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Article Addendum

A model for Arabidopsis class-1 *KNOX* gene function

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The Arabidopsis class-1 *KNOX* genes *STM*, *BP/KNAT1*, *KNAT2* and *KNAT6* encode homeodomain transcriptional regulators important for shoot apical meristem (SAM) and carpel development. During vegetative growth, *STM* is required to establish and maintain the stem cell pool of the SAM, a function replaceable by ectopic expression of *BP/KNAT1* but not of other class-1 *KNOX* genes. We recently demonstrated additional *STM* roles in the development of the central floral whorl and subsequent formation of carpels, a function replaceable by ectopic *KNAT2* expression. However, *STM* is normally required for *BP/KNAT1* and *KNAT2* expression, explaining why it is essential for both SAM and carpel development. We propose therefore that *STM* provides the critical KNOX function in these processes and that the SAM- and carpel-promoting activities of *STM* are redundantly duplicated in *BP/KNAT1* and *KNAT2* respectively. Here we show that ectopic *KNAT2* expression can restore carpel development to *stm* mutants, but fails to restore proper development of the central floral whorl, which requires a function analogous to the SAM-promoting activities of *STM* and *BP/KNAT1*. Similarly, we show that ectopic *KNAT2* expression does not restore normal meristem organisation to the SAM. We propose a model for discrete and overlapping class-1 *KNOX* gene function in Arabidopsis.

The *KNOTTED1*-like homeobox (*KNOX*) genes are found in all higher plant species and encode homeodomain transcription factors similar to those that regulate development in animals.[1] The class-1 *KNOX* gene subfamily in Arabidopsis comprises *SHOOT MERISTEMLESS* (*STM*), *BREVIPEDICELLUS/KNAT1*/ (*BP/KNAT1*), *KNAT2* and *KNAT6*, all of which are expressed in the shoot apical meristem (SAM) but not in lateral organs, where ectopic expression results in inhibited cell expansion and differentiation during leaf development.[2-6]

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Loss-of-function mutations in *STM* perturb or abolish the maintenance of the SAM because *STM* is required to maintain the stem cell pool, whereas mutation of other class-1 *KNOX* genes has no effect on SAM development (Fig. 1E).[7-9] Conversely, ectopic expression of *STM* leads to the formation of ectopic SAMs on the adaxial leaf surface, indicating that *STM* is sufficient to activate de novo SAM formation (Fig. 1B).[3,4] The closely related gene *BP/KNAT1* can also activate ectopic SAM formation when overexpressed, and can substitute for *STM* in SAM development if de-repressed or induced artificially when *STM* function is compromised (Fig. 1C and G).[10-12] However, loss of *BP/KNAT1* function does not affect SAM development but instead results in reduced growth of floral internodes, pedicels and the style during reproductive growth, consistent with its expression in these tissues/organs.[13,14]

KNOX genes are also involved in reproductive development. Ectopic expression of *KNAT2* (but not *BP/KNAT1* or *KNAT6*) during flowering induces the formation of ectopic carpels and the homeotic conversion of ovules to carpels,[5] although *KNAT2* is not essential for carpel formation in normal development because mutation of *KNAT2* confers no discernable phenotype.[12,15] Interestingly, we have recently shown that *STM* induces similar phenotypic changes to *KNAT2* when ectopically expressed in flowers.[16] Moreover, using inducible loss-of-function, we demonstrated that *STM* is required for the development of the central floral whorl, where it transiently maintains the central floral stem cells and subsequently directs the formation of carpels and the placental tissues from which ovules arise. These phenotypic changes are consistent with the expression of *STM* (and *KNAT2*) in the centre of the developing flower and in carpel placentae.[7,17] Thus, *STM* has an essential role in floral meristem (FM) and carpel development in addition to performing a critical function in the SAM.

We assessed the different functions of the class-1 *KNOX* genes in shoot and flower development using inducible ectopic expression in the wild-type (Landsberg *erecta*; L*er*) and *stm-1* mutant backgrounds (Fig. 1). Induced expression of *BP/KNAT1* fully restored SAM function to *stm* mutants, though the reproductive SAM was not as robustly maintained as the vegetative SAM (Fig. 1G). Interestingly, development of the two fused carpels of the gynoecium was variably restored in *stm-1* flowers overexpressing *BP/KNAT1*, with many anatomically normal gynoecia formed (Fig. 1O). However, no ectopic carpels or carpelloid ovules were observed, whereas these are seen with ectopic *STM* and *KNAT2* expression (Fig.1J, L, N and P). Conversely, ectopic expression of *KNAT2* failed to promote ectopic SAM formation, restore primary SAM function or proper central

