

The unique relationship between *tsh4* and *ra2* in patterning floral phytomers

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Phytomers are developmental compartments that display stereotypical patterns dependent on whether they are initiated during the vegetative phase or the floral phases. Differences in appearance result from differential partitioning mechanisms responsible for allocation of cells to different components of the phytomer. The *tasselsheath* loci of maize control cell partitioning within the phytomer, indirectly influencing growth and development of its individual components. The *tasselsheath4* (*tsh4*) gene accomplishes this through regulation of the *ramosa2* (*ra2*) meristem determinacy gene, whereas *tasselsheath1* (*tsh1*) appears to function differently.

Aerial plant development is organized into repeating units called phytomers, each consisting of three components, an axillary meristem, subtending leaf and associated internode.¹ While vegetative phytomers and floral phytomers have similar organization, there are differences in the degree of elaboration of the different components. For example, within vegetative phytomers the leaf portion is often noticeable and highly elaborated. In floral phytomers, however, the leaf portion is highly reduced and obscured, and the axillary meristems are elaborated, eventually forming the reproductive organs (Fig. 1A). Clonal analysis of maize development has shown that sectors tend to begin and end within phytomer boundaries, indicating that the phytomer behaves as a developmental compartment.² Thus, the cells of the phytomer share a common origin, and developmental mechanisms to partition these cells into the different components of the phytomer must exist.

Recently, a new class of mutants in maize has been described that confirmed the existence of these developmental mechanisms within the phytomer. The *tasselsheath* (*tsh*) mutants cause de-repression of bract leaf development within the floral phytomers.³ This bract leaf growth occurs at the expense of the axillary meristems of the inflorescence, including the branch meristems (BM) that produce the side branches of the tassel and the spikelet pair meristems (SPM) that produce the spikelets. Both of these meristems are either reduced in number in the *tasselsheath* mutants, or have reduced determinacy and thus produce fewer lateral primordia. To date, two *tasselsheath* loci have been described, *tsh1*,⁴ and *tsh4*.⁵ Loss-of-function mutants of both genes display nearly identical phenotypes. *tsh1* is a GATA class transcription factor that has conserved function and expression patterns in several grasses. *tsh1* is expressed at the base of the SPM in areas where repressed bract leaves exist, indicating that the gene functions to repress bract leaf development during the floral phase.⁴ *tsh4* is a microRNA regulated *SBP* box transcription factor that is also expressed in these areas (Fig. 1B), but has a wider expression pattern in the stem.⁵ Interestingly, both *tsh1* and *tsh4* mutants display numerous defects in axillary meristem development, and yet neither gene is expressed in meristems. Concerning *tsh4*, previous studies have shown that one explanation for this observation is that factors that repress it, including the miR156 microRNA, are expressed within meristems and function to turn it off. In support of this, double labeling experiments of miR156 RNA

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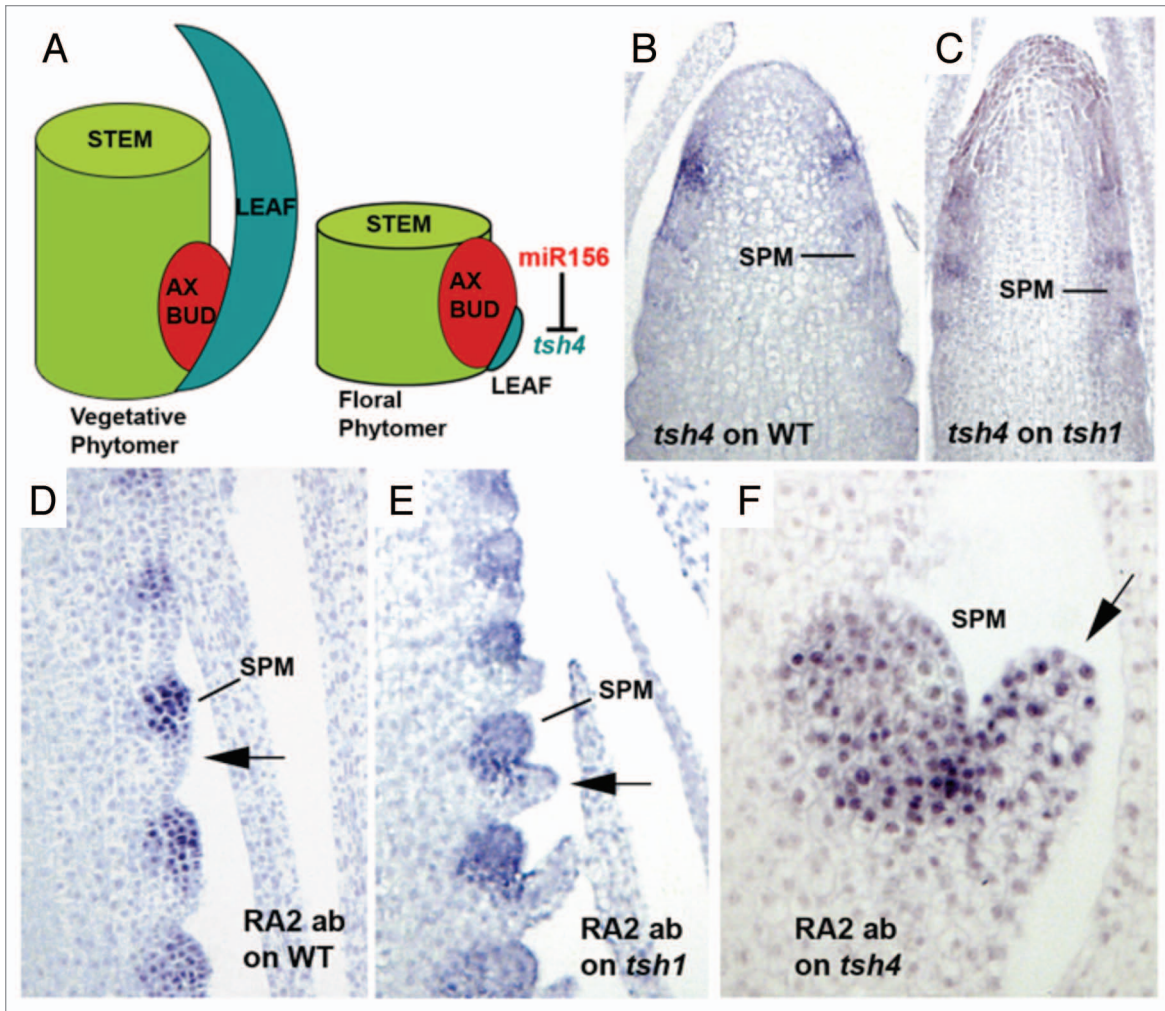


