The Role of PENNYWISE and POUND-FOOLISH in the Maintenance of the Shoot Apical Meristem in Arabidopsis[1][W][OA]

Nolan Ung, Shruti Lal, and Harley M.S. Smith*

Center for Plant Cell Biology, Institute for Integrative Genome Biology, Department of Botany and Plant Sciences (N.U., H.M.S.S.), and Genetics, Genomics, and Bioinformatics Graduate Program (S.L., H.M.S.S.), University of California, Riverside, California 92521

Growth of the aerial part of the plant is dependent upon the maintenance of the shoot apical meristem (SAM). A balance between the self-renewing stem cells in the central zone (CZ) and organogenesis in the peripheral zone (PZ) is essential for the integrity, function, and maintenance of the SAM. Understanding how the SAM maintains a balance between stem cell perpetuation and organogenesis is a central question in plant biology. Two related BELL1-like homeodomain proteins, PENNYWISE (PNY) and POUND-FOOLISH (PNF), act to specify floral meristems during reproductive development. However, genetic studies also show that PNY and PNF regulate the maintenance of the SAM. To understand the role of PNY and PNF in meristem maintenance, the expression patterns for genes that specifically localize to the peripheral and central regions of the SAM were examined in Arabidopsis (*Arabidopsis thaliana*). Results from these experiments indicate that the integrity of the CZ is impaired in *pny pnf* plants, which alters the balance of stem cell renewal and organogenesis. As a result, pools of CZ cells may be allocated into initiating leaf primordia. Consistent with these results, the integrity of the central region of *pny pnf* SAMs can be partially restored by increasing the size of the CZ. Interestingly, flower specification is also reestablished by augmenting the size of the SAM in *pny pnf* plants. Taken together, we propose that PNY and PNF act to restrict organogenesis to the PZ by maintaining a boundary between the CZ and PZ.

Postembryonic shoot development is dependent upon the shoot apical meristem (SAM), a highly organized group of self-renewing cells, which initiates leaves, axillary meristems, and structures such as internodes (Steeves and Sussex, 1989; Lyndon, 1998). The SAM is subdivided into cytohistological domains including the central zone (CZ), which is located at the apical tip of the SAM and is the site at which stem cells are maintained. Lateral organs are initiated at the peripheral zone (PZ), which surrounds the CZ on its flanks, while the rib meristem (RM) located beneath the CZ produces cells that differentiate into the internal stem tissue (Bernier et al., 1981; Steeves and Sussex, 1989; Lyndon, 1998). The maintenance of the SAM is achieved by a balance of stem cell renewal in the CZ and the allocation of cells into primordia in the PZ (Vollbrecht et al., 2000). To date, little is known about how the SAM regulates the balance of these two interdependent processes in the CZ and PZ.

In Arabidopsis (*Arabidopsis thaliana*), recent studies show that the integrity of the CZ and RM is maintained by the CLV-WUS negative feedback system (Sablowski, 2007; Tucker and Laux, 2007; Bleckmann and Simon, 2009). The *WUSCHEL* (*WUS*) homeobox gene, expressed in a small number of cells in the core of the SAM, acts to maintain stem cell identity in the CZ (Laux et al., 1996; Mayer et al., 1998). The *CLAVATA* (*CLV*) genes encode receptors, CLV1 and CLV2, and a secreted ligand, CLV3 (Clark, 2001; Fletcher, 2002). The *CLV3* expression domain marks the CZ, while *CLV1* is expressed in the core of the meristem (Clark et al., 1997; Fletcher et al., 1999). The CLV pathway functions to down-regulate and restrict the *WUS* expression domain to the cells in the core of the SAM. At the same time, WUS somehow signals to the apical cells to promote *CLV3* expression in the CZ. The negative feedback interaction displayed by CLV3 and WUS acts to maintain a stable population of stem cells (Brand et al., 2000, 2002; Schoof et al., 2000). Mathematical modeling predicts that an additional signaling mechanism(s) is required to maintain stem cells in the CZ and the *WUS* expression domain in the RM (Jönsson et al., 2005; Geier et al., 2008). Recent studies indicate that stem cells produce active cytokinins (CKs; Kurakawa et al., 2007), which regulate the *WUS* expression domain through CLV-dependent and independent pathways (Gordon et al., 2009). At the same

[1] This work was supported by the National Science Foundation (grant no. IOB–0615774), by the U.S. Department of Agriculture-Agricultural Experimental Station, and by the National Institutes of Health (grant no. GM062756 to N.U.).

* Corresponding author; e-mail harleys@ucr.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Harley M.S. Smith (harleys@ucr.edu).

[W] The online version of this article contains Web-only data.

[OA] Open Access articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.110.171462

time, WUS functions to down-regulate the CK negative RESPONSE REGULATOR5 (ARR5), ARR6, ARR7, and ARR15, creating a region of high CK response in the RM (Leibfried et al., 2005). Thus, CK and WUS form a positive feedback loop, which functions to specify the RM and the stem cells during shoot growth (Gordon et al., 2009).

The Arabidopsis KNOTTED1-like HOMEOBOX (KNOX) protein SHOOT MERISTEMLESS (STM) regulates the maintenance of the SAM during shoot development (Long et al., 1996). Phenotypic analysis of weak *stm* alleles indicates that this homeodomain protein maintains the central region of the SAM (Endrizzi et al., 1996) as well as organ boundaries (Barton and Poethig, 1993; Endrizzi et al., 1996; Kanrar et al., 2006). Experimental studies indicate that STM regulates lateral organ boundaries via the interplay between cytokinin and gibberellin (Jasinski et al., 2005; Yanai et al., 2005). Genetic analyses demonstrate that loss of CLV1 and CLV3 function partially restores shoot development in *stm* mutants (Clark et al., 1996). At the same time, *stm* suppresses the enlarged meristems produced in *clv1* and *clv3* plants. Therefore, results from this study indicate that STM and CLV proteins act in an opposite manner to regulate meristem maintenance and cell proliferation (Clark et al., 1996). In maize (*Zea mays*), the interplay between *knotted1* and *thick tassel dwarf1*, the orthologs of *STM* and *CLV1*, respectively, appear to also act oppositely in regulating SAM homeostasis, but only during reproductive development (Lunde and Hake, 2009). Interestingly, the hypomorphic mutant called *gorgon* represents a novel *stm* allele that leads to an increase in the size of the SAM due to the expansion of the central region, which includes the *CLV3* and *WUS* expression domains (Takano et al., 2010). It was postulated that the increase in the size of the central region of the SAM is attributed to a reduction in the allocation of cells into lateral organs and axillary meristems. Taken together, STM acts to control multiple processes that maintain the integrity and function of the SAM.

Genetic and molecular studies indicate that KNOX proteins are regulated in part by the interaction with specific BELL1-like homeodomain (BLH) proteins (Hake et al., 2004). In Arabidopsis, BLH proteins play crucial roles in regulating meristem function and fate during plant development (Hamant and Pautot, 2010). Genetic studies show that the *BLH* gene called *PENNYWISE* (*PNY*; also known as *BELLRINGER*, *REPLUMLESS*, *VAAMANA*, and *BLH9*) is required for internode patterning as well as flower and fruit development (Byrne et al., 2003; Roeder et al., 2003; Smith and Hake, 2003; Bao et al., 2004; Bhatt et al., 2004). Mutations in a gene closely related to *PNY* called *POUND-FOOLISH* (*PNF*) have no visible phenotypes (Smith et al., 2004; Rutjens et al., 2009). However, the *pny pnf* plants initiate compact shoots that fail to form flowers even though a subset of inflorescence meristem identity genes are expressed in the SAM (Smith et al., 2004; Rutjens et al., 2009). In the SAM, PNY is mainly expressed in the PZ (Smith and Hake, 2003; Cole et al., 2006), while PNF transcripts localize to the CZ and PZ (Smith et al., 2004). Genetic analyses show that PNY and PNF also act to regulate meristem maintenance. For example, the SAM of *pny pnf* plants frequently terminates during early stages of vegetative growth, which corresponds to a time at which the meristem is small (Smith et al., 2004; Rutjens et al., 2009). After termination of the main shoot, the outgrowth of axillary shoots from the axils of the rosette leaves restores shoot development. Once the development of the axillary shoots is established, the frequency of SAM termination decreases. Histological analyses demonstrate that *pny pnf* SAMs are smaller and narrower than wild-type SAMs, indicating that the integrity of the CZ and/or PZ is altered. The fact that internode patterning is impaired in *pny* and *pny pnf* plants suggests that these homeodomain proteins also regulate RM integrity (Byrne et al., 2003; Roeder et al., 2003; Smith and Hake, 2003; Bao et al., 2004; Bhatt et al., 2004). Genetic evidence indicates that the association of PNY and PNF with STM regulates meristem maintenance during shoot growth (Byrne et al., 2003; Bhatt et al., 2004; Kanrar et al., 2006; Rutjens et al., 2009). Currently, the role of PNY and PNF in regulating meristem maintenance is not understood.