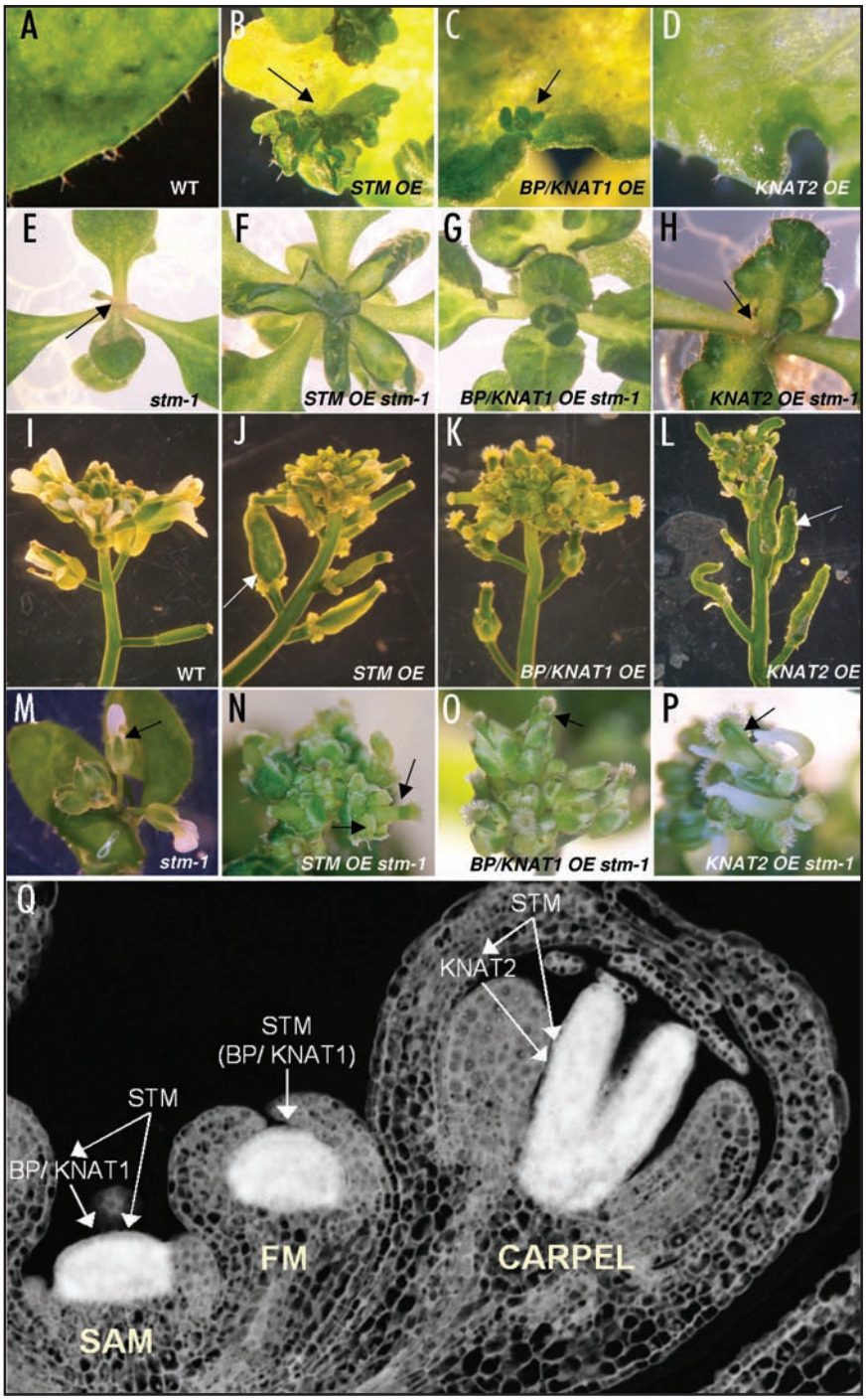

Figure 1. Discrete and overlapping roles of the Arabidopsis class-1 KNOX genes. (A–D) Overexpression of *KNOX* genes in leaves in the WT (L*er*) background. Induced *KNOX* gene expression levels were similar in the various lines. (A) WT leaf margin. (B) *STM* overexpressor (*STM OE*) leaf. Ectopic shoots are formed (arrow). (C) *BP/KNAT1* overexpressor (*BP/KNAT1 OE*) leaf. Ectopic shoots are formed (arrow). (D) *KNAT2* overexpressor (*KNAT2 OE*) leaf. No ectopic shoots are formed. (E–H) Overexpression of *KNOX* genes in *stm-1* shoot apices. (E) *stm-1* apex. Arrested shoot apex after the formation of a few adventitious leaves (arrow), as is often seen following 2–3 weeks growth of *stm-1* plants in vitro. (F) *STM OE stm-1* apex. Normal shoot activity is restored. (G) *BP/KNAT1 OE stm-1* apex. Normal shoot activity is restored. (H) *KNAT2 OE stm-1* apex. Shoot activity is not restored (arrow). (I–L) Overexpression of *KNOX* genes in WT (L*er*) inflorescences. (I) WT (L*er*) inflorescence. (J) *STM OE*, (K) *BP/KNAT1 OE* and (L) *KNAT2 OE* inflorescences. Ovule to carpel transformation[5,16] causes swelling of primary gynoecium in *STM OE* (J) and *KNAT2 OE* flowers (L) only (arrows). (M-P) Overexpression of *KNOX* genes in *stm-1* inflorescences. (M) *stm-1* inflorescence. Terminal flowers lack central carpels/ gynoecium (arrow). (N) *STM OE stm-1* inflorescence. Gynoecium and ectopic carpels develop (arrows). (O) *KNAT1 OE stm-1*. Gynoecium development is restored (arrow) but no ectopic carpels develop. (P) *KNAT2 OE stm-1*. Carpels form, but not the central gynoecium (arrow). (Q) Model for Arabidopsis class-1 *KNOX* gene function in the SAM, FM and carpel. *STM* is required for *BP/KNAT1* expression in the SAM (left), and both genes can promote SAM formation, although *BP/KNAT1* is not normally essential for SAM development. In the FM (centre), *STM* is required to maintain the central stem cells, and *BP/KNAT1* can also perform this function when ectopically expressed, although it is not normally expressed in this domain. *STM* and *KNAT2* promote carpel development (right). *STM* is normally required for *KNAT2* expression, but not when the FM stem cells are maintained by ectopic *BP/KNAT1* expression. *KNAT2* cannot maintain stem cells in the FM, and is not essential for carpel development.[12,15]

whorl formation in *stm* mutant flowers (Fig. 1 D, H and P). Although fused gynoecia were rarely formed, many ectopic carpels were initiated in *stm-1* flowers overexpressing *KNAT2*. These observations support the conclusion that *BP/KNAT1* has SAM-specific roles, whereas *KNAT2* has carpel specific roles, and demonstrate that these genes can functionally substitute for *STM* in these respective locations. Since *STM* is required for expression of both *KNAT1/BP* and *KNAT2* in vegetative and reproductive growth (Scofield and Murray, unpublished data),[16] the functional redundancy of *BP/KNAT1* and *KNAT2* is not apparent unless their expression is manipulated artificially.

