

# Companion Cells

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Companion cells are responsible for the life maintenance of the sieve element. Based on its ontogeny, the companion cell is an intrinsic part of the phloem.

## Introduction

Companion cells represent a major component of the phloem, the plant vascular tissue system responsible for long-distance transport of organic nutrients from the sites of synthesis (source organs) to sites of utilization (sink organs). The companion cells are intimately associated with phloem sieve elements, which make up the actual transport vessel (sieve tube) through which mass flow of assimilates occurs. Sieve elements are usually enucleate, although they are living cells, and are thus completely dependent upon the associated companion cells leading to the concept of the sieve element–companion cell complex (SE/CC). The companion cell is necessary not only for metabolic maintenance of sieve elements but also for the selective accumulation (phloem loading) of assimilates and macromolecules present in sieve elements and to be transported by the phloem.

## Ontogeny and Ultrastructure

Both companion cells and sieve elements originate from a single cambial mother cell by asymmetric cell division, after which both cells undergo their own developmental programmes and differentiation. Sieve elements undergo maturation by karyolysis, dissolution of vacuoles and loss of most organelles and by developing a parietal anastomosed endoplasmic reticulum system and sieve plates between sieve elements. The result of sieve element differentiation is a system of interconnected, enucleate, living cells with all organelles anchored to the plasma membrane, leaving the central volume of the cell available for mass flow of solution. In contrast, companion cells have a large nucleus, are rich in organelles and cytoplasm, and may divide once or twice before final differentiation, giving rise to one or more companion cells per sieve element. Chavan *et al.* (2000) analysed the relationship (number and length) between companion cells and sieve elements in 125 dicotyledonous species. A positive correlation was found between the number of companion cells and the length of sieve elements.

On the basis of ultrastructural features companion cells can be placed into three general classes: ordinary

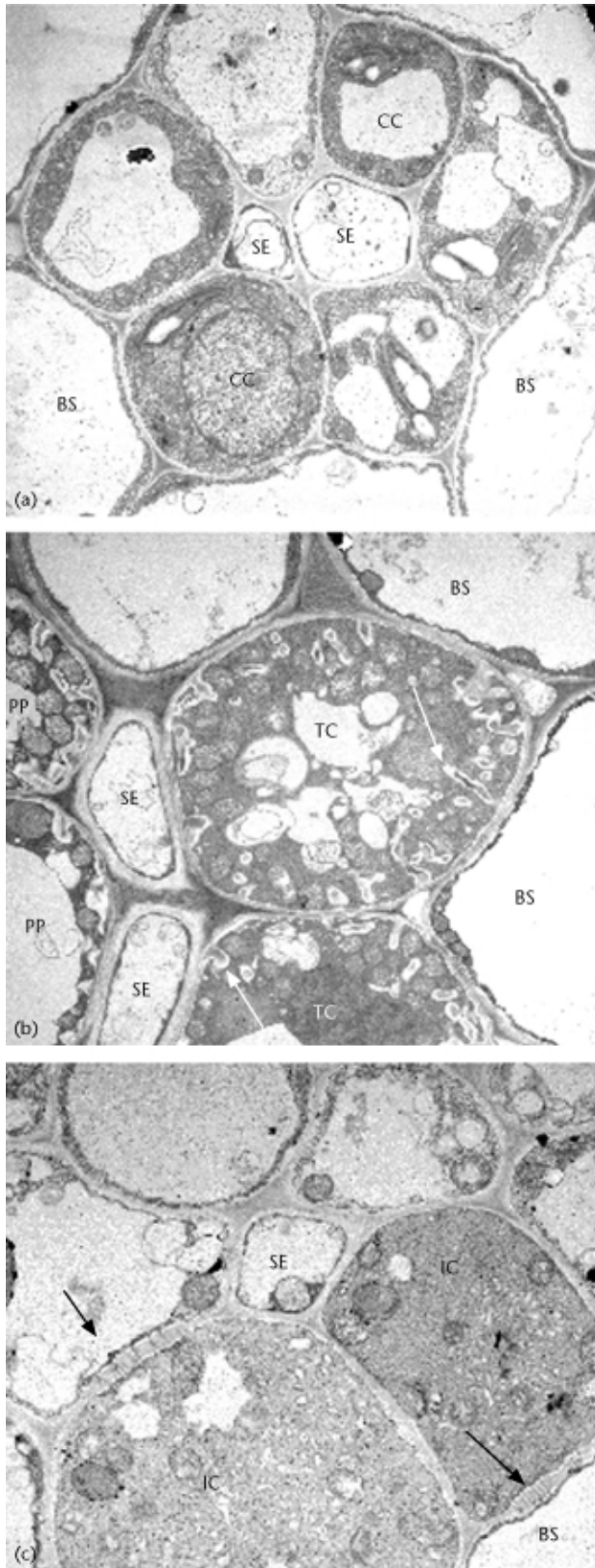
companion cells (CC), transfer cells (TC) and intermediary cells (IC) (**Figure 1**). All classes are characterized by an extraordinarily dense cytoplasm indicative of high metabolic rate, with numerous mitochondria and ribosomes, abundant smooth endoplasmic reticulum, and chloroplasts with more (ordinary CC and TC) or less developed thylakoids (IC). Intermediary cells contain many small vacuoles, while the two other types contain only one or two vacuoles. Diagnostic features of transfer cells are finger-like plasma membrane invaginations at the interface with bundle-sheath and/or vascular parenchyma cells. The invaginations drastically increase cell surface area, thus enhancing surface area to volume ratio, which is significant with respect to phloem loading. A function common to all transfer cells is a role in transport along tissue interfaces that require high fluxes. In the phloem of leaves, they potentially enhance the net flux of photoassimilates out of the leaves. Transfer cells are thus polarized and require mechanisms potentially similar to those of intestinal epithelia for creating polarity.