In this paper, the role of PNY and PNF in SAM integrity was investigated. Localization of a PZ-expressed gene indicates that the integrity of the meristem's central region is impaired in *pny pnf*. Consistent with this result, the localization pattern for genes expressed in the central region of the SAM, including *CLV3* and *WUS*, is altered in *pny pnf* SAMs. Interestingly, the expression patterns for WUS and CLV3 are reestablished in *pny pnf* plants lacking CLV function. The reproductive potential is also restored in *pny pnf* plants, with a reduction in CLV function or an increase in WUS function. We propose a model in which PNY and PNF regulate the integrity of the central region of the SAM by maintaining a boundary between the CZ and PZ, which restricts the allocation of cells into leaf primordial to the PZ.

RESULTS

A Role for PNY and PNF in Regulating the Integrity of the Central Region of the SAM

Histological studies indicate that the SAM of *pny pnf* plants is narrower than that of wild-type plants (Smith et al., 2004). To determine if PNY and PNF regulate the integrity of the SAM, the localization pattern of genes expressed in specific regions of the SAM was analyzed in 30-d-old wild-type and *pny pnf* SAMs. In wild-type inflorescence meristems, *STM* was expressed in the PZ and CZ (Fig. 1A; Long et al., 1996). On the flanks of wild-type shoot meristems, *STM* is down-regulated in a small group of cells that correspond to initial cells, which give rise to lateral organs (Fig. 1A; Long et al.,

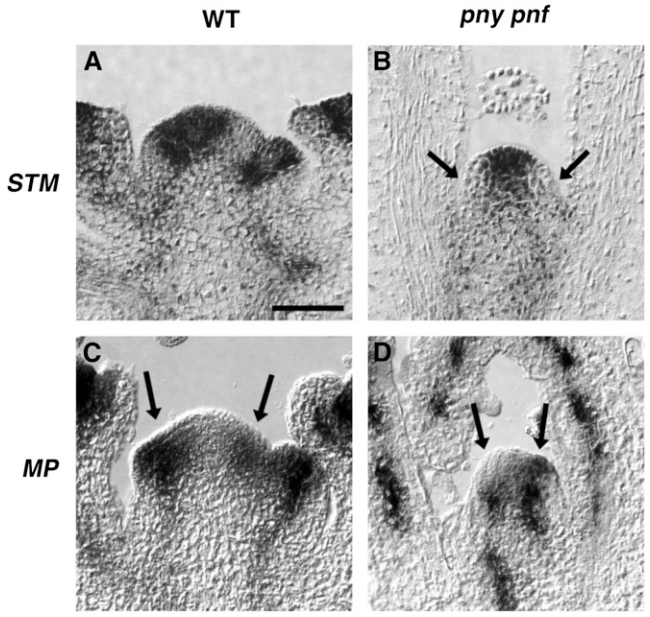

Figure 1. The integrity of the meristem's central region is impaired in *pny pnf*. Localization is shown for *STM* (A and B) and *MP* (C and D) transcripts in 30-d-old wild-type (WT) inflorescence (A and C) and *pny pnf* shoot (B and D) apices. In B, the arrows point to the leaf initial cells. In C and D, the arrows point to the *MP*-expressing cells. Bar = 50 μm.

1996). In *pny pnf* plants, *STM* was also expressed in the SAM (Fig. 1B; Smith et al., 2004). In contrast to the mRNA localization pattern in wild-type SAMs, the expression domain for *STM* was narrower in *pny pnf* (Fig. 1B; Smith et al., 2004). In addition, the cells in the periphery of *pny pnf* shoot meristems that fail to express *STM* appeared to impinge on the central region of the SAM, indicating that a higher proportion of meristem cells are allocated into leaf primordia (Fig. 1B, arrows). The fact that the size of the SAM and the expression domain for *STM* was narrower in *pny pnf* compared with the wild type indicates that the integrity of the central and/or peripheral regions of the SAM may be impaired. To address this, we first localized the transcripts for *MONOPTEROS* (*MP*), which was expressed in the PZ in wild-type inflorescence meristems (Fig. 1C; Hardtke and Berleth, 1998). *MP* encodes an auxin response factor, which regulates the positioning and organization of vascular cells required for patterning of the reproductive shoot (Przemeck et al., 1996). In *pny pnf* plants, cells expressing *MP* were detected on the flanks of the SAM (Fig. 1D). However, in contrast to the wild type, the central region separating the *MP* expression domains was reduced (Fig. 1D). These results indicate that the integrity of the central region of the SAM in *pny pnf* is impaired.

PNY and PNF Regulate Gene Expression Patterns in the Core of the SAM

To gain insight into the role of PNY and PNF in regulating the central region of the SAM, the expression pattern for three genes expressed in the core of the SAM was compared between wild-type and *pny pnf* shoot apices. In wild-type inflorescence meristems, *WUS* localizes to a small number of cells located in the core of the SAM, overlapping with the apical cells of the RM (Mayer et al., 1998; Fig. 2A). In contrast to the wild type, the *WUS* expression domain was diffuse and slightly expanded in *pny pnf* SAMs (Fig. 2B). Slight alterations in the *WUS* expression domain are observed during the floral transition (Geier et al., 2008). However, the perturbation of the *WUS* domain in *pny pnf* SAMs was not likely due to periodic fluctuations, since the diffuse pattern of expression for *WUS* was displayed in all the *pny pnf* shoot meristems analyzed (data not shown). To further characterize SAM integrity in *pny pnf*, two additional genes, *At3g59270* and *At1g26680*, expressed in the core of wild-type inflorescence meristems were examined (Yadav et al., 2009; Fig. 2, C and E). *At3g59270* and *At1g26680* encode a syntaxin-like protein and a B3 transcription factor, respectively. Unlike the diffuse localization pattern displayed by *WUS*, the *At3g59270* expression pattern was confined to fewer cells in the core of *pny pnf* SAMs

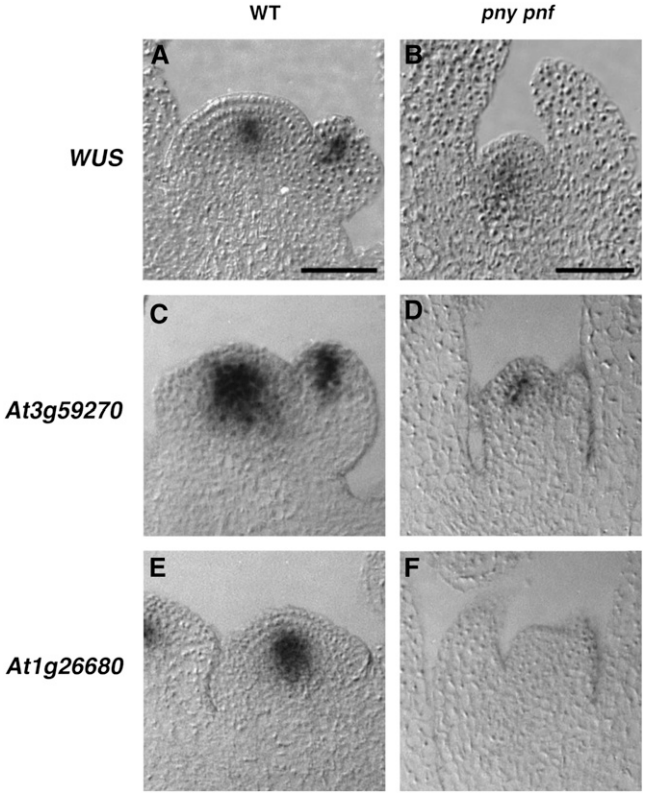

Figure 2. Alteration in the expression patterns for genes transcribed in the core *pny pnf* shoot meristems. In 30-d-old plants, in situ hybridization was utilized to determine the spatial expression patterns for *WUS* (A), *At3g59270* (C), and *At1g26680* (E) in wild-type (WT) inflorescence apices. The mRNA localization patterns for *WUS* (B), *At3g59270* (D), and *At1g26680* (F) were examined in 30-d-old *pny pnf* apices. Bars = 50 μm.

