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The bHLH transcription factor SPATULA is a key regulator of organ size in *Arabidopsis thaliana*

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Plant organ size and thus plant size is determined by both cell proliferation and cell expansion. The bHLH transcription factor SPATULA (SPT) was originally identified as a regulator of carpel patterning. It has subsequently been found to control growth of the organs of the shoot. It does this at least in part by controlling the size of meristematic regions of organs in parallel to gibberellic acid (GA). It also acts downstream of several environmental signals, influencing growth in response to light and temperature. We have recently demonstrated that SPT functions to repress the size of the root meristem and thus root growth and size. It appears to do this using a similar mechanism to its control of leaf size. Based on the recent work on SPT, we propose that it is a growth repressor that acts to limit the size of meristems in response to environmental signals, perhaps by regulating auxin transport.

Keywords: SPT, organ size, root growth, gibberellic acid, auxin, environmental signals

Abbreviations: SPT, SPATULA; bHLH, basic helix-loop-helix; GA, gibberellic acid; RAM, root apical meristem; QC, quiescent center; ALC, ALCATRAZ; PIF, PHYTOCHROME INTERACTING FACTOR; APB, active phytochrome binding domain

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The size of plant organs is controlled by both cell division and cell expansion and regulation of these processes is an area of active investigation. Many genes have been identified that influence final organ size either through control of cell division, expansion or both and the relationship between the two processes is complex (reviewed in refs. 1–3). Recently the bHLH transcription factor SPATULA (SPT) has emerged as an important regulator of organ size in *Arabidopsis thaliana*. Although first identified for its effects on pistil morphology,[4] SPT has emerged as a more general repressor of organ growth. Loss of function mutants in *SPT* have larger cotyledons, longer hypocotyls and larger leaves while overexpression leads to smaller organs.[5-7] Depending on the organ, the difference in size is the result of changes in cell number and/or cell size, suggesting that SPT can regulate both processes.

SPT functions in both cotyledons and leaves. In cotyledons it acts to repress expansion in parallel to the gibberellin (GA)-dependent DELLAs.[5] SPT and GA share some common target genes in this organ and SPT is negatively regulated by DELLAs, suggesting a complex relationship between GA and SPT. In contrast to the cotyledon, SPT restricts cell division in leaves. In this organ a proliferative zone is found between the developing blade and petiole.[8] This zone is established early in leaf development and produces cells that populate both blade and petiole. Expression of a *SPT* enhancer trap line is found in the marginal region of this proliferative zone.[6,8] Consistent with this expression, in *spt* leaves the meristematic region of the leaf primordia was found to have more cells than in wild type.[6] This data suggests that SPT is important for regulating the size of the meristematic region of leaves and that the larger leaf size seen in *spt* plants is a result of expanded meristematic identity.

Although it had previously been reported that *SPT* is expressed in the roots,[9] its function in this area of the plant had not been examined. *SPT* expression in Arabidopsis is first detected in the embryonic hypophysis, then the forming root

