Identifying the pathways that control resource allocation in higher plants

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A key feature in the evolution of multicellular organisms was the development of complex vascular systems to transport resources from sites of primary acquisition to sites of usage and storage. In plants, leaves generate the energy required to sustain multicellular growth through the reactions of photosynthesis, and much of that energy is used to reductively assimilate atmospheric CO2 into simple sugars. Assimilate partitioning refers to the physiological process by which carbohydrates generated by photosynthesis are transported from leaves to heterotrophic "sink" tissues (such as roots, stems, fruit, and seed) that are dependent on imported resources to support growth and development. As much as 80% of the carbon assimilated in photosynthesis is exported from the leaves to satisfy the needs of these heterotrophic tissue systems (1). In the results reported, Xu et al. (2) provide a significant step forward in our understanding of the regulation of assimilate partitioning.

Sucrose is the major carbohydrate transported in most plants. Sucrose synthesized in the cytoplasm of photosynthetic mesophyll cells in the leaves is transported to sink tissues via the phloem cells of the plant’s vascular system. Not unlike animal systems, this long-distance transport is mediated by pressure-driven mass flow. Unlike animals, however, hydrostatic pressure is not created by a physical pump (the heart) but by a large osmotic gradient that draws water into the phloem cells that are surrounded by an inelastic cell wall that restricts cell expansion, thereby generating high hydrostatic pressure (Fig. 1). The osmotic gradient across the plasma membrane is generated by a proton-sucrose symporter that draws water into the phloem cells to a substantial proton electrochemical potential across the plasma membrane that is created by a proton-pumping ATPase that can drive transport reactions three orders of magnitude away from equilibrium (3). Thus, the symporter in the leaf phloem is highly regulated by changes in protein abundance and by phosphorylation-mediated changes in $K_m$. Pressure-driven mass flow of solution moves sucrose from the leaf to heterotrophic sink tissue, where sucrose supports growth and development of nonphotosynthetic tissues. Modified with permission from ref. 13. Copyright American Society of Plant Biologists, www.plantphysiol.org.

In the broadest sense, the proton–sucrose symporter allows plants to function as multicellular organisms because of its pivotal role in assimilate partitioning, and, not surprisingly, its activity is highly regulated as part of a system-wide coordination of photosynthetic productivity in leaves and sucrose utilization by import-dependent "sink" tissues (4). Xu et al. (2) advance our understanding of the regulation of the phloem-loading sucrose symporter wherein the authors identify two enzymes that regulate the activity of the Arabidopsis SUCROSE TRANSPORTER 2 (SUC2) sucrose symporter by altering its turnover rate or by impacting the $K_m$ for sucrose by phosphorylating the transporter.

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Xu et al. (2) show that a 4-h shift into high light (100 to 400 μmol photon-m⁻²-s⁻¹) increased the rate of photosynthesis and sucrose export in Arabidopsis leaves. In parallel with these changes, they show an increase in the SUC2 symporter protein abundance in the phloem and an increase in SUC2 phosphorylation. A recent interactomics screen that used the mating-based yeast two-hybrid system to identify proteins that interact with plasma membrane proteins in Arabidopsis (5) identified several potential interaction partners of SUC2. One of these was the UBQUITIN-CONJUGATING ENZYME 34 (UBC34).

Xu et al. (2) use a couple of methods to demonstrate direct interaction between SUC2 and UBC34. Moreover, in ubc34 mutants, they measure higher levels of SUC2 protein abundance in the plasma membrane than in wild-type plants under the same conditions, thus supporting the notion that UBC34 ubiquitinates SUC2 and thereby targets it for degradation. Interestingly, glucose transport by the facilitated transporters, GLUT1 and GLUT4, in insulin-sensitive tissues are regulated, in part, by a structural analog to the E2 ubiquitin-conjugating enzyme that links sentrin to these transporters and regulates the abundance of these two carriers in opposite directions (6). Additional experiments by Xu et al. show that increased SUC2 protein levels are the result of lower rates of turnover versus higher rates of synthesis. They also show direct evidence for ubiquitination of SUC2 by UBC34. Significantly, the ubc34 mutants, under low-light conditions, had higher rates of photosynthesis and increased fresh weight and seed yield compared to those observed in wild-type plants. Taken together, these results suggest elevated levels of SUC2 protein increased sucrose export rates, thereby stimulating photosynthesis by decreasing negative feedback of sugars on carbon fixation (7).