(Fig. 2D). Unlike *WUS* or *At3g59270*, transcripts for *At1g26680* were not detected in *pny pnf* SAMs (Fig. 2F) even after a long period of exposure (data not shown). Although the functions of *At3g59270* and *At1g26680* have not been determined, this study shows that genes expressed in the core of the SAM respond differentially to the loss of PNY and PNF.

PNY and PNF Regulate the Integrity of the CZ

To determine if the integrity of the CZ is altered in *pny pnf* plants, the localization pattern of *CLV3* was determined. In wild-type inflorescence SAMs, *CLV3* transcripts localized to the CZ (Fig. 3A; Fletcher et al., 1999). In contrast to the wild type, *CLV3* transcript accumulation was not detected by in situ hybridization in *pny pnf* SAMs (Fig. 3B). The inability to detect *CLV3* transcript in the apical cells of *pny pnf* SAMs was surprising, since the shoot meristems of *pny pnf* plants are smaller than those of wild-type plants (Smith et al., 2004; Rutjens et al., 2009). A recent study indicates that the stability of GUS activity allows for the spatial detection of low-abundance transcripts that cannot be visualized by mRNA in situ hybridization (Shuai et al., 2002). Therefore, to examine the *CLV3* expression pattern further, *pCLV3*:*GUS* was crossed into *pny pnf*. In wild-type SAMs, the pattern of *pCLV3*:*GUS* activity was detected in the CZ of the SAM (Brand et al., 2002; Fig. 3C). In contrast to the wild type, *pCLV3*:*GUS* activity was detected throughout the SAM in *pny pnf* plants (Fig. 3D). The broad expression pattern detected

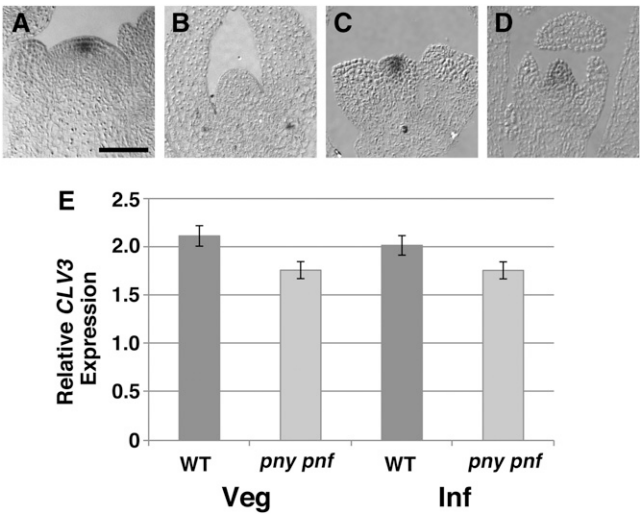

Figure 3. The *CLV3* expression domain is altered in *pny pnf* SAMs. A and B, *CLV3* transcripts were localized in 30-d-old wild-type inflorescence (A) and *pny pnf* shoot (B) apices. C and D, GUS activity was visualized in the apices of 30-d-old wild-type (C) and *pny pnf* (D) plants containing the *CLV3*:*GUS* transgene. Bar = *50* μm. E, The relative levels of *CLV3* transcripts were determine by q-PCR in the shoot apices of wild-type (WT) and *pny pnf* plants during vegetative (Veg) and inflorescence (Inf) development. In the q-PCR analysis, *UBIQUITIN10* (*AT4G05320*) was used as a reference.

for *pCLV3*:*GUS* in *pny pnf* plants was not due to a diffusion of the signal, as the conditions used for this experiment were identical to those used to detect the expression of organ boundary genes (Shuai et al., 2002). To determine if the relative levels of *CLV3* were similar in wild-type and *pny pnf* SAMs, quantitative (q)-PCR was performed. The results showed that transcript levels for *CLV3* were comparable between wild-type and *pny pnf* apices (Fig. 3E). Taken together, we propose that a decrease in the integrity of CZ in *pny pnf* SAMs alters the expression domain of *CLV3*. Furthermore, the impaired CZ may explain the aberrant expression patterns for genes expressed in the core of *pny pnf* SAMs, including *WUS*.

Restoration of Floral Specification in *pny pnf clv3* Plants

Mutations in *clv* genes result in a lateral expansion of the CZ and RM during shoot development (Brand et al., 2000; Schoof et al., 2000). Therefore, to determine if the alteration in the integrity of the CZ is attributed to the *pny pnf* phenotype, *clv3-2* was crossed to *pny pnf* and the *pny pnf clv3* plants were examined. In the wild type, *pny*, and *clv3*, the inflorescences initiated flowers (Fig. 4, A–C). The inflorescence phenotypes of *pny clv3* plants are additive (Byrne et al., 2003), and the loss of these genes had no effect on floral specification (Fig. 4D). In addition, the inflorescence shoots produced by *pnf clv3* were morphologically similar to *clv3* (data not shown). In *pny pnf* plants, the compact shoots initiated leaves and displayed a non-flower-producing phenotype when plants were grown in long or short days (Smith et al., 2004; Rutjens et al., 2009; Fig. 4E). Interestingly, under long-day photoinductive conditions, the SAMs of *pny pnf clv3* underwent the floral transition, producing compact inflorescence shoots (Fig. 4, F–I) that initiated flower-like structures composed mostly of unfused carpels and some sepals (Fig. 4, J–L). The inflorescence shoots of *pny pnf clv3* initiated under short-day growth conditions were similar in morphology to those in long-day conditions (data not shown). Floral specification also occurred in *pny pnf clv1*; however, the shoots were less compact and petals as well as stamen-like organs were initiated in addition to carpels and sepals (Supplemental Fig. S1; Supplemental Materials and Methods S1). These results demonstrate that loss of CLV function partially restored the floral specification potential of *pny pnf* shoots.

LEAFY Is Up-Regulated in the Reproductive Shoots of *pny pnf clv3*

In Arabidopsis, environmental and endogenous cues converge and activate the key flower meristem identity gene *LEAFY* (*LFY*; Blázquez and Weigel, 2000; Yamaguchi et al., 2009). LFY functions downstream of PNY and PNF, as flower specification is restored in *pny pnf* plants overexpressing *LFY* (Kanrar et al., 2008). Therefore, to determine if the floral specification phenotype of *pny pnf clv3* is attributed to the up-regulation

