The sink-source transition in leaves - new insights

A.G. Roberts, S. Santa Cruz, P. Boevink, I.M. Roberts, N. Sauer & K.J. Oparka

All leaves on a plant undergo a transition from a sink (a net carbon importer) to a source (a net carbon exporter) during their development. The early growth of a leaf is supported by carbohydrate imported from other sources in the plant. These sources are usually other mature leaves or photosynthetic organs on the plant, or in the case of a seedling, the cotyledons. As the lamina expands and the leaf matures, levels of photosynthesis increase until the leaf can support itself. When the amount of carbon accumulated by photosynthesis is greater than the requirement of respiration and growth, a positive carbon balance is achieved by that leaf. The leaf then becomes an exporter of carbon. In dicotyledonous plants, the conversion from sink to source begins shortly after the leaf begins to unfold, and is known as the sink-source transition. The transition begins at the leaf tip and moves basipetally as the leaf matures.

In *Nicotiana benthamiana*, five vein classes have been identified (Fig. 1). The class I vein (the midrib) branches at frequent intervals to form class II veins. These class IIs frequently interconnect at the leaf margins, providing a loop between adjacent class II veins. Class III veins are derived from class IIs and subdivide at regular intervals to form the class III veinal network. These three classes of veins are collectively termed the major veins, and previous autoradiographic evidence has shown that these are involved in unloading of photoassimilates from the phloem. Vein classes IV and V, the minor veins, are found within the islands of the class III vein network and are involved in phloem loading once the leaf has become a source.

Figure 1 The veination of N. benthamiana. The class I vein (brown) branches to produce class II (green) and then class III veins (yellow). Minor veins (red) are found within the class III network.

Non invasive imaging of the sink-source transition

Until recently, the only way to image the sink-source transition was by using autoradiography. However, this method is destructive, technically demanding and time consuming. Recent studies carried out in the Unit of Cell Biology have shown that the sink-source transition can be studied more simply, and in real-time, with fluorescent tracers[1]. These techniques have enabled us to study the unloading pattern of photoassimilates using the phloem-mobile, fluorescent probe carboxyfluorescein (CF). This probe is loaded onto the abraded surface of source leaves as carboxyfluorescein diacetate (CFDA), which is then cleaved by plant esterases to produce the impermeant CF moiety. This is translocated through the phloem and unloaded in

sink leaves along with the flow of photoassimilate. After phloem import of CF, the leaves can be detached and their petioles placed into a solution of 3 kDa Texas Red dextran. This dye is transpired through the xylem network of the leaf and labels all veins, allowing the vein classes to be easily identified. The fluorescence in sink tissue is then detected and imaged using a confocal laser scanning microscope (CLSM).

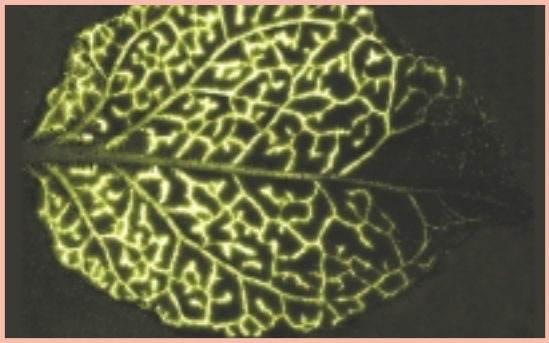

Figure 2 CF unloading from major veins in a leaf that has just started to undergo the sink-source transition.

The pattern of CF transport (Fig. 2) was dictated to a large extent by the phyllotaxy of the plant, with leaves directly above the loaded leaf becoming more uniformly labelled than leaves on the opposite side of the plant. In sink leaves displaying phloem transport of CF, dye was seen first in class I and II veins but was not observed to unload from these vein classes. In contrast, after entry into the class III veinal network, CF was unloaded into the mesophyll. In sink leaves in which the sink-source transition was absent, or was only recently commenced, dye unloading was widespread and the subtending tissues became highly fluorescent. However, in tissue close to the sink-source transition, CF unloading was greatly reduced and the dye was restricted to the class III veins. CF was not unloaded from the phloem in any source tissue or from class IV or V veins.

