The Plant Cell, Vol. 11, 2061, November 1999, www.plantcell.org

IN THIS ISSUE

Tissue Development in a New Vein

The organs of dicots and monocots have traditionally been summarized in terms of dermal, ground, and vascular tissues. In contrast, over 40 different plant cell types have been identified (see Zhong and Ye, 1999, in this issue). In this era of molecular biology, where many of the mechanisms that underlie gene regulation have been elucidated, the reduction of all plant material into a mere three categories thus seems somehow quaint. This simplification, however, which students will continue to find anatomically instructive (if not always cytologically accurate), points out an important gap in our understanding of plant development that continues to demand bridging, especially in this era of molecular biology. Specifically, how does the impressive array of plant vegetative and reproductive structures, along with the tissue patterns and cell types on which they are based, arise from the protoderm, ground meristem, and procambium?

Significant progress has been made in answering this question within the contexts of specific organ systems. As an example, diverse studies continue to disclose the complexities of flower development that, in the case of Arabidopsis, have already been shown to emanate from the specific expression of more than 60 genes (Levy and Dean, 1998). At the center of such advances is a concern for the organization and differentiation of meristem tissues, which give rise to all three canonical tissue types throughout the life cycle of the plant.

Investigations of gene functions that are particular to specific cell fates, as opposed to the analysis of loci and genes (including homeotic genes) that affect the larger issues of organogenesis (e.g., flowering), have also met with some success. Epidermal cell fate in the elaboration of Arabidopsis root hairs, for example, rests on specific "root hairless" genes from among several complementation groups (Schneider et al., 1997). Another instance where genetic control of differentiation has been analyzed at the suborgan level, also involving epidermal tissues, lies in the identification of mutants in which stomatal patterning is disrupted (Geisler et al., 1998).

In contrast, vascular development represents an area in which molecular and genetic analyses are relatively lacking. Such nescience is surprising, not only in consideration of the role leaf venation has played in plant systematics, but also in light of the evolutionary significance of the plant vascular system as reflected by the common interchangeability of the terms "land plant" and "vascular plant." In terms of plant developmental studies, our present ignorance of vascular formation is especially unfortunate, given the essential role the plant vasculature plays in the systemic delivery of signals that may themselves regulate differentiation throughout the plant body in general.

On pages 2123–2137 of this issue, Carland et al. describe their efforts to further our molecular understanding of plant vascular development by isolating Arabidopsis mutants with altered vasculatures. Rationalizing that relatively simple venation established early after seed germination would be more easily screened, and that subtle perturbations of plant development would be the most informative, the authors painstakingly analyzed the vascular morphologies of thousands of mutagen-treated seedling cotyledons. Two stable mutant lines that showed altered cotyledonary venation patterns (*cvp1* and *cvp2*) were chosen for subsequent study by virtue of their ability to undergo normal growth rates and attain generally normal adult morphology; complementation analysis established the two mutations to represent distinct genes. The identification of mutants with such characteristics is in itself something of a coup, because mathematical models have suggested that the random flow of auxin, a positive regulator of vascularization, could in theory establish leaf venation patterns (see Nelson and Dengler, 1997). Accordingly, the researchers showed that neither mutation was associated with alteration of auxin content, perception, or transport, thereby corroborating a genetic basis for venation.

Upon inspection of a few hundred 7-day-old wild-type cotyledons, the authors determined that vascular patterns predictably consist of four closed loops, two loops emanating from either side of the midvein. The vascular circuits typical of *cvp1* and *cvp2* mutants, on the other hand, typically fail to close, preferentially forming discontinuous loops and freely ending veins, respectively. The two genes additionally affected early vein patterning in other distinct ways. Vascular strands of *cvp1* cotyledons appear thickened, for example, characterized by unusually large numbers of xylem tracheary cells, whereas the *cvp2* mutation tended to increase the number of secondary veins relative to wild-type cotyledons. Root morphologies of the 7-day-old mutant seedlings did not appear to deviate from the wild type.

To determine whether the *cvp* mutations differentially affected the cell types of the vascular bundles, numerous histological characteristics were screened. Significantly, staining patterns for both xylem and phloem tissues proved to be identical, suggesting that the mutations

IN THIS ISSUE

affected vascular development quite early, most likely in the procambial tissue that gives rise to both xylem and phloem cells. Indeed, the authors confirmed the altered patterns to be reflected in the embryonic procambial tissue. Additional histological investigations showed that cotyledon anatomy and, ultimately, leaf anatomy were otherwise unperturbed; the *cvp* mutations were thus found to be specific to early differentiation of vascular cells.

