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

Srilakshmi Makkena¹,† and Rebecca S. Lamb¹,²,*

¹Plant Cellular and Molecular Biology Graduate Program; Ohio State University; Columbus, OH USA; ²Department of Molecular Genetics; Ohio State University; Columbus, OH USA

†Current Affiliation: Indiana Crop Improvement Association; Lafayette, IN USA

---

**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

---

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.

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...
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. 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 and recovery of the spt carpel phenotype by application of auxin transport inhibitors.

The role of SPT in carpel and fruit development has been extensively analyzed. 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. GA positively regulates SPT in this organ independently of DELLA proteins. In carpels and fruits SPT is partially redundant with its paralog ALCATRAZ (ALC). However, ALC-like genes are confined to a subset of angiosperms consisting of at least the Brassicaceae. 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. 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. 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
differes in having lost the active phytochrome-binding domain (APB). SPT is involved in regulating growth in response to both light and temperature in seeds, leaves and carpels. 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. 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, similar to the regulation of organ size in plants. Curr Biol 2012; 22:R360-7; PMID:22575478; http://dx.doi.org/10.1016/j.cub.2012.02.010

In conclusion, SPT is 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.

References
1. Gonzalez N, Vanhaeren H, Inzé D. Leaf size control: complex coordination of cell division and expansion. Trends Plant Sci 2012; 17:332-40; PMID:22401845; http://dx.doi.org/10.1016/j.tplants.2012.02.003
2. Powell AE, Lenhard M. Control of organ size in plants. Curr Biol 2012; 22:R360-7; PMID:22575478; http://dx.doi.org/10.1016/j.cub.2012.02.010
3. Krizek BA, Anderson JT. Control of flower size. J Exp Bot 2013; PMID:23404902; http://dx.doi.org/10.1093/jxb/ert025
4. Alvarez J, Smyth DR. CRABS CLAW and SPATULA, two Arabidopsis genes that control carpel development in parallel with AGAMOUS. Development 1999; 126:2377-86; PMID:10225997
5. Josse EM, Gan Y, Bou-Torrent J, Stewart KL, Gilday AD, Jefferie CE, et al. A DELLA in disguise: SPATULA restrains the growth of the developing Arabidopsis seedling. Plant Cell 2011; 23:1357-51; PMID:21478445; http://dx.doi.org/10.1093/tpc/tpn082
6. Ichikashi Y, Horiguchi G, Glessberg S, Tsukaya H. The bHLH transcription factor SPATULA controls final leaf size in Arabidopsis thaliana. Plant Cell Physiol 2010; 51:252-61; PMID:20040585; http://dx.doi.org/10.1093/pcphysic/51.2.184
7. Sidaway-Lee K, Josse EM, Brown A, Gan Y, Halliday KJ, Graham IA, et al. SPATULA links daytime temperature and plant growth rate. Curr Biol 2010; 20:1493-7; PMID:20705468; http://dx.doi.org/10.1016/j.cub.2010.07.028
8. Ichikashi Y, Kameda K, Usami T, Horiguchi G, Takahashi T, Tsukaya H. Key proliferative activity in the junction between the leaf blade and leaf petiole of Arabidopsis. Plant Physiol 2011; 157:1151-62; PMID:21880932; http://dx.doi.org/10.1104/pp.111.185066
9. Grozmann M, Bystraa Y, Lampugnani ER, Smyth DR. Regulation of tissue-specific expression of SPATULA, a bHLH gene involved in carpel development, seedling germination, and lateral organ growth in Arabidopsis. J Exp Bot 2010; 61:1495-508; PMID:20176890; http://dx.doi.org/10.1093/jxb/erp051
10. Makkena S, Lamb RS. The bHLH transcription factor SPATULA regulates root growth by controlling the size of the root meristem. BMC Plant Biol 2013; 13:1; PMID:23280064; http://dx.doi.org/10.1186/1471-2229-13-1
11. Girin T, Paicu T, Stephenson P, Fuentes S, Körner E, O’Brien M, et al. INDEHISCENT and SPATULA interact to specify carpel and valve margin tissue and thus promote seed dispersal in Arabidopsis. Plant Cell 2011; 23:3641-53; PMID:21990939; http://dx.doi.org/10.1105/tpc.111.099044
12. Nemhauser JL, Feldman IJ, Zambrisky PC. Auxin and ETTIN in Arabidopsis gynoecium morphogenesis. Development 2000; 127:58770-88
13. Reymond MC, Bruonnod G, Chaavet A, Martinez-Garcia JF, Martin-Magniette ML, Monéger F, et al. A light-regulated generic module was recruited to carpel development in Arabidopsis following a structural change to SPATULA. Plant Cell 2012; 24:2812-25; PMID:22851763; http://dx.doi.org/10.1105/tpc.112.103192
14. Foreman J, White J, Graham I, Halliday KJ, Josse EM. Shedding light on flower development: phytochrome B regulates gynoecium formation in association with the transcription factor SPATULA. Plant Signal Behav 2011; 6:471-6; PMID:21364353; http://dx.doi.org/10.4161/psb.6.4.14496
15. Groszmann M, Paicu T, Alvarez JP, Swain SM, Smyth DR, SPATULA and ALCATRAZ, are partially redundant, functionally-diverging bHLH genes required for Arabidopsis gynoecium and fruit development. Plant J 2011; 68:816-29; PMID:21801252; http://dx.doi.org/10.1111/j.1365-313X.2011.04732.x
16. Fuentes S, Liqiu K, Sorefan K, Alvey E, Harberd NP, Östergaard L. Fruit growth in Arabidopsis occurs via DELLA-dependent and DELLA-independent gibberellin responses. Plant Cell 2012; 24:3982-96; PMID:23064323; http://dx.doi.org/10.1105/tpc.112.103192
17. Penfield S, Josse EM, Kannangara R, Gilday AD, Halliday KJ, Graham IA. Cold and light control seed germination through the bHLH transcription factor SPATULA. Curr Biol 2005; 15:1998-2006; PMID:16035588; http://dx.doi.org/10.1016/j.cub.2005.11.010
18. Salisbury FJ, Hall A, Grieron CS, Halliday KJ. Phytochrome coordinates Arabidopsis shoot and root development. Plant J 2007; 50:429-38; PMID:17419844; http://dx.doi.org/10.1111/j.1365-313X.2007.03059.x