Looking deep inside the phloem and xylem cell wall composition by synchrotron FTIR and Raman spectroscopy

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Identity crisis: a molecular network switching protophloem cell fate

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The developmental trajectory of plant cell files encompasses gradual cell identity transitions, which are influenced by their relative cell position within the plant. In *Arabidopsis thaliana* roots, protophloem sieve elements (PSE) originate from a stem cell whose daughter cell undergoes two consecutive periclinal divisions to give rise to PSE and metaphloem sieve elements (MSE). Owing to its specialized ontogenesis, PSE survival depends on the metabolic support of companion cells (CC), two PSE-adjacent cells whose developmental trajectory is poorly described. The activity of two phloem-specific 5' phosphatases, *COTYLEDON VASCULAR PATTERN2 (CVP2)* and its homologous *CVP2-LIKE1 (CVL1)* are required to maintain PSE identity. Transcriptomic analysis combined with live imaging revealed the presence of CC-associated transcripts in PSE of *cvp2 cvl1* roots, suggesting that these phloem cells transit between PSE and CC identities. Remarkably, perturbing the function of *RECEPTOR LIKE KINASE 2 (RPK2)* in *cvp2 cvl1* double mutants restores this hybrid identity to a wild-type situation. By perceiving CLAVATA3/EMBRYO SURROUNDING REGION 45 (CLE45) peptide at the CC, RPK2 excludes protophloem identity from the surrounding cells and, in turn, contributes to prime a functional phloem pole. Together, our data suggest that the CLE45-RPK2 module is required to maintain a reservoir of uncommitted phloem cells that will acquire distinct cell identities upon positional information.

Key words: companion cells, protophloem, cell fate, CLE45 signaling.

Illuminating the translocation stream

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The study of phloem transport has long been hampered by a lack of suitable tools. Many of the existing best methods rely on radiolabeled carbon sources, which lack resolution in small seedlings like Arabidopsis, or require expensive equipment, such as MRI mediated velocimetry. As an alternative, we developed a suite of fluorescent probes which enable the high-throughput study of phloem transport in the roots of live seedlings (Knoblauch et al, 2015). We recently used one of these probes, the coumarin glucoside esculin, to demonstrate that flow velocity is regulated in response to environmental factors (Knox et al, 2018). These results revealed important insights into the regulation of phloem transport, how such regulation might integrate with the Mass Flow Hypothesis and ultimately, the control of carbon partitioning. Furthermore, we have screened for and synthesized an array of probes with which to dissect the 'rules of engagement' governing which substances can enter the phloem.

Key Words: Phloem, fluorescent probes, phloem loading, xenobiotics

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Cell walls are highly complex structures that are modified during plant growth and development[1]. For example, the development of phloem and xylem vascular cells, which participate in the transport of sugars and water as well as providing support, can be influenced by cell-specific wall composition. We used synchrotron radiation-based Fourier-transform infrared (SR-FTIR) and Raman spectroscopy to analyze the cell wall composition of floral stem vascular tissues of wild-type Arabidopsis and the double-mutant *sweet11-1 sweet12-1*, which has impaired sugar transport[2,3]. The SR-FTIR spectra showed that in addition to modified xylem cell wall composition, phloem cell walls in the double mutant line were characterized by modified hemicellulose composition. Combining Raman spectroscopy with a classification and regression tree (CART) method identified combinations of Raman shifts that could distinguish xylem vessels and fibers. In addition, the disruption of the *SWEET11* and *SWEET12* genes impacted on xylem wall composition in a cell-specific manner, with changes in hemicelluloses and cellulose observed at the xylem vessel interface[4]. These results suggest that the facilitated transport of sugars by transporters that exist between vascular parenchyma cells and conducting cells is important in ensuring correct phloem and xylem cell wall composition.

References

1. M. R. Tucker, H. Lou, M. K. Aubert, L. G. Wilkinson, A. Little, K. Houston, S. C. Pinto and N. J. Shirley *Plants* **7**, pii: E42. doi: 10.3390/plants7020042 (2018).
2. L. Q. Chen, X. Q. Qu, B. H. Hou, D. Sosso, S. Osorio, A. R. Fernie, W. B. Frommer *Science* **335**, 207-211 (2012).
3. R. Le Hir, L. Spinner, P. A. Klemens, D. Chakraborti, F. De Marco, F. Vilaine, N. Wolff, R. Lemoine, B. Porcheron, C. Géry, E. Téoulé, S. Chabout, G. Mouille, H. E. Neuhaus, S. Dinant and C. Bellini *Mol. Plant* **8**, 1687-1690 (2015).
4. S. Dinant, N. Wolff, F. De Marco, F. Vilaine, L. Gissot, E. Aubry, C. Sandt, C. Bellini and R. Le Hir, *J. Exp. Bot.* https://doi.org/10.1093/jxb/ery396.

Controlling intercellular flow through mechanosensitive plasmodesmata nanopores

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In plants, plasmodesmata (PD) are nanopores that serve as channels for molecular cell-to-cell transport. Precise control of PD permeability is essential to regulate phloem loading and unloading; moreover it is involved in growth, tissue patterning, and defense against pathogens. Callose deposition modulates PD transport but little is known of the rapid events that lead to PD closure in response to tissue damage or osmotic shock. We propose a new mechanism of PD closure as a result of mechanosensing. Pressure forces cause the cell wall, or the dumbbell-shaped ER-desmotubule-complex, to be displaced from the equilibrium position, thus closing the PD aperture. Cell wall elasticity and the filamentous protein tethers that link the plasma membrane (PM) to the ER complex play a key role in determining the selectivity of the PD pore. This model of PD control compares favorably with experimental data on the pressure-generated closure of PD.