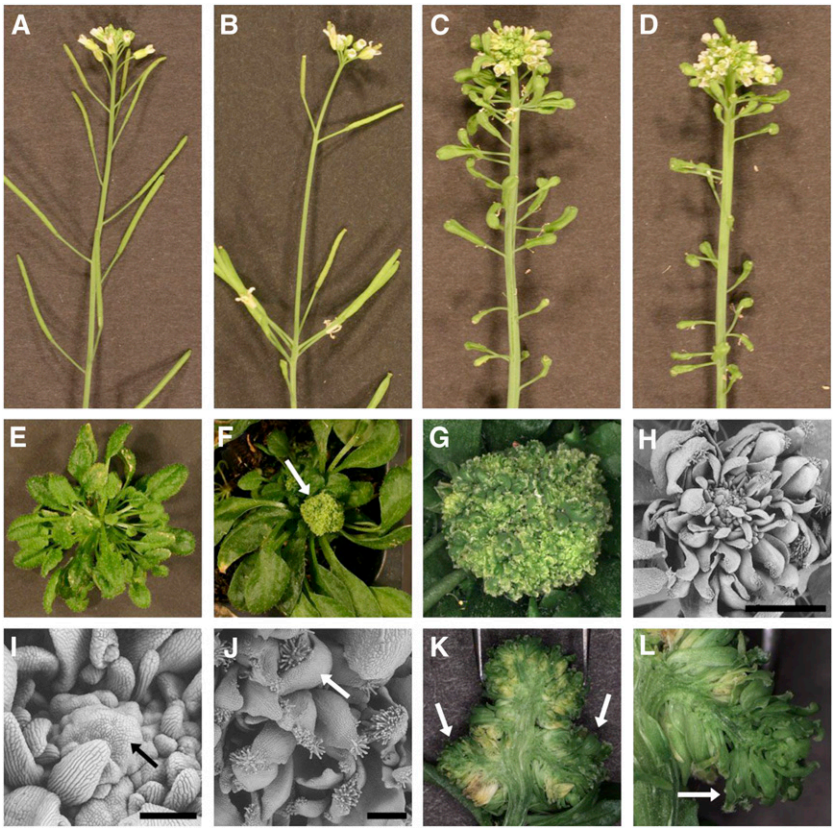

Figure 4. Restoration of floral specification during *pny pnf clv3* shoot development. A to D, Inflorescence shoots of wild-type (A), *pny* (B), *clv3* (C), and *pny clv3* (D) plants. E and F, Images of *pny pnf* (E) and *pny pnf clv3* (F) plants. In F, the arrow points to the compact inflorescence shoot produced in *pny pnf clv3* plants. G, Closeup of a *pny pnf clv3* inflorescence. H and I, Scanning electron microscopy images of a reproductive *pny pnf clv3* apex. In H, bar = 1 mm. In I, the arrow points to the shoot meristem. Bar = 100 μm. J, Scanning electron microscopy image of unfused carpels produced during later stages of reproductive development. The arrow points to one of the unfused carpels. Bar = 200 μm. K, A mature reproductive shoot of *pny pnf clv3* was dissected with a razor blade to display the growth patterns. The arrows point to the reproductive shoots that produce the carpel-like organs. L, Closeup view of a carpel-bearing shoot. The arrow points to a carpel-like organ.

of *LFY*, the expression pattern of this flower meristem identity gene was examined. In wild-type inflorescence apices, *LFY* was expressed in a small group of cells in the PZ of the SAM as well as in the floral meristem (Weigel et al., 1992; Fig. 5A). During *clv3* inflorescence development, *LFY* was expressed in the floral meristem and small groups of cells in the peripheral region near the base of the SAM (Fig. 5C). In addition, *LFY* expression was not detected in the central region of the *clv3* inflorescence meristem, which corresponds to the enlarged CZ (Fig. 5C). In contrast to the wild type and *clv3*, *LFY* transcripts failed to accumulate in *pny pnf* shoot meristems under conditions that promote reproductive development (Kanrar et al., 2008; Fig. 5B). In *pny pnf clv3*, *LFY* was expressed in the reproductive shoot meristems, demonstrating that the floral specification potential was restored in *pny pnf clv3* SAMs (Fig. 5D).

The Integrity of the *WUS* Expression Domain Is Restored in *pny pnf clv3* Plants

To determine if PNY and PNF control the expression patterns for *WUS*, *At3g59270*, and *At1g26680* by maintaining the integrity of the CZ, the mRNA localization patterns for these genes were examined in the *pny pnf clv3* triple mutant. Mutations in *clv3* led to the formation of enlarged shoot meristems in which the *WUS* domain expanded laterally in the SAM (Fig. 6A; Brand et al., 2000, 2002; Schoof et al., 2000). In *clv3* SAMs, the expression domains also expanded for *At3g59270* (Fig. 6C; Aggarwal et al., 2010) and *At1g26680* (Fig. 6E). Interestingly, in *pny pnf clv3*, the *WUS* expression domain appeared to be restored such that *WUS* transcripts were easily detected in a small group of cells in the core of the SAM (Fig. 6B). In addition, the mRNA localization pattern for *At3g59270* expanded in *pny pnf clv3* SAMs compared with the expression pattern observed in *pny pnf* (Fig. 6D). However, unlike *WUS* and *At3g59270*, transcripts for *At1g26680* could not be detected in *pny pnf clv3* SAMs (Fig. 6F) even after a long exposure period (data not shown). Taken together, these results indicate that functional integrity of the central region of the SAM is partially restored in *pny pnf clv3* plants. The fact that the *WUS* expression domain was reestablished in *pny pnf clv3* indicates that PNY and PNF regulate the *WUS* expression domain by maintaining the integrity of the CZ. That the expression pattern for *At1g26680* was not restored in *pny pnf clv3* SAMs suggests that PNY and PNF control a subset of "core expressed genes" independent of CZ regulation.

Restoration of the *CLV3* Expression Domain in *pny pnf clv1*

Although the expression domains do not completely coincide, experimental observations indicate that WUS

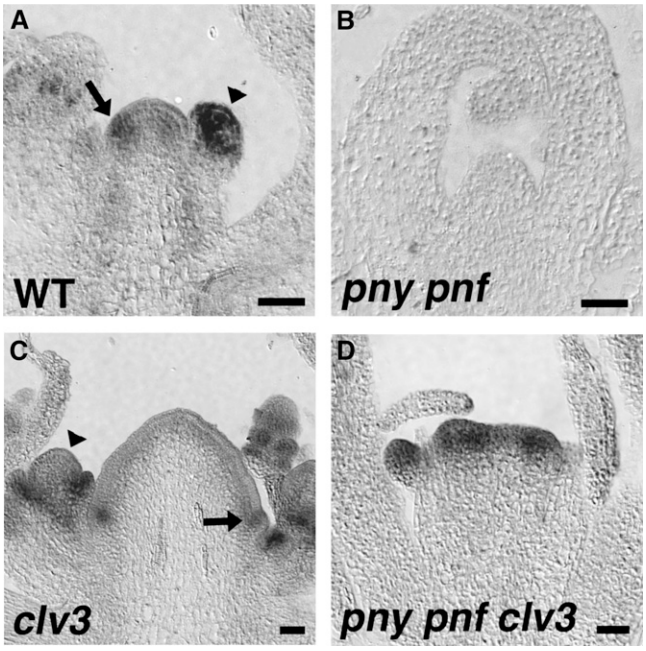

Figure 5. Expression of *LFY* in *pny pnf clv3* reproductive shoots. The spatial expression pattern for *LFY* was determined in wild-type (WT; A), *pny pnf* (B), *clv3* (C), and *pny pnf* (D) apices after 30 d of growth. The inflorescence meristem of *clv3* (C) is much larger than the wild-type meristems (A), due to the expanded CZ. In A and C, the arrows point to *LFY*-expressing cells in the PZ of the shoot meristem. The arrowheads point at the floral meristems. Bars = 50 μm.

positively regulates *CLV3* (Brand et al., 2000; Schoof et al., 2000; Yadav et al., 2010). Since *clv1* produces a similar phenotype as *clv3* (Clark et al., 1995), we asked if restoration of the *WUS* expression domain correlated with the accumulation of *CLV3* transcripts in the apical cells of *pny pnf clv1-8* shoot meristems. In the SAMs of *clv1*, both the *WUS* and *CLV3* expression domains were expanded compared with the wild type (Fig. 7, A and C). Similar to *pny pnf clv3*, transcripts for *WUS* were detected at relatively high levels in the cells of the RM in *pny pnf clv1-8* (Fig. 7B). Moreover, the *CLV3* expression domain was also reestablished in the apical cells of the SAM in *pny pnf clv1-8* (Fig. 7D). Therefore, results from this experiment show that the loss of CLV signaling in *pny pnf* restores the ability of WUS to positively activate *CLV3* in the apical cells of the SAM.

