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Regulation of shoot meristem integrity during Arabidopsis vegetative development

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Shoot growth and development is mediated by the activity of the shoot meristem, which initiates leaves and axillary meristems. Meristem maintenance is achieved by a poorly understood process that functions to sustain the balance of stem cell perpetuation in the central zone (CZ) and organogenesis in the peripheral zone (PZ). A recent study showed that two related homeodomain transcription factors, PENNYWISE (PNY) and POUND-FOOLISH (PNF), regulate meristem maintenance by controlling the integrity of the CZ. The non-flower producing phenotype displayed by *pny pnf* plants can be rescued by genetically increasing the size of the shoot meristem. In this addendum, we show that augmenting the size of the central region of *pny pnf* shoot meristems partially rescues the meristem termination phenotype that occurs during early stages of vegetative development. Thus, regulation of CZ integrity by PNY and PNF is crucial for vegetative and reproductive development.

Homeodomain Proteins Function to Regulate the Maintenance of the Shoot Meristem

Small self-organizing structures located at the tips of shoots, called shoot meristems, mediate the growth and development of the aerial parts of the plant.[1] Shoot meristems are subdivided into discrete functioning zones.[2] The apical stem cells are located and maintained in the central zone (CZ). Stem cells supply the morphogeneic regions of the shoot meristem, the peripheral (PZ) and rib meristem (RM), with cells that are eventually incorporated into axillary meristems, leaves and stems. The CLV-WUS negative feedback loop functions in part to maintain a stable population of stem cells in the CZ.[3] The enlarged shoot meristems displayed in *clv1* and *clv3* plants are due to a lateral expansion of CZ/stem cells.[4,5] In contrast, the failure to maintain shoot growth in *wus* is due to the inability of the shoot meristem to sustain the stem cell population.[6] In addition, the interplay between WUS and cytokinin (CK) signaling is thought to maintain region of high CK response in the core of the shoot meristem.[7-9]

Genetic studies indicate that meristem maintenance involves the balance of stem cell perpetuation in the central apical cells in the CZ and organogenesis in the PZ.[10] Although the mechanism(s) of meristem maintenance is poorly understood, studies in maize and Arabidopsis indicate that two orthologous homeodomain transcription factors KNOTTED1 (KN1) and SHOOT MERISTEMLESS (STM), respectively, regulate this process.[11,12] The expression patterns for STM and KN1, suggest these homeodomain proteins function in CZ, PZ and RZ. Moreover, in the appropriate genetic background, the shoot meristem terminally differentiates in plants harboring null alleles of *kn1* and *stm*. Interestingly, shoot growth can be partially rescued in *stm* plants by increasing the size of the CZ/stem-cell population, indicating that STM may regulate the integrity of the CZ.[13]