Another major difference, with functional implications, between the different classes of companion cells resides in the degree of plasmodesmal connectivity with surrounding cells (**Figure 2**). Plants with few interconnections between the SE/CC complex and the surrounding cells are generally thought to be apoplasmic loaders, while the presence of more plasmodesmata was used as an argument for symplasmic loading. Gamalei (1989) classified more than 700 plant species according to their plasmodesmal frequencies in minor veins between SE/CC complex and surrounding cells. Plants with numerous plasmodesmata were classified as type 1, plants with fewer interconnections as type 2. Type 2 plants were divided further into 2a, having ordinary companion cells and few plasmodesmata of deltoid shape and 2b, having transfer cells and few plasmodesmata. A third group, type 1-2a, has a number of plasmodesmal connections in between type 1 and type 2a. Among type 1 plants, species are found with intermediary cells where plasmodesmata are typically branched on both interfaces. Intermediary cells are never found in the middle of a vascular bundle but rather in a peripheral position where loading of assimilates is presumably occurring. Other species have only ordinary

## Secondary article

### Article Contents

- Introduction
- Ontogeny and Ultrastructure
- Functions
- Conclusion



companion cells and no intermediary cells. As a corollary, minor vein structure has also been related to the type of sugar transported; for example, species with ordinary companion cells or transfer cells and little continuity between SE/CC complex and surrounding cells mainly seem to transport sucrose, while species with more connections, like intermediary cells, seem to transport raffinose-type sugars and sugar-alcohols, although, some species of the type 1 without intermediary cells transport sucrose and raffinose to some extent (Turgeon, 1996). However, phloem is highly dynamic, and cell structure can vary with tissue function. Collecting phloem contains ordinary CCs, TCs or ICs. However as the phloem changes into the translocation pathway, the nature of companion cells seems to change. For example, in *Cucurbita pepo* intermediary cells are found in collecting phloem, whereas the translocation path phloem has ordinary companion cells. In many species, more than one type of companion cell can be observed, all being connected to their adjacent sieve elements by numerous plasmodesmata of a unique deltoid type (branched on the companion cell side).