An Increase in WUS Function Promotes Reproductive Development in *pny pnf* Plants

The fact that loss of CLV signaling in *pny pnf* restored floral specification and the *WUS* expression domain led us to examine whether the levels of this homeobox gene could trigger reproductive development in *pny pnf* plants. Loss-of-function *wus* plants produced terminal shoots during vegetative and reproductive development due to the failure to maintain stem cells in the shoot and floral meristems (Fig. 8A; Laux et al., 1996; Mayer et al., 1998). Genetic studies showed that *clv3* and *clv1* phenotypes are dependent upon WUS function, since *wus clv3* and *wus clv1* mutants display a phenotype similar to *wus* single mutants during vegetative and reproductive development (Schoof et al., 2000). To determine if WUS function is required for reproductive development in *pny pnf clv3*, *pny pnf clv3 wus-1* plants were created and analyzed. During the vegetative mode of development, the plants of *pny pnf clv3 wus-1* produced terminal shoots that were morphologically similar to *wus-1* single mutants (data not shown). However, in contrast to *wus-1*, inflorescence shoots and flowers were never produced in *pny pnf clv3 wus-1* (Fig. 8B). Thus, these results showed that WUS function was required for reproductive development in *pny pnf clv3* plants.

The fusion of WUS to the glucocorticoid receptor-binding domain (GR) has been successfully used to induce WUS function in order to identify genes regulated by this homeodomain protein in a dexamethasone (DEX)-dependent manner (Brand et al., 2002;

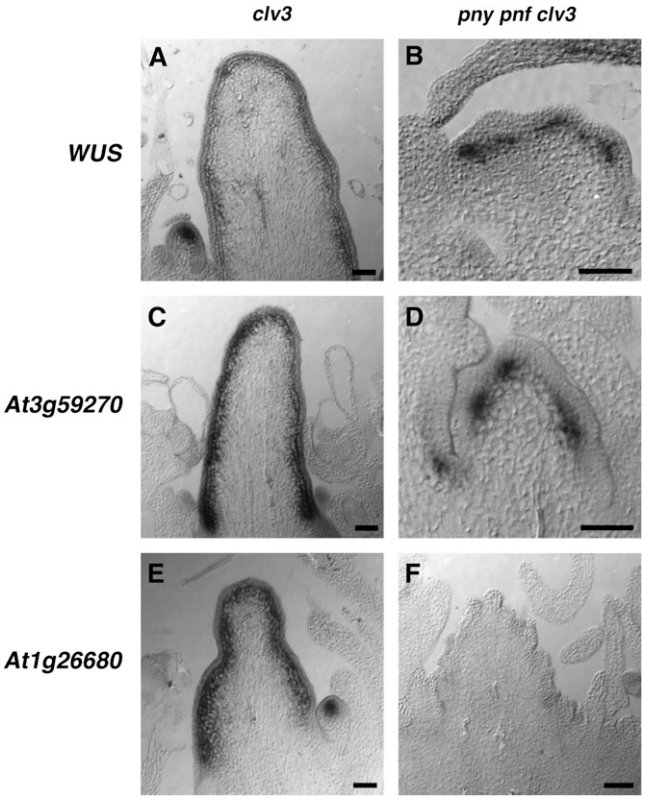

Figure 6. Reestablishment of the *WUS* and *At3g59270* expression domains in *pny pnf clv3* shoot meristems. In 30-d-old plants, in situ hybridization was utilized to determine the spatial expression patterns for *WUS* (A), *At3g59270* (C), and *At1g26680* (E) in *clv3* inflorescence apices. The mRNA localization patterns for *WUS* (B), *At3g59270* (D), and *At1g26680* (F) were examined in 30-d-old *pny pnf clv3* apices. Bars = 50 μm.

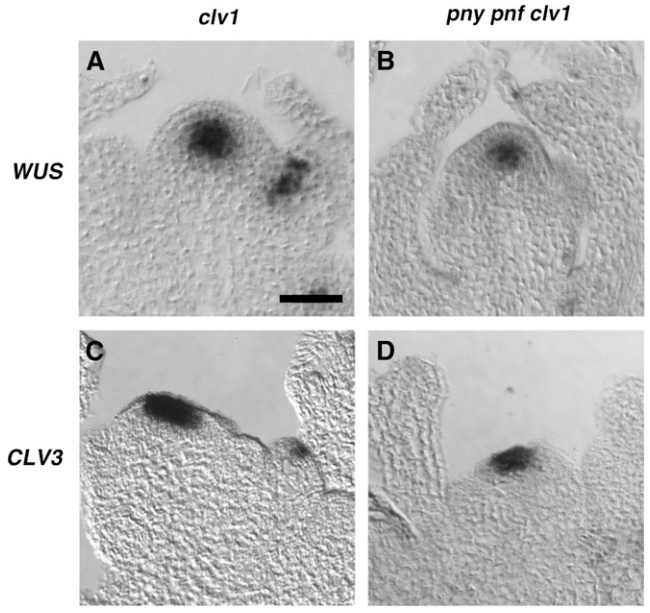

Figure 7. Restoration of the *CLV3* expression domain in *pny pnf clv1* plants. The spatial expression pattern for *WUS* was determined in 30-d-old *clv1* (A) and *pny pnf clv1* (C) inflorescence shoot apices. An expanded *CLV3* expression domain is detected in *clv1* (B) and *pny pnf clv1* (D) inflorescence apices. Bar = 50 μm.

Lenhard et al., 2002; Leibfried et al., 2005; Busch et al., 2010). Applications of 1 μM DEX to wild-type inflorescences have mild effects on flower development (Kieffer et al., 2006; data not shown). To determine if an increase in WUS function can promote the floral specification in *pny pnf* plants, *35S:WUS-GR* was crossed to *pny pnf* to create *35S:WUS-GR pny pnf* plants. In the absence of DEX, the shoots of *35S:WUS-GR pny pnf* plants treated with a control solution continued to produce leaves without any visible signs of flower formation (Fig. 8C). However, repeated applications of 1 μM DEX to *35S:WUS-GR pny pnf* produced inflorescences that terminated with sepal- and carpel-like organs approximately 43% of the time (Fig. 8, D and E). In addition, a terminal flower often formed after *35S:WUS-GR pny pnf* plants were treated with a single application of 1 μM DEX (Fig. 8F). These results showed that an increase in WUS function promotes the floral specification in *pny pnf* plants.

DISCUSSION

The function of the SAM is dependent upon the balance between the self-renewing activities of the stem cells and the allocation of PZ cells into lateral organs and axillary meristems (Vollbrecht et al., 2000). An alteration in the balance between stem-cell perpetuation and organogenesis is predicted to disrupt meristem function. In this paper, we provide evidence that PNY and PNF act to control meristem maintenance by regulating the integrity of the central region of the SAM. First, the expression pattern for *MP* indicates that the central region of the SAM is reduced in *pny pnf* plants. Second, the expression patterns for *WUS*, *At3g59270*, *At1g26680*, and *CLV3* are altered. Third, the loss of CLV signaling, which increases the size of the central region of the SAM, restores the expression patterns for *WUS*, *At3g59270*, and *CLV3* in *pny pnf* plants. Experimental evidence indicates that PNY/PNF-STM complexes act to regulate meristem maintenance (Byrne et al., 2003; Bhatt et al., 2004; Kanrar et al., 2006; Rutjens et al., 2009). Furthermore, genetic analyses indicate that STM also regulates the central region of the SAM (Clark et al., 1996; Endrizzi et al., 1996; Long et al., 1996). Therefore, the association of PNY and PNF with STM may act to regulate the integrity of the CZ during shoot development.