Figure 1. (A) Schematic of phytomer organization within the vegetative and floral phases. The two phases differ by the degree of elaboration of the three components. Within the floral phase of maize, the leaf component is repressed by the action of the *tsh4* gene, which itself is repressed by meristem specific expression of miR156. (B) *tsh4* in situ hybridization on wildtype tassels showing expression subtending SPMs. (C) *tsh4* in situ hybridization on *tsh1* tassels showing expression similar to wildtype. (D) Immunolocalization of RA2 in wildtype tassels. RA2 is found throughout the SPM, but not in the subtending repressed bract (arrow). (E) Immunolocalization of RA2 in *tsh1* tassels. No ectopic RA2 is found in the de-repressed bract (arrow). (F) Immunolocalization of RA2 in *tsh4* tassels. Ectopic RA2 is found in the de-repressed bract (arrow).

and TSH4 protein show a complementary expression pattern,⁵ where the microRNA is localized in the axillary meristem and TSH4 is localized to the suppressed bract leaf (Fig. 1A). *tsh1/tsh4* double mutants appear indistinguishable from the single mutants (data not shown), making epistasis difficult to establish. *tsh4* shows a normal pattern of expression within *tsh1* inflorescences (Fig. 1C), indicating that *tsh1* is not required for *tsh4* expression.

In order to understand how *tsh4* can affect axillary meristems without being expressed in them, the expression of lateral meristem markers was observed in *tsh4* mutants. One such marker is the *ra2* gene that functions to specify SPM determinacy.

ra2 is a LOB domain transcription factor expressed within the SPM, but not in the bract subtending it⁶ (Fig. 1D). Expression of RA2 protein within *tsh1* appeared normal (Fig. 1E). However, ectopic RA2 protein was observed within the de-repressed bracts of the *tsh4* mutant (Fig. 1F). These results indicate that *tsh4* plays a specific role in repressing *ra2* expression within bracts that is distinct from *tsh1*. The ectopic RA2 protein within the *tsh4* bracts reflect lost axillary meristem potential, indicating that de-repressed bract growth occurs at expense of axillary meristem growth, i.e., cells normally destined for the SPM are reallocated into the bract. This observation may explain how *tsh4*

can affect axillary meristem growth without actually being expressed within them. How *tsh1* is able to accomplish the same function independent of *ra2* is unclear.

Overall, these results show that a limited number of cells initially form the phytomer compartment, and that competition for these cells exists between the individual phytomer components. For example, the bract component competes with the axillary meristem component for cells. When the bract is de-repressed, its growth re-allocates cells normally destined to form the axillary meristem, as shown by the fact that bracts in *tsh4* mutants ectopically express meristem markers such as *ra2* (Fig. 1D). Since previous studies of *tsh4*

have shown that the antagonism between cells of the axillary bud and the subtending bract may be mediated by miR156, the possibility remains that meristem specific genes such as *ra2* may regulate microRNA expression.

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References

1. Rutishauser R, Sattler R. Complementarity and heuristic value of contrasting models in structural botany. *Bot Jahrb Syst Pflanzengesch Planzengeogr* 1985; 107:415-55.
2. Johri MM, Coe EH. Clonal analysis of corn plant development. I. The development of the tassel and the ear shoot. *Dev Biol* 1983; 97:154-72.
3. McSteen P, Hake S. *barren inflorescence2* regulates axillary meristem development in the maize inflorescence. *Development* 2001; 128:2881-91.
4. Whipple C, Hall D, DeBlasio S, Taguchi-Shiobara F, Schmidt R, Jackson D. A conserved mechanism of bract suppression in the grass family. *Plant Cell* 2010; In press.
5. Chuck G, Whipple C, Jackson D, Hake S. The maize SBP-box transcription factor encoded by *tasselsheath4* regulates bract development and the establishment of meristem boundaries. *Development* 2010; 137:1243-50.
6. Bortiri E, Chuck G, Vollbrecht E, Rocheford TF, Martienssen R, Hake S. *ramosa2* encodes a LATERAL ORGAN BOUNDARY domain protein that determines the fate of stem cells in branch meristems of maize. *Plant Cell* 2006; 18:574-85.