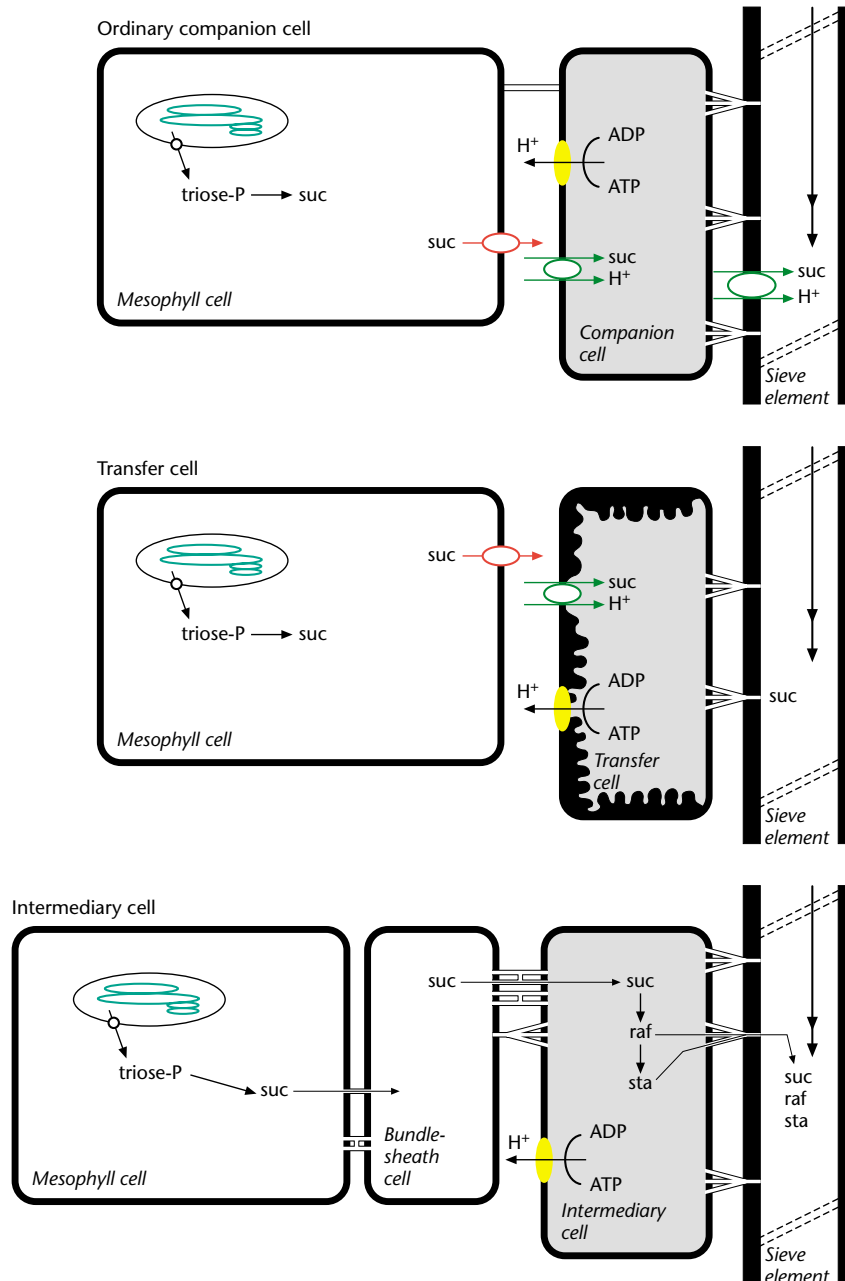
In gymnosperms, the functional equivalents to angiosperm companion cells are Strassburger cells, which are also characterized by dense cytoplasm, numerous mitochondria and well-developed endoplasmic reticulum. The ontogeny of these cells is less clear than that of angiosperm companion cells. The intercellular connections between Strassburger cells and the sieve elements originate from branched plasmodesmata on both cellular interfaces, forming a sieve pore on the sieve element side. Based on their position, Strassburger cells are considered to have similar functions to companion cells.

## Functions

### Transport functions

The dense cytoplasm of companion cells argues for a particularly active status, a situation to be expected if the companion cell also has to support the functions of its fully dependent partner, the sieve element. While there is a range of vein sizes in a leaf, phloem loading occurs primarily in

**Figure 1** Companion cells. Electron micrographs of leaf minor vein companion cells in different species. (a) Tomato (*Lycopersicon esculentum* Mill.). Minor veins from tomato are classified as type 2a with ordinary companion cells and few plasmodesmata at the interface between sieve element-companion cell complex and surrounding cells;  $\times 6000$  (b) Onion (*Allium cepa* L.). Type 2b species like onion have transfer cells with cell wall ingrowths (white arrowheads) and very few plasmodesmata;  $\times 6000$ . (c) Squash (*Cucurbita maxima*). Squash is characterized by intermediary cells showing fields of plasmodesmata (black arrowheads). Note small vacuoles and dense cytoplasm;  $\times 8000$ . BS, bundle-sheath cell; CC, companion cell; IC, intermediary cell; PP, phloem parenchyma; TC, transfer cell.



