., 2002; Yin et al., 2002; Zhao et al., 2002). As with BIN2, the exact link between the POL/PLL1 and the upstream receptor kinase remains unclear. Whether CLV1 directly modulates POL/PLL1 activity and what are the targets of POL/PLL1 phosphatase activity are crucial issues to pursue.

pol pll1 mutants have pleiotropic phenotypes, with evidence for POL/PLL1 regulation of basal embryo development, of pedicel development and of leaf vascular patterning (Song and Clark, 2005). These activities may represent the function of POL/PLL1 downstream of other receptor kinases. The redundant nature of many receptor kinases may explain why the corresponding receptors have not yet been identified (Cano-Delgado et al., 2004; DeYoung et al., 2006; Shpak et al., 2004; Zhou et al., 2004).

It is of interest to consider that the CLV1-related BAM receptors in Arabidopsis may also utilize POL and related proteins as signaling intermediates. bam mutant combinations exhibit many developmental defects, including effects on leaf vascular patterning similar to those that result from the overexpression of PLL1 (DeYoung et al., 2006; Song and Clark, 2005), raising the possibility that BAM receptors may regulate leaf development by signaling through POL/PLL1 or related phosphatases. The observation that CLV1 can replace BAM function during leaf, stem and floral-organ development suggest a common signaling pathway in each of these different tissues (DeYoung et al., 2006). Given the large receptor-kinase gene family in Arabidopsis, it will be crucial to test the association of specific receptors with potential downstream intermediates.

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Supplementary material
Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/133/23/4691/DC1

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