During early stages of vegetative growth, the SAM of *pny pnf* plants frequently terminates after several

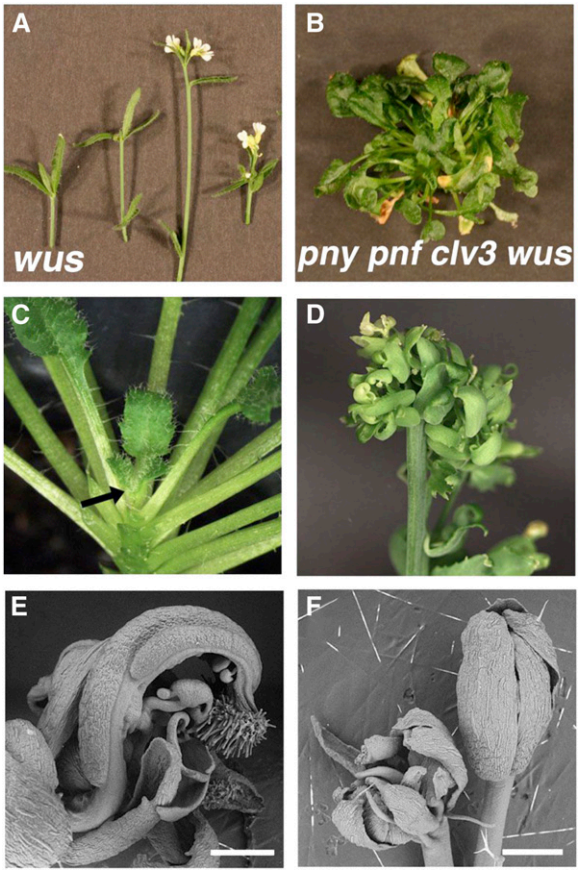

Figure 8. The levels of WUS control floral specification in plants with reduced PNY and PNF function. A, Image of *wus* inflorescence shoots. B, The shoots of *pny pnf clv3 wus* fail to initiate flowers. C, The shoot apex of a *35S:WUS-GR pny pnf* plant treated with a mock solution without DEX. The arrow points to the leaf-bearing shoot apex. D, Repeated applications of DEX to *35S:WUS-GR pny pnf* plants promotes inflorescence development. E, Closeup of a carpel-like floral organ in a *35S:WUS-GR pny pnf* inflorescence. F, A single application of DEX to *WUS-GR pny pnf* induced the formation of terminal flowers. Bars = 0.5 mm.

leaves are initiated (Smith et al., 2004; Rutjens et al., 2009). Shoot development is reestablished by the outgrowth of axillary shoots, which results in a bushy plant phenotype. The frequency of meristem termination decreases as the shoots grow and mature, presumably due to an increase in meristem size, which may render the SAM to be less susceptible to termination. This hypothesis is supported by studies showing that a null allele of the *STM* ortholog in maize called *knotted1* (*kn1*) displays a terminal shoot phenotype in inbred lines that typically produce smaller embryonic meristems (Vollbrecht et al., 2000). However, in inbred lines that initiate larger meristems, the SAM is readily maintained in *kn1* mutants (Kerstetter et al., 1997; Vollbrecht et al., 2000). How might PNY and PNF regulate the integrity of the central region of the SAM? mRNA in situ hybridization experiments indicate that PNY and PNF expression patterns overlap in the PZ (Smith and Hake, 2003; Smith et al., 2004; Cole et al., 2006). Therefore, these homeodomain proteins may act in the PZ to mediate the allocation of cells into initiating leaf primordia. Based on the expression patterns of *STM*, leaf initial cells appear to impinge on the central region of *pny pnf* shoot meristems. Thus, in the absence of PNY and PNF, the balance of organogenesis and stem cell renewal is altered, leading to the allocation of CZ cells into developing leaf primordia. Based on genetic analyses between *clv* and *stm* mutants, it was hypothesized that STM functions to maintain a boundary between the CZ and PZ in the SAM (Clark et al., 1996). In support of this hypothesis, we propose that PNY/PNF-STM complexes act to maintain a boundary between the CZ and PZ, which restricts organogenesis to the peripheral region of the SAM. During early stages of vegetative development, the small size of the *pny pnf* vegetative SAM combined with the failure to maintain the CZ/PZ boundary may result in the allocation of all of the CZ or stem cells into leaf primordia, resulting in the termination of the SAM. However, once shoot growth is established, the SAM is able to maintain itself, because the size of the meristem increases to a point at which a small pool of CZ or stem cells can be readily maintained. In *pny pnf clv* mutants, the central region of the meristem expands, leading to an increase in the size of the SAM. Expression analyses of *WUS*, *At3g59270*, and *CLV3* in *pny pnf clv3* suggest that the integrity of the SAM's central region is partially restored, indicating that the CZ/PZ boundary is reestablished.

The CLV3-WUS negative feedback loop plays a pivotal role in maintaining a stable population of stem cells in the CZ (Sablowski, 2007; Tucker and Laux, 2007; Bleckmann and Simon, 2009). To date, the mechanism by which WUS controls the spatial expression pattern of *CLV3* is poorly understood. However, the CZ/PZ boundary may provide positional cues that direct the transcription of *CLV3* to the CZ. In this study, experimental evidence suggests that the transcript levels for *CLV3* are similar between wild-type and *pny pnf* apices. However, we were unable to detect the *CLV3*-expressing cells by in situ hybridization in *pny pnf* SAMs. Furthermore, analysis of the *CLV3*:*GUS* expression pattern in *pny pnf* shoot meristems suggests that the *CLV3* expression domain is expanded throughout the SAM. Based on our results, we propose that in the absence of PNY and PNF, the CZ/PZ boundary is not properly maintained. Therefore, the positional cues required for confining the spatial expression pattern of *CLV3* to the CZ are absent. As a result, WUS activates *CLV3* in all the cells of the meristem. However, since the CLV pathway acts to negatively regulate *WUS*, transcript levels for *CLV3* are expected to be significantly lower in the meristem cells of *pny pnf* than in the CZ cells of the wild type. Therefore, the cells of *pny pnf* shoot meristems express *CLV3* at a level that cannot be detected by in situ hybridization.

Recent studies show that a mutation in the *BLH* gene *ARABIDOPSIS THALIANA HOMEOBOX1* (*ATH1*) enhances the terminal shoot phenotype of *pny pnf* that occurs shortly after germination (Rutjens et al., 2009). *ATH1* appears to be structurally and phylogenetically distinct from PNY and PNF (Quaedvlieg et al., 1995; Mukherjee et al., 2009). Genetic studies indicate that ATH1 acts to maintain the basal boundary between the stem and lateral organ (Gómez-Mena and Sablowski, 2008; Rutjens et al., 2009). Similar to PNY and PNF, ATH1 interacts with STM to regulate meristem maintenance events (Hackbusch et al., 2005; Rutjens et al., 2009). However, it is unclear if ATH1 acts redundantly with PNY and PNF. As postulated by Cole et al. (2006), distinct KNOX-BLH complexes may regulate specific meristem maintenance pathways in discrete regions of the SAM. Therefore, the function of STM may be modulated by interaction with specific BLH proteins in the SAM. Consequently, ATH1-STM complexes may control the basal organ boundaries, while PNY/PNF-STM complexes act to restrict organogenesis to the PZ. Thus, the terminal SAM phenotype of *pny pnf ath1* plants may be due to combinatorial loss of multiple meristem maintenance pathways controlled by STM.

The fact that *pny pnf clv* mutants specify floral cell fate is quite intriguing. In *pny pnf* plants, leaf organogenesis is not perturbed, while the formation of flowers and the development of internodes are severely impaired. Clonal analysis studies indicate that a small number of meristem initial cells give rise to lateral organs (Stewart and Dermen, 1975; Poethig, 1989). During the allocation processes, PNY and PNF may function in the PZ to establish positional cues in the initial cells that give rise to floral meristems and internodes. In the absence of PNY and PNF, the initial cells may not be competent for floral specification and internode patterning cues. In addition, fewer initial cells may be produced due to the smaller size of the *pny pnf* SAM. As a result, a reduction in the number of initial cells combined with a loss of positional information may have a dramatic effect on floral specification and internode patterning. However, in *pny pnf clv* shoots, the increase in the size of the SAM may

augment the number of cells that are responsive to or competent for the cues that promote floral specification. Future studies are aimed at understanding how PNY and PNF act to establish positional cues in the PZ of the SAM.