**Figure 2** Schematic representation of different transport mechanisms in the phloem in relation to the classes of companion cells. Depending on the density of plasmodesmata and the type of companion cell, respectively, assimilates are loaded into the phloem via apoplastic routes (upper and middle panels). In plants with intermediary cells, where plasmodesmata are frequent at the bundle-sheath cell–intermediary cell interface, assimilates are supposed to diffuse through plasmodesmata to the intermediary cell. Here, sucrose is transformed enzymatically to raffinose-type sugars, which are potentially too big to diffuse back via plasmodesmata. fru, fructose; glu, glucose; sta, stachyose; triose-P, triose phosphate; suc, sucrose.

minor veins. Companion cells are essential for this process by supplying energy (**Figure 2**). A finding supporting this role is that in minor veins companion cells are twice the diameter of the sieve element and represent the major phloem plasma membrane surface area, while in major

veins companion cells are smaller than sieve elements. This is consistent with loading in minor veins and long-distance translocation in major veins. Many genes are specifically expressed in phloem, serving as excellent tools for functional analyses (**Table 1**). While companion cells are

**Table 1** A list of some phloem-localized RNAs or proteins

Compound	Phloem	Companion cell	Sieve element	Phloem sap
Actin				Protein
Amino acid transporter (AtAAP2)	Promoter			
β-Amylase	Protein			
Aquaporin (SunTIP7)	RNA			
Aquaporin (ZmTIP1)	RNA	RNA		
Calreticulin	Promoter			
Cell wall invertase (wound-inducible)	RNA (wound area)			
CmNACP	RNA			RNA
Coconut foliar decay virus	Promoter			
<i>Commelina yellow mottle virus</i>	Promoter			
Cystatin				Protein
Endotransglycosylase (Bru1)	RNA			
Extensin	Protein/Promoter			
Farnesyltransferase	Promoter			
Flavonoid 3',5'-hydrolase	Protein (cotyledons)			
Galactinol synthase		Protein		
Glutamine synthetase1 (GS1)	RNA			
Glutaredoxin				Protein
Glyoxylase			Protein	
Homeobox protein (PttHB2)	RNA			
Hydroxyproline-rich glycoprotein (HRGP4.1)	Promoter			
Hydroxyproline-rich glycoprotein (SbHRGP3)	Promoter			
K <sup>+</sup> channel (AKT3)	RNA			
Leghaemoglobin (GLB3)	Promoter			
Lipoxygenase (LOX97)				Protein
Mannitol dehydrogenase (MTD)	Protein			
Patatin	Protein			
Peptide transporter (NaNTR1)	RNA			
Peroxidase (MsPxdD)	RNA			
Phaseolin	Promoter			
Phloem protein (CmPP1)	Protein	RNA/Protein	Protein	
Phloem protein (PP2)	Promoter			Protein
Phosphoenolpyruvate decarboxylase (ppcB)	Promoter			
Plasma membrane ATPase (PMA2)	Promoter			
Plasma membrane ATPase (PMA4)	Promoter	Promoter		
Profilin				Protein
Proline transporter (AtProT1)	RNA (flower)			
Proline-rich protein (SbPRP2)	RNA			
Putative cell wall protein (TED2)	RNA			
Δ <sup>1</sup> -pyrroline-5-carboxylase (P5CR)	RNA			
Ribonuclease-like pathogen-related protein (Ypr10)	Promoter			
RoIC	Promoter	Promoter		