As noted earlier, high-light conditions increased both SUC2 protein abundance and the phosphorylation level of the symporter. The interactome data identified several kinases that interact with SUC2. Förster resonance energy transfer analysis reported by Xu et al. (2) confirms these protein–protein interactions. However, loss-of-function mutant analysis of each kinase shows that only one, WALL-ASSOCIATED KINASE LIKE 8 (WAKL8), decreased the ratio of phloem sucrose concentrations to SUC2 protein abundance and thereby targets it for degradation. Interestingly, glucose transport by the facilitated transporters, GLUT1 and GLUT4, in insulin-sensitive tissues are regulated, in part, by a structural analog to the E2 ubiquitin-conjugating enzyme that links sentrin to these transporters and regulates the abundance of these two carriers in opposite directions (6).

The impact of WAKL8 phosphorylation on SUC2 transport activity was explored with coexpression in yeast, a well-established, functional model of a plant cell when investigating plant plasma membrane transporters. Yeast growth on a medium with sucrose as the sole carbon source was faster when WAKL8 was coexpressed with SUC2. In addition, ¹³C-labeled sucrose uptake kinetics showed a significant decrease in Kₚ by 40%, while Vₘₐₓ remained virtually unchanged when both proteins were coexpressed.

Taken together, the Xu et al. (2) report provides evidence for direct regulation of the SUC2 symporter by controlling symporter protein abundance and by phosphorylation. These results provide a mechanistic link to previous publications that showed the symporter is dynamically regulated to coordinate assimilate partitioning in the face of changing physiological and environmental conditions (4, 8). For example, Kühn et al. (9) previously provided evidence that symporter turnover is regulated in potato leaves, when they showed a diurnal pattern of symporter protein abundance with decreased levels in the night.

Chiou and Bush (10) provided the first evidence that regulation of sucrose symporter activity might be a key regulatory step in the systemic distribution of photoassimilates. They showed that increases in sugar beet leaf sucrose levels decreased Vₘₐₓ symporter transport activity and lowered symporter message levels. They also showed that the impact of sucrose on symporter activity was reversible. They concluded sucrose is a signal molecule that regulates assimilate partitioning. Subsequent work by Vaughn et al. (11) showed that decreased transport activity in the presence of high sucrose was caused by a reduction in the abundance of symporter protein. In addition, RNA gel blot analysis revealed that symporter message levels also declined, and nuclear run-on experiments demonstrated that this was the result of decreased transcription. Vaughn et al. also showed that symporter protein and message are both degraded rapidly. Finally, Ransom-Hodgkins et al. (12) used protein phosphatase and kinase inhibitors to provide evidence that a protein phosphorylase is involved in sucrose regulation of symporter transcription. Taken together, these data suggest phloem loading is regulated by sucrose-mediated changes in transcription of the sucrose symporter in a regulatory system that plays a pivotal role in balancing photosynthetic activity with resource utilization.