Virus movement follows the 'rules' of solute unloading Using the CF unloading studies as a basis, unloading of GFP-tagged viruses was then studied. Potato virus X (PVX) carrying the green fluorescent protein (GFP) was inoculated onto *Nicotiana benthamiana* leaves. The GFP allows the spread of the virus to be monitored in both whole plants and tissues when illuminated by UV light. PVX spread throughout the inoculated leaf and entered the phloem where it was carried in the flow of photoassimilate into sink tissues. The first indication of virus entry into sink leaves was the appearance of fluorescent flecks on the lamina, indicating that the virus was unloaded from discrete foci rather than uniformly along leaf veins (Fig. 3). Flecks of virus were first visible in sink leaves approximately 9 days after the plants were inoculated. After the appearance of fluorescent flecks, the mesophyll tissue between veins also became infected. Fluorescent images of five leaves from one systemically infected plant are shown in Figure 4. In sink leaves near the apex, the virus was present throughout the entire leaf, although the tips of these leaves often showed more intense fluorescence than did the base. By contrast, after the onset of the sink-source transition, the apical (source) region of the leaf showed no fluorescence, indicating an absence of virus. When Texas Red was introduced into the transpiration stream of systemically infected leaves, virus unloading was found to occur predominantly from the class III vein network; the same vein class used to unload solutes (Fig. 3).

Sink leaves can unload macromolecules We have been collaborating with N. Sauer's group in Erlangen, Germany. These researchers have expressed GFP in companion cells of source leaves using the promoter of the *Arabidopsis* sucrose transport protein, AtSUC2[2]. The GFP was produced in companion cells of source leaves, entered sieve elements, and was found to unload subsequently from the phloem in sink leaves. GFP was also found to spread from cell to cell in sink tissues. GFP is a 27 kDa protein and therefore much larger than the size exclusion limit (SEL) of plasmodesmata (see SCRI Ann. Rep. 1993,

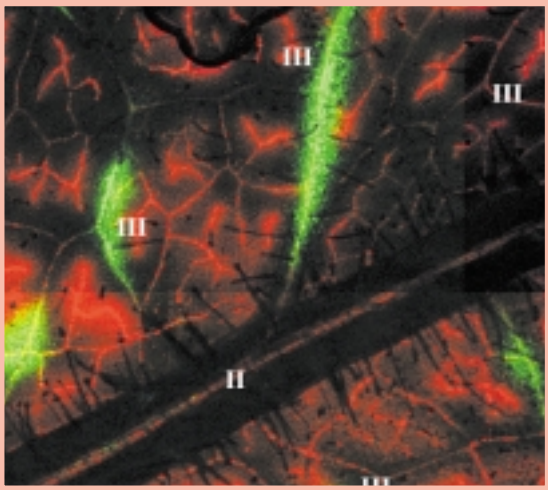

Figure 3 Unloading of GFP (green) occurs from the class III vein network and produces elongated flecks. The vascular system (shown in red) is labelled with Texas Red dextran.

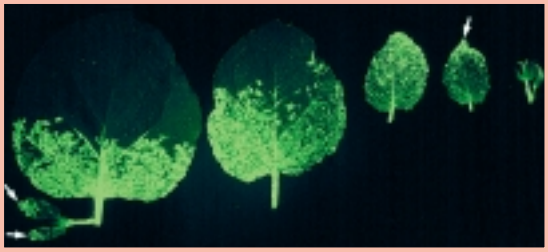

Figure 4 Five apical leaves from a plant systemically infected with PVX.GFP. In small sink leaves, the tip of the leaf becomes more fluorescent than the base (arrowed). The largest two leaves were undergoing the sink-source transition at the time of virus entry.

50-53). Thus, some plant tissues have the capacity to traffick macromolecules.

The distribution of GFP allowed the sink-source transition to be studied non-invasively in intact leaves. GFP has a half-life in living cells of approximately 4 hours. This meant that, as the transition moved, alterations in the distribution of GFP between sink and source areas of leaves could be seen (Fig. 5). In source tissues, punctate fluorescence from individual companion cells could be seen along the veins due to the restriction of GFP to this cell type (Fig. 6). In contrast, sink leaves showed diffuse unloading of GFP from major veins (Fig. 7), characteristic of solute unloading (cf. Fig. 2).