*continued*

Table 1 – continued

Compound	Phloem	Companion cell	Sieve element	Phloem sap
S-adenosyl-L-methionine synthetase (SAM-S)	Promoter			
Sporamin	Promoter			
Stachyose synthase		Protein		
Sucrose binding protein		Protein		
Sucrose synthase (RSuS1)	Protein (roots)			
Sucrose synthase (RSuS2)	Protein (mesophyll)	Protein		
Sucrose transporter (AtSUC2)	Protein/Promoter	Protein		
Sucrose transporter (SUT1)	Promoter	RNA	RNA/Protein	RNA
Sucrose transporter (SUT2)	Promoter		Protein	
Sucrose transporter (SUT4)	Promoter		Protein	
Superoxide dismutase	Promoter			
Thioredoxin h (RPP13-1)		RNA	Protein	Protein
Tyrosine decarboxylase (TYDC)	Promoter/RNA			
$\beta$ -Tubulin (AtTUB8)	Promoter			
Ubiquitin	RNA			

similar ultrastructurally throughout the plant, molecular studies have begun to reveal distinct functional domains. Certain companion cell-specific promoters are exclusively expressed in companion cells of the loading zone (minor veins), such as the low-affinity sucrose transporter SUT4; others are present in the translocation phloem as well, while some are only active in phloem of sink organs (Weise *et al.*, 2000). Furthermore, functional differentiation seems to have occurred between the different companion cells within a vascular bundle and also between different bundles, i.e. abaxial, adaxial and extrafascicular phloem (Hirner *et al.*, 1998).

Companion cells provide the gateway for entry of materials into the phloem translocation system. Companion cells supply the energy for loading of nutrients into the phloem and, ultimately, the driving force for long-distance transport between organs. In this sense, an analogy can be made between companion cells and animal hearts (pumps), although the mechanism for generating a driving force is a complex chemiosmotic rather than a biomechanical process. Accumulation of assimilates and, in response, influx of water generates a pressure gradient powering mass flow of solution through the associated sieve tubes. Besides assimilates, the phloem also transports signals for communication between organs. Various phytohormones are found in phloem sap, as are systemic defence signals, such as salicylic acid or systemin, all of which either have to be generated in companion cells or to pass through them en route to the sieve elements. Recent data lead to the hypothesis that macromolecules such as RNA and proteins may act as signalling molecules affecting many processes, potentially representing signals such as the mysterious florigen (Ruiz-Medrano *et al.*, 1999).

In comparison to ordinary companion cells and transfer cells, intermediary cells are less well understood functionally, though they are found only in plants transporting raffinose-type sugars, such as cucurbits. Galactinol synthase, a key enzyme in synthesis of raffinose-series sugars, has been found in high levels in intermediary cells, providing a mechanism for synthesis of raffinose-type sugars directly adjacent to the sieve elements. Turgeon (1991) has proposed a polymer-trap hypothesis for the way intermediary cells function in phloem loading, which relies on differential size exclusion limits of plasmodesmata between the intermediary cell and adjacent mesophyll and sieve elements. This hypothesis proposes that sucrose enter intermediary cells from mesophyll cells via plasmodesmata, where, after addition of a galactinol moiety, it is trapped in the IC/SE complex because it is too large to diffuse back to the mesophyll (Figure 2). This allows for the observation of loading of the phloem with raffinose, seemingly against a concentration gradient.

## Macromolecular transport

Since mature sieve elements are devoid of nuclei, they have to be provided with proteins by companion cells to accommodate long-term function and turnover. Of particular relevance to this relationship is the recently emerging evidence for the capacity of plant cells to transport macromolecules, such as transcription factors, between each other. Companion cells seem to be even more sophisticated in that they are able to sustain the neighbouring sieve element by supplying a full set of mRNAs and proteins through the highly specialized plasmodesmata that connect the two cells. Plasmodesmata

permit the passage not only of sugars and amino acids, but also of macromolecules such as proteins or RNA molecules (Ruiz-Medrano *et al.*, 1999). Most of the proteins in **Table 1** have been shown to move using heterograft experiments. Proteins as large as 200 kDa and large RNAs are known to traffic through the deltoid plasmodesma, indicating sophisticated mechanisms that help the passage of such proteins. However, trafficking through plasmodesmata (not only those connecting companion cells to sieve elements but all the others as well) has to be regulated to maintain specialization of adjacent cells. It is hypothesized that, similarly to intracellular targeting, signals that are recognized by receptors selectively guide macromolecules to plasmodesmata and mediate transport through these pores, in principle similarly to type IV secretion systems between bacteria and hosts that allow macromolecular transport processes to occur (Baker *et al.*, 1997). This analogy is supported by observations that membrane proteins like the sucrose transporters SUT1, SUT2 and SUT4 are present on the plasma membrane of sieve elements (Kühn *et al.*, 1997; Barker *et al.*, 2000; Weise *et al.*, 2000). Since at least SUT1 turns over rapidly, it has to be synthesized constantly in companion cells (Kühn *et al.*, 1997). The mRNA of SUT1 is present at both orifices of the deltoid plasmodesma, suggesting that such membrane proteins are synthesized from mRNA produced in companion cells, guided to plasmodesmata in an untranslatable form, moved through the plasmodesmal channels and translated in sieve elements (Lalonde *et al.*, 1999).