The D.R.B. laboratory’s working hypothesis is that sucrose utilization in the sinks feeds back on symporter activity in the leaf, thereby controlling phloem loading and, ultimately, photosynthesis. For example, high rates of mass flow occur in the phloem to actively growing sinks as sucrose is rapidly removed to satisfy metabolic needs. Rapid removal of sucrose from the sink phloem maintains a large pressure gradient between the leaf and the sink, thereby driving the high rates of mass flow. Under these conditions, sucrose is rapidly transported out of the leaf, effectively lowering sucrose levels in the leaf phloem and thereby stimulating high rates of symporter transcription and high levels of symporter protein abundance to maximize phloem loading. In contrast, if sink utilization drops, sucrose removal at the sinks slows and mass flow decreases, because the pressure gradient drops as high sucrose levels remain in the sink phloem. Therefore, sucrose transport out of the leaf slows. Initially, however, photosynthesis in the mesophyll is unaffected, and newly synthesized sucrose is still actively loaded into the leaf phloem. Because mass flow out of the leaf is slowed, and active loading by the symporter continues, sucrose levels build up in the leaf phloem. The D.R.B. laboratory hypothesizes that a sucrose sensor detects this increase in leaf phloem sucrose levels and sets off a signaling cascade that decreases symporter transcription and, in the presence of high symporter turnover rates, lowers phloem loading capacity as symporter abundance drops. As loading slows, sucrose then backs up in the photosynthetic mesophyll cells, and that increases glucose levels that trigger hexokinase mediated decreases in photosynthesis (7).

The Xu et al. (2) report illuminates the molecular details of two pathways that impact phloem loading capacity in the leaf by controlling the activity and/or abundance of the sucrose symporter. Xu et al. also demonstrate that these pathways are linked to changes in photosynthetic activity and assimilate partitioning. The challenge for the future is to fill in the gap
between earlier work demonstrating sucrose-mediated regulation of symporter activity (4) and the molecular mechanisms described by Xu et al. (2). It seems clear we’re on the threshold of a comprehensive understanding of the dynamic process of carbon allocation between sites of primary assimilation and sink utilization in plants as complex, multicellular organisms.

1 W. Kalt-Torres, P. S. Kerr, H. Usuda, S. C. Huber, Diurnal changes in maize leaf photosynthesis: I. Carbon exchange rate, assimilate export rate, and enzyme activities. Plant Physiol. 83, 283–288 (1987).
2 Q. Xu et al., Carbon export from leaves is controlled via ubiquitination and phosphorylation of sucrose transporter SUC2. Proc. Natl. Acad. Sci. U.S.A. 117, 6223–6230 (2020).
3 D. R. Bush, Proton-coupled sugar and amino acid transporters in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44, 513–542 (1993).
4 E. A. Ainsworth, D. R. Bush, Carbohydrate export from the leaf: A highly regulated process and target to enhance photosynthesis and productivity. Plant Physiol. 155, 64–69 (2011).
5 A. M. Jones et al., Border control—A membrane-linked interactome of Arabidopsis. Science 344, 711–716 (2014).
6 F. Giorgino et al., The sentrin-conjugating enzyme mUbcs9 interacts with GLUT4 and GLUT1 glucose transporters and regulates transporter levels in skeletal muscle cells. Proc. Natl. Acad. Sci. U.S.A. 97, 1125–1130 (2000).
7 J. C. Jang, J. Sheen, Sugar sensing in higher plants. Plant Cell 6, 1665–1679 (1994).
8 Q. Xu, S. Chen, R. Yunjuan, S. Chen, J. Liesche, Regulation of sucrose transporters and phloem loading in response to environmental cues. Plant Physiol. 176, 930–945 (2018).
9 C. Kühn, V. R. Franceschi, A. Schulz, R. Lemoine, W. B. Frommer, Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. Science 275, 1298–1300 (1997).
10 T. J. Chiou, D. R. Bush, Sucrose is a signal molecule in assimilate partitioning. Proc. Natl. Acad. Sci. U.S.A. 95, 4784–4788 (1998).
11 M. W. Vaughn, G. N. Harrington, D. R. Bush, Sucrose-mediated transcriptional regulation of sucrose symporter activity in the phloem. Proc. Natl. Acad. Sci. U.S.A. 99, 10876–10880 (2002).
12 W. D. Ransom-Hodgkins, M. W. Vaughn, D. R. Bush, Protein phosphorylation plays a key role in sucrose-mediated transcriptional regulation of a phloem-specific proton-sucrose symporter. Planta 217, 483–489 (2003).
13 T.-J. Chiou, D. R. Bush, Molecular cloning, immunochromatic localization to the vacuole, and expression in transgenic yeast and tobacco of a putative sugar transporter from sugar beet. Plant Physiol. 110, 511–520 (1996).