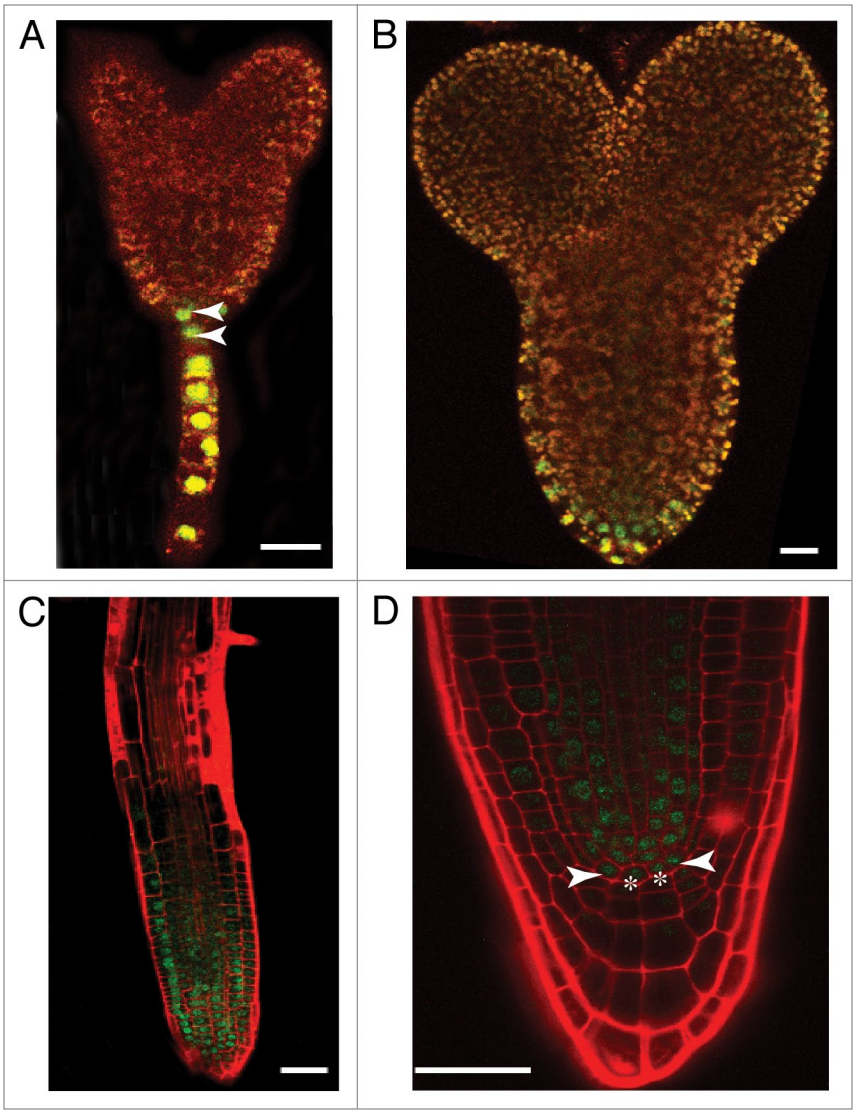

Figure 1. *SPT* is expressed in the root starting in embryogenesis. Confocal micrographs of *SPTp::gSPT-GFP* embryos (**A and B**) and seedlings (**C and D**) stained with propidium iodide as in Reference 10. (**A**) Transition stage embryo. Arrowheads indicate expression in the upper and lower hypophyseal cells. (**B**) Torpedo stage embryo with expression in the presumptive RAM. (**C**) 7 d after germination (DAG) root. SPT is expressed throughout the division zone. (**D**) Root tip of a 7DAG seedling. Asterisks indicate QC cells while arrowheads indicate initial cells. Scale bars indicate 50 μm in (**A and B**) and 100 μm in (**C and D**).

apical meristem (RAM). It continues to be expressed in the RAM and stele after germination (**Fig. 1** and ref. 9). Our studies revealed that *spt* mutants have longer roots and an increase in the number of cortical cells around the diameter of the root,[10] suggesting that SPT represses growth in all axes of the root. However, overall patterning of the root is not disrupted. The size increase is due to an expanded region of cell proliferation in the RAM. Closer examination of *spt* roots revealed that the number of cells in the quiescent center (QC) is increased as examined both morphologically and with molecular markers. The changes seen in *spt* roots is similar to those seen in *spt* leaves, i.e., an increase in the size of the meristematic region leading to increased cell proliferation and increased organ size and expands the role of SPT as a growth repressor throughout the plant.

In the root SPT acts in parallel to GA.[10] This is consistent with results in the shoot,[5] supporting the idea that the molecular mechanism by which SPT represses growth may be similar in these regions. SPT regulates at least one DELLA target gene in the root suggesting that co-regulation by SPT and the GA pathway maybe a feature throughout the plant. *spt* mutant roots have broader auxin maxima at their tips and altered expression of the auxin efflux carrier PIN4.[10] Although *spt* roots respond normally to exogenous auxin, they are hypersensitive to auxin transport inhibitors. This suggests that SPT regulates auxin transport. This is consistent with its regulation of genes related to this transport in the flower[11] and recovery of the *spt* carpel phenotype by application of auxin transport inhibitors.[12]