MATERIALS AND METHODS

Expression Analyses

Wild-type inflorescence and *pny pnf* apices from Arabidopsis (*Arabidopsis thaliana*) were harvested and fixed 30 d after germination under long-day growth conditions. Tissue fixation, processing, and in situ hybridization were performed as described previously (Jackson, 1991). Preparation of *WUS*, *CLV3*, *At3g59270*, and *At1g26680* in situ probes was described previously (Yadav et al., 2009). Preparation of *STM* and *MP* in situ probe was published previously (Long et al., 1996; Zhao et al., 2010). For each genotype examined by in situ hybridization, at least three biological replicas were fixed and sectioned. Two to three sectioned apices from each genotype were adhered to a single microscope slide. In this analysis, we performed at least one hybridization experiment for each replica. Therefore, each genotype was hybridized with a specific in situ probe six or more times.

For q-PCR, apices from wild-type and *pny pnf* plants were dissected before and after floral induction. Note that transcripts for the flower meristem identity gene, *APETALA1*, were only detected in the inflorescence shoot apices of the wild type (data not shown). RNA was isolated using the RNeasy kit from Qiagen. cDNA synthesis and q-PCR procedures were described by Kanrar et al. (2008). The *CLV3-F* (5′-ATGGATTCGAAGAGTTTTCTG-3′) and *CLV3-R* (5′-CAAGGGAGCTGAAAGTTGTTTC-3′) primers were used to amplify *CLV3*. *UBIQUITIN10* (*AT4G05320*) was used as a reference gene (Kanrar et al., 2008). Genotype determination for *pny-40126* and *pnf-33879* was described previously (Smith and Hake, 2003; Smith et al., 2004). The procedure used to visualize GUS activity in plant tissues was described previously (Sundaresan et al., 1995; Springer, 2000).

Genetic Analyses

The *pny-40126* and *pnf-33879* alleles used in this study were identified previously in the Columbia ecotype (Smith and Hake, 2003; Smith et al., 2004). *clv1-8*, which is in the Columbia background, was crossed to *pny PNF/pnf* in order to characterize the *pny pnf clv1-8* phenotype. The *clv3-2* and *wus-1* alleles, both in the Landsberg ecotype, were backcrossed to wild-type Columbia plants two times before crossing to *pnf*, *pny*, *pny PNF/pnf*, and *pnf clv PNY/pnf* plants. *PNF* and *CLV3* are located on chromosome 2 approximately 255 kb apart. To generate *pny pnf clv3* plants, we first crossed *pnf* with *clv3* and screened F3 for *clv3* plants that were heterozygous for *pnf*. After allowing these plants to self-pollinate, *pnf clv3* plants were crossed to *pny*. The phenotypes for *pny pnf clv3*, *pny pnf clv1*, and *pny pnf clv3 wus* plants were characterized by maintaining *pnf clv3 PNY/pnf*, *pny clv1 PNF/pnf*, and *pnf clv3 PNY/pny WUS/wus* plants. F3 *pny clv3 WUS/wus* and *pny WUS/wus* plants were used to characterize *pny clv3 wus* and *pny wus* plants. The *35S:WUS-GR pny pnf* plants were characterized by maintaining *35S:WUS-GR pny PNF/pnf* plants. To examine the spatial patterns of *CLV3*, wild-type plants containing *pCLV3:GUS* were crossed to *pny PNF/pnf* plants. Seeds derived from *pny PNF/pnf* plants containing *pCLV3:GUS* were used to analyze the expression pattern for *CLV3* in *pCLV3:GUS pny pnf* plants. *CLV3:GUS* was created by fusing the *CLV3* promoter and 3′ enhancer region to *GUS* (Brand et al., 2002).

All DEX induction experiments were performed 4 weeks after germination, to allow sufficient time for shoot maturation. In these experiments, 1 μM DEX was applied once or every other day for a 10-d period. The TM-1000 Tabletop Microscope from Hitachi (www.hitachi.com) was used for scanning electron microscopy (Kanrar et al., 2008).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Flower specification occurs in *pny pnf clv1* plants.

Supplemental Materials and Methods S1. Formation of inflorescence-like structures in *pny pnf clv1*.

ACKNOWLEDGMENTS

We are grateful to Drs. Patricia Springer, Sarah Hake, Venugopala Reddy, and Alon Samach for critical reading of the manuscript. We thank Jennifer Fletcher for the *clv1-8* allele, Rudiger Simon for *CLV3:GUS*, and Detlef Weigel for the *pDW124* plasmid used to synthesize the *LFY* in situ probe. We also thank Venugopala Reddy for providing us with the *wus-1* allele, *35S:WUS-GR* seed, and plasmids containing *CLV3*, *WUS*, *At3g59270*, and *At1g26680*, which were used to synthesize the antisense UTP-digoxigenin-labeled probes. We thank Dr. David Carter for help with scanning electron microscopy.

Received December 20, 2010; accepted March 12, 2011; published April 19, 2011.

LITERATURE CITED

Aggarwal P, Yadav RK, Reddy GV (2010) Identification of novel markers for stem-cell niche of *Arabidopsis* shoot apex. Gene Expr Patterns **10:** 259–264

Bao X, Franks RG, Levin JZ, Liu Z (2004) Repression of *AGAMOUS* by BELLRINGER in floral and inflorescence meristems. Plant Cell **16:** 1478–1489

Barton MK, Poethig RS (1993) Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the *shoot meristemless* mutant. Development **119:** 823–831

Bernier G, Kinet JM, Sachs RM (1981) The Physiology of Flowering, Vol 2. CRC Press, Boca Raton, FL

Bhatt AM, Etchells JP, Canales C, Lagodienko A, Dickinson H (2004) VAAMANA, a BEL1-like homeodomain protein, interacts with KNOX proteins BP and STM and regulates inflorescence stem growth in *Arabidopsis*. Gene **328:** 103–111

Blázquez MA, Weigel D (2000) Integration of floral inductive signals in Arabidopsis. Nature **404:** 889–892

Bleckmann A, Simon R (2009) Interdomain signaling in stem cell maintenance of plant shoot meristems. Mol Cells **27:** 615–620

Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R (2000) Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by *CLV3* activity. Science **289:** 617–619

Brand U, Grünewald M, Hobe M, Simon R (2002) Regulation of *CLV3* expression by two homeobox genes in Arabidopsis. Plant Physiol **129:** 565–575

Busch W, Miotk A, Ariel FD, Zhao Z, Forner J, Daum G, Suzaki T, Schuster C, Schultheiss SJ, Leibfried A, et al (2010) Transcriptional control of a plant stem cell niche. Dev Cell **18:** 849–861

Byrne ME, Groover AT, Fontana JR, Martienssen RA (2003) Phyllotactic pattern and stem cell fate are determined by the *Arabidopsis* homeobox gene *BELLRINGER*. Development **130:** 3941–3950

Clark SE (2001) Meristems: start your signaling. Curr Opin Plant Biol **4:** 28–32

Clark SE, Jacobsen SE, Levin JZ, Meyerowitz EM (1996) The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in *Arabidopsis*. Development **122:** 1567–1575

Clark SE, Running MP, Meyerowitz EM (1995) *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. Development **121:** 2057–2067

Clark SE, Williams RW, Meyerowitz EM (1997) The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. Cell **89:** 575–585

Cole M, Nolte C, Werr W (2006) Nuclear import of the transcription factor SHOOT MERISTEMLESS depends on heterodimerization with BLH proteins expressed in discrete sub-domains of the shoot apical meristem of *Arabidopsis thaliana*. Nucleic Acids Res **34:** 1281–1292

Endrizzi K, Moussian B, Haecker A, Levin JZ, Laux T (1996) The *SHOOT MERISTEMLESS* gene is required for maintenance of undifferentiated cells in *Arabidopsis* shoot and floral meristems and acts at a different regulatory level than the meristem genes *WUSCHEL* and *ZWILLE*. Plant J **10:** 967–979

Fletcher JC (2002) Shoot and floral meristem maintenance in *Arabidopsis*. Annu Rev Plant Biol **53:** 45–66

Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM (1999) Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems. Science **283:** 1911–1914

Geier F, Lohmann JU, Gerstung M, Maier AT, Timmer J, Fleck C (2008) A quantitative and dynamic model for plant stem cell regulation. PLoS ONE **3:** e3553