It is intriguing that *BP/KNAT1* is able to restore gynoecium development to *stm* mutant flowers, given that it is not normally expressed in the centre of the FM and its ectopic expression does not result in carpelloid features, and at first sight this appears to contradict the model of specific roles. However, the roles of *STM* in floral development are in fact two fold. Its first function is to sustain a stem cell population in the central zone of the floral meristem, and this function, directly analogous to its maintenance role for the stem cell population of the SAM, can be compensated by ectopic *BP/KNAT1* in *stm* flowers which maintains the central zone of floral stem cells during the initial stages of floral meristem development. This creates a domain in which *KNAT2* expression can occur and induce carpel formation. In agreement with this hypothesis, we detected normal levels of *KNAT2* expression in *stm* mutant flowers overexpressing *BP/KNAT1* (data not shown), indicating that *KNAT2* is probably functional.

We propose a general model for class-1 *KNOX* gene function in Arabidopsis (Fig. 1Q). *STM* is essential for SAM formation and maintenance during both vegetative and reproductive growth, as well

as for central floral whorl establishment and carpel formation during flowering. The vegetative SAM is maintained because *STM* inhibits the cellular differentiation normally associated with organogenesis and permits the *WUS-CLAVATA* feedback loop to maintain the central stem cells.[18,19] This function can also be performed by *BP/KNAT1* when de-repressed or ectopically expressed (Fig. 1G).[11-12] In floral meristems, the stem cells must be transiently maintained in order to generate the central whorl of carpels. *STM* is critical not only for this initial maintenance of the stem cells, a function that as show here can also be performed by *BP/KNAT1*, but also for the subsequent formation of carpels and carpel placentae following the termination of stem cell maintenance by *AGAMOUS*.[20,21] This second function can also be performed by *KNAT2*. Since *STM* is required for the expression of both *BP/KNAT1* and *KNAT2*, the redundant functions of these genes are not performed when *STM* function is lost, resulting in the *stm* phenotype. We propose that *KNOX* genes, and *STM* in particular, are key conserved factors in several types of meristematic tissue in the shoot: those that generate vegetative organs and flowers (SAM), those that generate reproductive organs (FM central zone) and those that generate female gametes (carpel placentae). In addition, *BP/KNAT1* plays roles in the growth of certain structures such as the floral stalk. Thus, our work shows that various different types of meristematic tissue share a common molecular regulatory mechanism. Interaction with growth phase- or tissue-specific factors, or accessibility to downstream target genes, likely determines the type of meristematic tissue produced by *KNOX* genes, and interaction with such factors is probably conserved for *STM* and *BP/KNAT1* in the SAM and *STM* and *KNAT2* in the carpel.