STM and KN1 interact with members of the BELL1-like Homeodomain

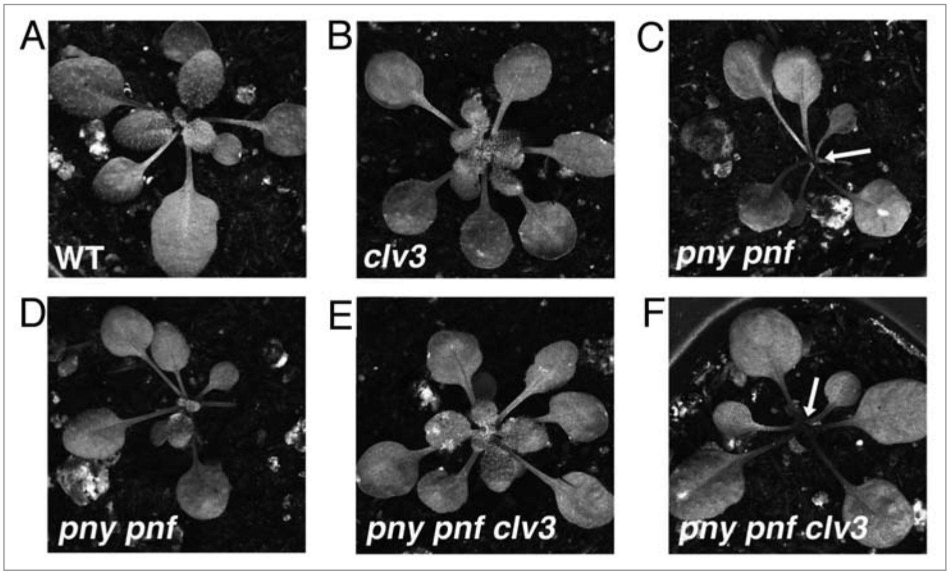

Figure 1. Analysis of vegetative shoot growth under long-day growth conditions. (A) Wild-type and (B) *clv3* plants maintained shoot growth during the vegetative phase of growth. However, (C) 78% of *pny pnf* shoots terminated after 3–5 leaves were produced (arrow points at terminated shoot in which leaf formation ceased), while (D) 22% of *pny pnf* shoots maintained shoot growth from the main axis. (E) 58% of *pny pnf clv3* maintained vegetative shoot growth, while (F) the remaining 42% of the shoots terminated growth after 3–5 leaves were produced (arrow points at the terminated shoot).

(BLH) family of transcription factors.[11,14] In Arabidopsis, the expression patterns described for some BLH genes in the shoot meristem suggest that STM regulates distinct meristem maintenance pathways in association with specific BLH proteins.[15] *PENNYWISE* (*PNY:* also known as *BELLRINGER*, *REPLUMLESS*, *VAAMANA* and *BLH9*) and *POUND-FOOLISH* (*PNF*) encode two related *BLH* genes that associate with STM.[14] During early stages of vegetative development, the shoot apical meristem in *pny pnf* plants frequently terminates after 3–5 leaves are initiated.[16,17] Subsequently, axillary shoots grow out from the axils of rosette leaves. However, the mature shoots of *pny pnf* plants fail to initiate axillary and floral meristems; therefore, *pny pny* shoots display a non-flower producing phenotype. The role of PNY and PNF in meristem maintenance was recently investigated and results show that these homeodomain proteins regulate the integrity of the CZ.[18] In addition, increasing the size of the CZ in *pny pnf* plants by creating *pny pnf clv* triple mutants allows these plants to enter the reproductive phase of growth. In this addendum, results showed that the early vegetative shoot meristem termination phenotype displayed in *pny pnf* plants is only partially restored in *pny pnf clv3* plants.

Loss of *CLV3* Function Partially Rescues the Shoot Meristem Termination Phenotype in *pny pnf* Plants

In this analysis, growth from the main shoot was analyzed during vegetative development. Results show that the shoot apical meristems of wild-type and *clv3* plants sequentially initiated leaves until these plants transitioned to reproductive growth (**Fig. 1A and B**). In contrast to wild-type, 78% of *pny pnf* shoot apical meristems terminated after the production of 3–5 leaves (**Fig. 1C**).[16,17] Subsequently, shoot development was restored by the outgrowth of axillary shoots from the axils of rosette leaves (data not shown).[16,17] However, the remaining 22% of *pny pnf* plants maintained shoot growth. In *pny pnf clv3*, 58% of the central shoots maintained vegetative growth, while 42% of the shoots terminated growth after 3–5 leaves were produced (**Fig. 1E and F**). These results indicate that loss of *clv* function in *pny pnf* plants partially restores the maintenance of the shoot apical meristem, during early stages of vegetative growth.