Gómez-Mena C, Sablowski R (2008) *ARABIDOPSIS THALIANA HOMEOBOX GENE1* establishes the basal boundaries of shoot organs and controls stem growth. Plant Cell **20:** 2059–2072

Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM (2009) Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. Proc Natl Acad Sci USA **106:** 16529–16534

Hackbusch J, Richter K, Müller J, Salamini F, Uhrig JF (2005) A central role of *Arabidopsis thaliana* ovate family proteins in networking and subcellular localization of 3-aa loop extension homeodomain proteins. Proc Natl Acad Sci USA **102:** 4908–4912

Hake S, Smith HM, Holtan H, Magnani E, Mele G, Ramirez J (2004) The role of knox genes in plant development. Annu Rev Cell Dev Biol **20:** 125–151

Hamant O, Pautot V (2010) Plant development: a TALE story. C R Biol **333:** 371–381

Hardtke CS, Berleth T (1998) The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J **17:** 1405–1411

Jackson D, editor (1991) *In Situ* Hybridisation in Plants. Oxford University Press, Oxford

Jasinski S, Piazza P, Craft J, Hay A, Woolley L, Rieu I, Phillips A, Hedden P, Tsiantis M (2005) KNOX action in *Arabidopsis* is mediated by coordinate regulation of cytokinin and gibberellin activities. Curr Biol **15:** 1560–1565

Jönsson H, Heisler M, Reddy GV, Agrawal V, Gor V, Shapiro BE, Mjolsness E, Meyerowitz EM (2005) Modeling the organization of the WUSCHEL expression domain in the shoot apical meristem. Bioinformatics (Suppl 1) **21:** i232–i240

Kanrar S, Bhattacharya M, Arthur B, Courtier J, Smith HM (2008) Regulatory networks that function to specify flower meristems require the function of homeobox genes *PENNYWISE* and *POUND-FOOLISH* in *Arabidopsis*. Plant J **54:** 924–937

Kanrar S, Onguka O, Smith HMS (2006) *Arabidopsis* inflorescence architecture requires the activities of KNOX-BELL homeodomain heterodimers. Planta **224:** 1163–1173

Kerstetter RA, Laudencia-Chingcuanco D, Smith LG, Hake S (1997) Loss-of-function mutations in the maize homeobox gene, knotted1, are defective in shoot meristem maintenance. Development **124:** 3045–3054

Kieffer M, Stern Y, Cook H, Clerici E, Maulbetsch C, Laux T, Davies B (2006) Analysis of the transcription factor WUSCHEL and its functional homologue in *Antirrhinum* reveals a potential mechanism for their roles in meristem maintenance. Plant Cell **18:** 560–573

Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, Nagato Y, Sakakibara H, Kyozuka J (2007) Direct control of shoot meristem activity by a cytokinin-activating enzyme. Nature **445:** 652–655

Laux T, Mayer KF, Berger J, Jürgens G (1996) The WUSCHEL gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development **122:** 87–96

Leibfried A, To JP, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU (2005) WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature **438:** 1172–1175

Lenhard M, Jürgens G, Laux T (2002) The WUSCHEL and SHOOTMERISTEMLESS genes fulfil complementary roles in *Arabidopsis* shoot meristem regulation. Development **129:** 3195–3206

Long JA, Moan EI, Medford JI, Barton MK (1996) A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. Nature **379:** 66–69

Lunde C, Hake S (2009) The interaction of knotted1 and thick tassel dwarf1 in vegetative and reproductive meristems of maize. Genetics **181:** 1693–1697

Lyndon RF (1998) The Shoot Apical Meristem: Its Growth and Development. Cambridge University Press, Cambridge, UK

Mayer KF, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T (1998) Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell **95:** 805–815

Mukherjee K, Brocchieri L, Bürglin TR (2009) A comprehensive classification and evolutionary analysis of plant homeobox genes. Mol Biol Evol **26:** 2775–2794

Poethig S (1989) Genetic mosaics and cell lineage analysis in plants. Trends Genet **5:** 273–277

Przemeck GK, Mattsson J, Hardtke CS, Sung ZR, Berleth T (1996) Studies on the role of the Arabidopsis gene MONOPTEROS in vascular development and plant cell axialization. Planta **200:** 229–237

Quaedvlieg N, Dockx J, Rook F, Weisbeek P, Smeekens S (1995) The homeobox gene *ATH1* of *Arabidopsis* is derepressed in the photomorphogenic mutants *cop1* and *det1*. Plant Cell **7:** 117–129

Roeder AH, Ferrándiz C, Yanofsky MF (2003) The role of the REPLUMLESS homeodomain protein in patterning the *Arabidopsis* fruit. Curr Biol **13:** 1630–1635

Rutjens B, Bao D, van Eck-Stouten E, Brand M, Smeekens S, Proveniers M (2009) Shoot apical meristem function in Arabidopsis requires the combined activities of three BEL1-like homeodomain proteins. Plant J **58:** 641–654

Sablowski R (2007) The dynamic plant stem cell niches. Curr Opin Plant Biol **10:** 639–644

Schoof H, Lenhard M, Haecker A, Mayer KFX, Jürgens G, Laux T (2000) The stem cell population of Arabidopsis shoot meristems in maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell **100:** 635–644

Shuai B, Reynaga-Peña CG, Springer PS (2002) The *lateral organ boundaries* gene defines a novel, plant-specific gene family. Plant Physiol **129:** 747–761

Smith HM, Campbell BC, Hake S (2004) Competence to respond to floral inductive signals requires the homeobox genes *PENNYWISE* and *POUND-FOOLISH*. Curr Biol **14:** 812–817

Smith HM, Hake S (2003) The interaction of two homeobox genes, *BREVIPEDICELLUS* and *PENNYWISE*, regulates internode patterning in the *Arabidopsis* inflorescence. Plant Cell **15:** 1717–1727

Springer PS (2000) Gene traps: tools for plant development and genomics. Plant Cell **12:** 1007–1020

Steeves TA, Sussex IM (1989) Patterns in Plant Development, Ed 2. Cambridge University Press, Cambridge, UK

Stewart RN, Dermen H (1975) Flexibility in ontogeny as shown by the contribution of the shoot apical layers to leaves of periclinal chimeras. Am J Bot **62:** 935–947

Sundaresan V, Springer P, Volpe T, Haward S, Jones JDG, Dean C, Ma H, Martienssen R (1995) Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. Genes Dev **9:** 1797–1810

Takano S, Niihama M, Smith HM, Tasaka M, Aida M (2010) gorgon, a novel missense mutation in the *SHOOT MERISTEMLESS* gene, impairs shoot meristem homeostasis in Arabidopsis. Plant Cell Physiol **51:** 621–634

Tucker MR, Laux T (2007) Connecting the paths in plant stem cell regulation. Trends Cell Biol **17:** 403–410

Vollbrecht E, Reiser L, Hake S (2000) Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, *knotted1*. Development **127:** 3161–3172

Weigel D, Alvarez J, Smyth DR, Yanofsky MF, Meyerowitz EM (1992) *LEAFY* controls floral meristem identity in *Arabidopsis*. Cell **69:** 843–859

Yadav RK, Girke T, Pasala S, Xie M, Reddy GV (2009) Gene expression map of the *Arabidopsis* shoot apical meristem stem cell niche. Proc Natl Acad Sci USA **106:** 4941–4946

Yadav RK, Tavakkoli M, Reddy GV (2010) WUSCHEL mediates stem cell homeostasis by regulating stem cell number and patterns of cell division and differentiation of stem cell progenitors. Development **137:** 3581–3589

Yamaguchi A, Wu MF, Yang L, Wu G, Poethig RS, Wagner D (2009) The microRNA-regulated SBP-box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. Dev Cell **17:** 268–278

Yanai O, Shani E, Dolezal K, Tarkowski P, Sablowski R, Sandberg G, Samach A, Ori N (2005) Arabidopsis KNOXI proteins activate cytokinin biosynthesis. Curr Biol **15:** 1566–1571

Zhao Z, Andersen SU, Ljung K, Dolezal K, Miotk A, Schultheiss SJ, Lohmann JU (2010) Hormonal control of the shoot stem-cell niche. Nature **465:** 1089–1092