Plasmodesmata in sink leaves have a SEL of approximately 50 kDa In order to probe the SEL of plasmodesmata in sink tissue, leaves were biolistically

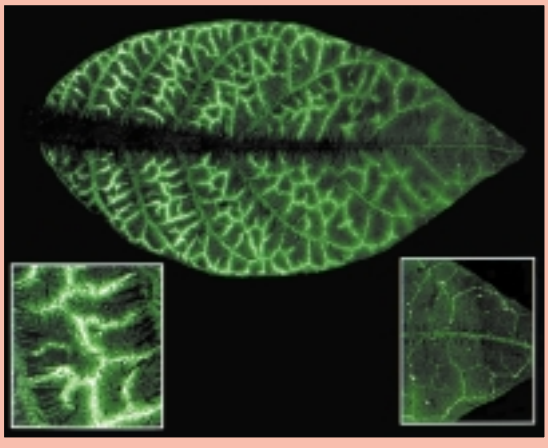

Figure 5 A leaf unloading GFP expressed from the AtSUC2 promoter. The sink source transition has just started at the leaf tip. Boxed areas from the tip and base of the leaf highlight the differences in GFP distribution in sink or source areas.

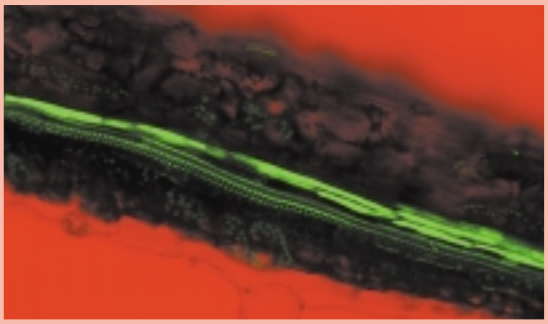

Figure 6 In source tissue, GFP is found only in companion cells, seen here in a section through a leaf. The xylem is autofluorescent.

bombarded with plasmids encoding various GFP-fusion proteins of different sizes (Fig. 8). Fusions were made between GFP and the storage proteins sporamin (mass 47 kDa), and patatin (mass 67 kDa). A third fusion was created between GFP and a truncated version of the patatin protein (GFP-patati), which had a mass of 61 kDa. When leaves were bombarded with a plasmid expressing only the *gfp* gene, intense fluorescence was detected in the initial bombarded epidermal cell and also in several cells surrounding the 'hit' cell. GFP was detected in neighbouring cells within 7h, and 2 days later had spread into approximately 200 cells (Fig. 8A), moving upwards into leaf trichomes and also downwards into the mesophyll. In contrast, when GFP was bombarded into epidermal cells of source leaves, the spread of fluorescence was greatly restricted (Fig. 8B). However, trace movement was often observed in cells immediately adjoining the bombarded cell. In sink leaves, the GFP-sporamin fusion moved several cells away from the bombarded cells, but not as extensively as GFP alone. In contrast, no cell-to-cell movement of GFP-sporamin was

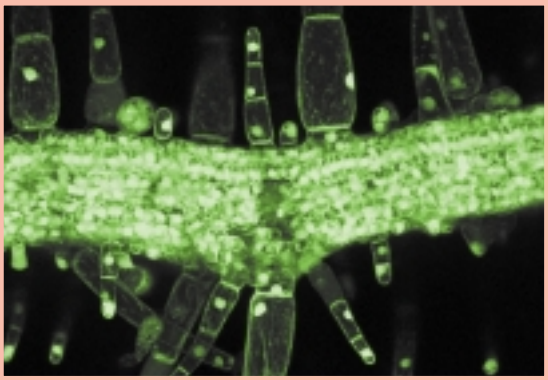

Figure 7 In sink tissue, unloaded GFP is present throughout the cytoplasm of each cell in the leaf.

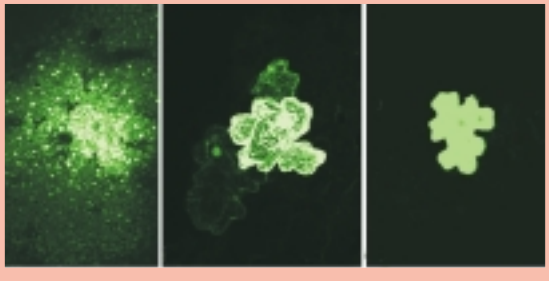

Figure 8 Leaf tissue bombarded with GFP and GFP-fusion proteins. (A) In sink tissue, GFP moves rapidly from cell to cell. (B) In source tissue, GFP is much more restricted to the bombarded cell. (C) In sink tissue, GFP-patatin does not move out of the bombarded cell.

observed in source leaves. Both the GFP-patatin (Fig. 8C) and the GFP-patati fusion proteins failed to move from cell to cell in either sink or source leaf tissues. These results demonstrate that proteins of at least 47 kDa, but not as large as 61 kDa, can move freely through sink-leaf plasmodesmata. This plasmodesmatal permeability decreased in response to the developmental switch from sink to source status.