The tendency of *cvp2* mutant patterns to produce vascular "dead ends" was associated with a gradual reduction in the number of vascular cells along the vascular strand. The authors therefore conclude that the *cvp2* mutation reduces the number of cells recruited into the vascular tissue, which, along with observations in leaf (as opposed to cotyledon) morphologies, was regarded as a sign of premature vein termination (as opposed to late initiation). The reduced cell elongation observable in the thickened *cvp1* mutant, on the other hand, was taken to reflect a failure in cell axialization. More specific knowledge about the roles of *CVP1* and *CVP2* will await their cloning, but the authors speculate compellingly on their potential involvement in the perception of positional signals in the developing embryo.

A second paper in this issue also addresses the genetic basis of vascular development in Arabidopsis. **On pages 2139–2152 Zhong and Ye** report on the molecular characterization of a gene, *INTERFASCICULAR FIBERLESS1* (*IFL1*), the mutation of which has been noteworthy for the strikingly pendant stems that result (Zhong et al., 1997). As a result of the successful cloning of *IFL1*, which forms the basis for their present report in this issue of THE PLANT CELL, the authors have been able to determine the specific expression of the gene in the vascular tissues of roots, leaves and cotyledons.

Significantly, *ifl1* mutants were found to be dramatically compromised in the formation of vascular bundles. The differentiation of secondary xylem was especially affected, such that a reduction in vessel element formation was noted. Unlike the case of the *cvp* mutations noted by Carland et al. (1999; see above), however, the genetic lesion in the case of *ifl1* mutants cannot be primarily attributed to an effect on procambial or vascular tissue per se. Rather, the gene exerts its most fundamental function in the differentiation of those fibers (i.e., cell types that are traditionally relegated to the ground tissue) not associated with xylem or phloem. The pendant stem phenotype associated with *ifl1* mutants represents the most obvious effect of the absence of interfascicular fibers.

In addition to establishing the gene's (secondary) effect on vascular tissue, Zhong and Ye have also determined the gene product to be directed to the cell nucleus. Sequence comparisons, in fact, strongly suggest the protein encoded by *IFL1* to be a transcription factor of the homeodomain–leucine zipper (HD-ZIP) class, particular to plants. (Interestingly, another Arabidopsis gene that falls into this class, *Athb8*, is specifically expressed in procambial cells [Sessa et al., 1998].) It is thus not so surprising, perhaps, that IFL1 affects multiple tissue types, establishing not only the proper spacing of interfascicular fibers, but also of vascular development. Whether the pleiotropic effects due to *ifl1* mutations may be associated with alterations in polar flow of auxin along the stems is a question that the authors consider worthy of further investigation. Intriguingly, mutation of *IFL1* reduces xylem differentiation much more distinctly in basal stem regions, reminiscent of decreased auxin concentrations associated with its polar flow (Aloni, 1987).

The findings presented in this issue that concern *IFL1*, *CVP1*, and *CVP2* will be welcome contributions to the field of vascular organization in plants. They certainly confirm the existence of multiple genetic factors. Clearly, the identification of other regulators of venation and vascular development will be necessary for establishing the context into which these three genes fit.

Harry B. Smith

REFERENCES

Aloni, R. (1987). Differentiation of vascular tissues. Ann. Rev. Plant Physiol. **38,** 179–204.

Carland, F.M., Berg, B.L., FitzGerald, J.N., Jinamomphongs, S., Nelson, T., and Keith, B. (1999). Genetic regulation of vascular tissue patterning in Arabidopsis. Plant Cell **11,** 2123–2137.

Geisler, M., Yang, M., and Sack, F.D. (1998). Divergent regulation of stomatal intitiation and patterning in organ and suborgan regions of the Arabidopsis mutants *too many mouths* and *four lips.* Planta **205,** 522–530.

Levy, Y.Y., and Dean, C. (1998). The transition to flowering. Plant Cell **10,** 1973–1989.

Nelson, T., and Dengler, N. (1997). Leaf vascular pattern formation. Plant Cell **9,** 1121–1135.

Schneider, K., Wells, B., Dolan, L., and Roberts, K. (1997). Structural and genetic analysis of epidermal cell differentiation in Arabidopsis primary roots. Development **124,** 1789–1798.

Sessa, G., Steindler, C., Morelli, G., and Ruberti, I. (1998). The Arabidopsis Athb-8, -9, and -14 genes are members of a small gene family coding for highly related HD-ZIP proteins. Plant Mol. Biol. **38,** 609–622.

Zhong, R., and Ye, Z.-H. (1999). IFL1, a gene regulating interfascicular fiber differentiation in Arabisopsis, encodes a homeodomain-leucine zipper protein. Plant Cell **11,** 2139–2152.

Zhong, R., Taylor, J.J., and Ye, Z.-H. (1997). Disruption of interfascicular fiber differentiation in an Arabidopsis mutant. Plant Cell **9,** 2159–2170.