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References

1. Scofield S, Murray JAH. *KNOX* gene function in plant stem cell niches. Plant Mol Biol 2006; 60:929-46.
2. Lincoln C, Long J, Yamaguchi J, Serikawa K, Hake S. A *knotted1*-like homeobox gene in Arabidopsis is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants. Plant Cell 1994; 6:1859-76.
3. Brand U, Grunewald M, Hobe M, Simon R. Regulation of *CLV3* expression by two homeobox genes in Arabidopsis. Plant Physiol 2002; 129:565-75.
4. Lenhard M, Jurgens G, Laux T. The *WUSCHEL* and *SHOOTMERISTEMLESS* genes fulfil complementary roles in Arabidopsis shoot meristem regulation. Development 2002; 129:3195-206.
5. Pautot V, Dockx J, Hamant O, Kronenberger J, Grandjean O, Jublot D, Traas J. *KNAT2*: Evidence for a link between *knotted*-like genes and carpel development. Plant Cell 2001; 13:1719-34.
6. Dean G, Casson S, Lindsey K. *KNAT6* gene of Arabidopsis is expressed in roots and is required for correct lateral root formation. Plant Mol Biol 2004; 54:71-84.
7. Long JA, Moan EI, Medford JI, Barton MK. A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of Arabidopsis. Nature 1996; 379:66-9.
8. 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.
9. Endrizzi K, Moussian B, Haecker A, Levin JZ, Laux T. 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 1996; 10:967-79.
10. Chuck G, Lincoln C, Hake S. *KNAT1* induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis. Plant Cell 1996; 8:1277-89.
11. Byrne ME, Barley R, Curtis M, Arroyo JM, Dunham M, Hudson A, Martienssen RA. *Asymmetric leaves1* mediates leaf patterning and stem cell function in Arabidopsis. Nature 2000; 408:967-71.
12. Byrne ME, Simorowski J, Martienssen RA. *ASYMMETRIC LEAVES1* reveals *knox* gene redundancy in Arabidopsis. Development 2002; 129:1957-65.
13. Douglas SJ, Chuck G, Dengler RE, Pelecanda L, Riggs CD. *KNAT1* and *ERECTA* regulate inflorescence architecture in Arabidopsis. Plant Cell 2002; 14:547-58.
14. Venglat SP, Dumonceaux T, Rozwadowski K, Parnell L, Babic V, Keller W, Martienssen R, Selvaraj G, Datla R. The homeobox gene *BREVIPEDICELLUS* is a key regulator of inflorescence architecture in Arabidopsis. Proc Natl Acad Sci USA 2002; 99:4730-5.
15. Belles-Boix E, Hamant O, Witiak SM, Morin H, Traas J, Pautot V. *KNAT6*: An Arabidopsis homeobox gene involved in meristem activity and organ separation. Plant Cell 2006; 18:1900-7.
16. Scofield S, Dewitte W, Murray JAH. The *KNOX* gene *SHOOT MERISTEMLESS* is required for the development of reproductive meristematic tissues in Arabidopsis. Plant J 2007; 50:767-81.
17. Long J, Barton MK. Initiation of axillary and floral meristems in Arabidopsis. Dev Biol 2000; 218:341-53.
18. Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R. Dependence of stem cell fate in Arabidopsis on a feedback loop regulated by *CLV3* activity. Science 2000; 289:617-9.
19. Schoof H, Lenhard M, Haecker A, Mayer KF, Jurgens G, Laux T. The stem cell population of Arabidopsis shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell 2000; 100:635-44.
20. Lenhard M, Bohnert A, Jurgens G, Laux T. Termination of stem cell maintenance in Arabidopsis floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. Cell 2001; 105:805-14.
21. Lohmann JU, Hong RL, Hobe M, Busch MA, Parcy F, Simon R, Weigel D. A molecular link between stem cell regulation and floral patterning in Arabidopsis. Cell 2001; 105:793-803.