The sink-source transition is accompanied by a change from simple to branched plasmodesmata We have shown that, during the sink-source transition in tobacco leaves, simple plasmodesmata give rise to more branched forms as the leaf matures. This provided an ideal system to study the functional differences between simple and branched plasmodesmata. To examine the structure of plasmodesmata during the sink-source transition, leaf samples were taken from each leaf on AtSUC2 transgenic tobacco plants expressing GFP. Before excision of the samples, the position of the sink-source transition was recorded by imaging each intact leaf under the CLSM using low magnification objectives. The sink-source transition was demarcated by the change in appearance of vein classes that were unloading GFP and those that showed CC-specific expression of GFP (see Fig. 5). Counts of plasmodesmal types (simple versus branched) revealed a marked reduction in the proportion of simple plasmodesmata in source tissues during the progression of the sink-source transition (Fig. 9). Tissue samples taken at the base of the leaf showed predominantly simple plasmodesmata, while those at the tip were mainly branched. In samples studied by electron microscopy, the simple plasmodesmata had conspicuous wall collars surrounding the neck of the pore. Within the region of the sink-source transition, 'pairs' of simple plasmodesmata were observed routinely in both longitudinal and oblique sections of the

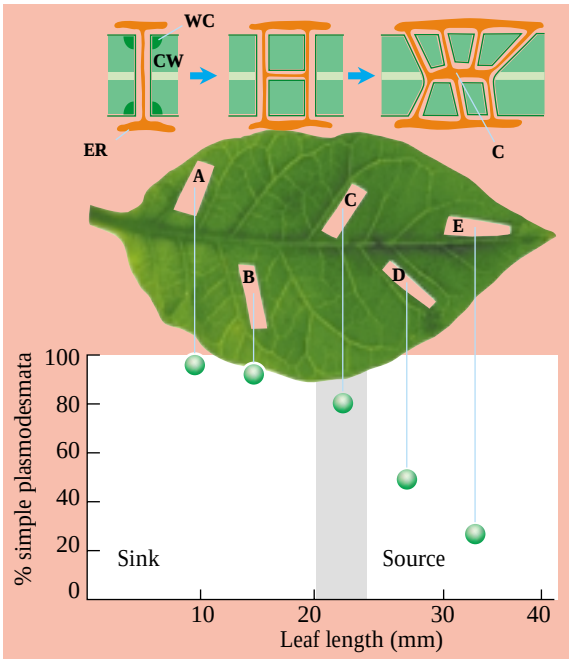

Figure 9 The sink-source transition is accompanied by a change from simple to branched plasmodesmata (top). The graph (bottom) shows the percentage of simple plasmodesmata in different sectors of the leaf (A-E). The transition zone is shown within the shaded region. C, central cavity; CW, cell wall; ER, endoplasmic reticulum; WC, wall collar.

wall, giving rise to 'H'-shaped plasmodesmal branching patterns. Branched plasmodesmata also displayed conspicuous median cavities. Unlike the simple plasmodesmata in sink tissues, the branched plasmodesmata found in source regions of the leaf did not display electron-dense wall collars.

Our studies have shown that the sink-source transition is a dynamic change that occurs in all leaves and that it has an enormous effect on both the structure and function of leaf tissues. Sink leaves are capable of non-specific trafficking of molecules of at least 47 kDa through simple plasmodesmata. On the other hand, the branched plasmodesmata in source tissues have a low size exclusion limit and restrict macromolecular trafficking. This suggests that the leaf utilises a 'downregulation' of plasmodesmal conductance to restrict intercellular communication during the sink-source transition. It thus appears that the plant uses plasmodesmata as 'control centres' for macromolecular trafficking.

References

[1] Roberts, A.G., Santa Cruz, S., Roberts, I.M., Prior, D.A.M., Turgeon, R. & Oparka, K.J. (1997). *Plant Cell* 9, 1381-1396.

[2] Imlau, A., Truernit, E. & Sauer, N. (1999). *Plant Cell* 11, 309-322.