The role of SPT in carpel and fruit development has been extensively analyzed,[4,11,13-15] In this context SPT does not seem to act merely as a growth repressor but to regulate patterning of the septum, style and stigma in the carpel and subsequently dehiscence zone development in the silique.[4,15] Interestingly, GA positively regulates SPT in this organ independently of DELLA proteins.[16] In carpels and fruits SPT is partially redundant with its paralog *ALCATRAZ (ALC)*.[15] However, ALC-like genes are confined to a subset of angiosperms consisting of at least the Brassicaceae.[13,15] The function of ALC outside of the flower has not been examined; however, *ALC* is expressed in hypocotyls, the lateral margins of leaves and in leaf vasculature.[9] Some of this expression overlaps that of *SPT*, especially in the leaf margins, suggesting that the functional redundancy between *ALC* and *SPT* could extend to control of leaf growth. *ALC* is expressed in emerging lateral roots and the root-lateral root junction, and in the stele but not the root tip.[9] Earlier expression in the embryo has not been reported. Since *ALC* is not expressed in the root tip, it seems unlikely that *SPT* is functionally redundant with this gene in controlling the size of the RAM. However, a function in the stele that is masked in *spt* mutants by the presence of a wild type *ALC* locus is possible. Examination of non-floral phenotypes of *spt; alc* plants should be undertaken to determine to what extent these two genes are redundant.

SPT is related to a group of bHLH proteins, the PHYTOCHROME INTERACTING FACTORS (PIFs), but

differs in having lost the active phytochrome-binding domain (APB).[13] SPT is involved in regulating growth in response to both light and temperature in seeds, leaves and carpels.[7,13,14,17] Recently it has been suggested that a light-regulated module functioning in shade avoidance was recruited to carpel development after the loss of the APB domain from SPT-like genes.[13] Roots, like carpels, develop in dark and shaded conditions, supporting the view that loss of the APB domain allowed expansion of expression and function of SPT-like genes into non-light regulated pathways. Phytochromes have been implicated in regulation of root architecture, controlling the emergence of lateral roots partly by regulating auxin distribution,[18] similar to the regulation of root growth by SPT via control of auxin transport.[10] It is possible that light, temperature or other environmental signals are integrated into root growth by SPT. This hasn't been examined as all work in our paper was done under standard long day conditions and normal temperature regimes.[10]

In conclusion, SPT is a central hub in the regulation of organ size in *Arabidopsis* (**Fig. 2**). It integrates environmental signals, most prominently light, with GA and controls the size of meristematic regions by restricting cell proliferation as well as controlling cell expansion in some organs. SPT regulates auxin transport, directly or indirectly, and shares a subset of GA targets. Further experimentation is necessary to determine the exact mechanisms by which SPT functions and the origins of its organ specific functions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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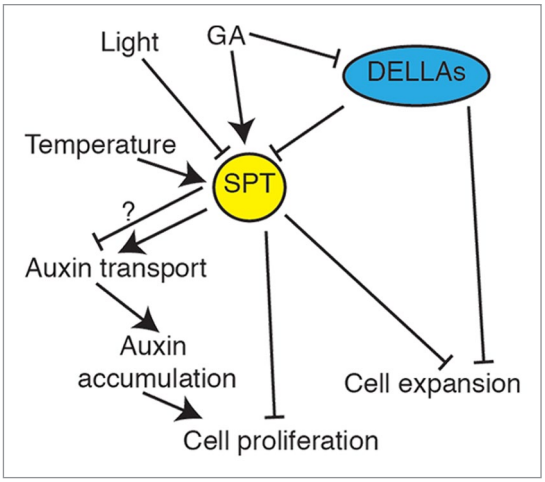

Figure 2. A model for SPT action. SPT acts as a central hub in the control of organ size. Hormonal and environmental inputs regulate the expression and stability of SPT which in turn regulates auxin transport, either directly or indirectly, as well as other genes to negatively regulate cell division and expansion in parallel to the GA-regulated DELLAs.