Maintaining the Integrity of the CZ in Shoot Meristem

A recent study showed that PNY and PNF maintain the integrity of the CZ, which is crucial for regulating the *CLV3* and *WUS* expression domains in the shoot apical meristem.[18] Augmenting the size of the CZ by creating *pny pnf clv* mutants reestablishes the *CLV3* and *WUS* expression domains and rescues the non-flower producing phenotype. In this addendum, we determined whether loss of *clv3* function could suppress the *pny pnf* shoot meristem termination phenotype, which is displayed during early stages of vegetative growth. Experimental evidence shows that the *pny pnf* shoot meristems are considerably smaller than wild type. In addition, only 22% of *pny pnf* shoot meristems maintain growth during early stages of vegetative development. It was speculated that the small size of *pny pnf* shoot meristems combined with the loss of CZ integrity contribute the meristem termination phenotype during vegetative growth.[18] During early stages of shoot development, 58% of *pny pnf clv3* shoot meristems maintain an incessant pattern of growth, indicating the loss of CLV function in *pny pnf* plants partially restores meristem maintenance. In contrast, all *pny pnf clv3* plants produce reproductive shoots. To explain these differences, we propose that the increased CZ in *clv3* vegetative shoot meristems may not be sufficient to fully rescue the vegetative meristem termination phenotype produced in plants with reduced PNY and PNF function. For example, *clv3* the embryonic shoot meristem is only 1.4 times larger than wild type.[5] However, during later stages of *clv3* shoot development, the inflorescence meristems can increase up to 1,000-fold larger than wild type. Therefore, the complete rescue reproductive shoot formation in *pny pnf clv3* and *pny pnf clv1* plants is likely caused by the excess production of meristematic cells, which enables these shoot meristems to respond to floral inductive cues and produce flower-like structures.

References

1. Steeves TA, Sussex IM. Patterns in Plant Development. Cambridge: Cambridge University Press 1989:387.
2. Bernier G, Kinet JM, Sachs RM. The Physiology of Flowering. Boca Ranton; CRC 1981:2.
3. Bleckmann A, Simon R. Interdomain signaling in stem cell maintenance of plant shoot meristems. Mol Cells 2009; 27:615-20.
4. Clark SE, Running MP, Meyerowitz EM. *CLAVATA1*, a regulator of meristem and flower development in Arabidopsis. Development 1993; 119:397-418.
5. Clark SE, Running MP, Meyerowitz EM. *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting he same processes as *CLAVATA1*. Development 1995; 121:2057-67.
6. Laux T, Mayer KF, Berger J, Jürgens G. The *WUSCHEL* gene is required for shoot and floral meristem integrity in Arabidopsis. Development 1996; 122:87-96.
7. Leibfried A, To JP, Busch W, Stehling S, Kehle A, Demar M, et al. *WUSCHEL* controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature 2005; 438:1172-5.
8. Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM. Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem. Proc Natl Acad Sci USA 2009; 106:16529-34.
9. Zhao Z, Andersen SU, Ljung K, Dolezal K, Miotk A, Schultheiss SJ, et al. Hormonal control of the shoot stem-cell niche. Nature 2010; 465:1089-92.
10. Vollbrecht E, Reiser L, Hake S. Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, *knotted1*. Development 2000; 127:3161-72.
11. Hake S, Smith HM, Holtan H, Magnani E, Mele G, Ramirez J, et al. The role of knox genes in plant development. Annu Rev Cell Dev Biol 200; 20:125-51.
12. Barton MK. Twenty years on: The inner workings of the shoot apical meristem, a developmental dynamo. Dev Biol 2009; 341:95-113.
13. Clark SE, Jacobsen SE, Levin JZ, Meyerowitz EM. The *CLAVATA and SHOOT MERISTEMLESS* loci competitively regulate meristem activity in Arabidopsis. Development 1996; 122:1567-75.
14. Hamant O, Pautot V. Plant development: a TALE story. C R Biol 2010; 333:371-81.
15. Cole M, Nolte C, Werr W. 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 2006; 34:1281-92.
16. Smith HM, Campbell BC, Hake S. Competence to respond to floral inductive signals requires the homeobox genes *PENNYWISE and POUND-FOOLISH*. Curr Biol 2004; 14:812-7.
17. Rutjens B, Bao D, van Eck-Stouten E, Brand M, Smeekens S, Proveniers M. Shoot apical meristem function in Arabidopsis requires the combined activities of three BEL1-like homeodomain proteins. Plant J 2009; 58:641-54.
18. Ung N, Lal S, Smith HM. The role of PENNYWISE and POUND-FOOLISH in the maintenance of the shoot apical meristem in Arabidopsis. Plant Physiol 2011; 15:605-14.