## Functions in defence

The degree of plasmodesmal interconnection of companion cells to other cell types may also have a foundation in the fact that companion cells are highly important for defence reactions. Viruses spread from cell to cell via plasmodesmata, thus providing an ideal path for systemic infection if they reach the SE/CC complex. Plants are highly efficient in pathogen defence even in the absence of a classical immune system. One of the reasons is their capability to restrict infections; for example, by limiting the number of plasmodesmata or their permeability, companion cells can be envisaged as strategic players in defence reactions. If a virus infects a leaf, but cannot enter the phloem, significant spread is less likely, leading only to the loss of a single infected leaf. On the other hand, if virus enters through the phloem, e.g. via aphid stylets, a restriction in the ability to leave the phloem will prevent systemic spread. This process must have led to the evolution of defence and response mechanisms on the plant side to prevent movement and, on the viral side, the search for means to overcome plasmodesmata – first by acquiring ‘movement proteins’ allowing transport through plasmodesmata, later by overcoming barriers. Besides the movement of signals such as salicylic acid or systemin,

small double-stranded RNAs have been found that are crucial in blocking infection by means of PTGS/SAS (posttranscriptional gene silencing/systemic acquired gene silencing) (Hamilton and Baulcombe, 1999). Thus, companion cells represent a key cell type in the plant regarding defence as well as regulation of transport and partitioning of assimilates.

## Conclusion

Companion cells are highly specialized plant cells whose function is integrated with that of sieve elements by a truly unique relationship among biological systems. Companion cells are responsible for sustaining the living status of enucleate sieve elements over months to years, while assisting in the function of long-distance transport. All three types of companion cells play crucial roles in the long-distance transport of nutrients, morphogenetic factors and defence signals. In addition, they appear to be directly involved in some defence reactions (restriction of domains, synthesis of defence signalling agents) against intruders. Biochemical and molecular dissection of the companion cells will be necessary to fully understand the various functions of this cell type. An increasing collection of differentially expressed promoters represents the basis to modify metabolism and transport, to label the different classes of companion cells with green fluorescent protein and to study development, cell biology and molecular physiology using single cell sampling, array technology and electrophysiology, and to unravel the mechanisms of macromolecular trafficking.

## References

- Baker B, Zambryski P, Staskawicz B and Dinesh Kumar SP (1997) Signalling in plant–microbe interactions. *Science* **276**: 726–733.
- Barker L, Kühn C, Weise A *et al.* (2000) A sucrose transporter homolog in sieve elements is a candidate gene for QTLs controlling starch. *Plant Cell* **12**: 1153–1164.
- Chavan RR, Braggins JE and Harris PJ (2000) Companion cells in the secondary phloem of Indian dicotyledon species: a quantitative survey. *New Phytologist* **146**: 107–118.
- Gamalei Y (1989) Structure and function of leaf minor veins in trees and herbs. A taxonomic review. *Trees* **3**: 96–110.
- Hamilton AJ and Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **286**: 950–952.
- Hirner B, Fischer WN, Rentsch D, Kwart M and Frommer WB (1998) Developmental control of H<sup>+</sup>/amino acid permease gene expression during seed development of *Arabidopsis*. *Plant Journal* **14**: 535–544.
- Kühn C, Franceschi VR, Schulz A, Lemoine R and Frommer WB (1997) Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. *Science* **275**: 1298–1300.
- Lalonde S, Boles E, Hellmann H *et al.* (1999) The dual function of plant sugar carriers in transport and in nutrient sensing. *Plant Cell* **11**: 707–726.

- Ruiz-Medrano R, Xoconostle-Cázares B and Lucas WJ (1999) Phloem long-distance transport of *CmNACP* mRNA: implications for supracellular regulation in plants. *Development* **126**: 4405–4419.
- Turgeon R (1996) Phloem loading and plasmodesmata. *Trends in Plant Science* **1**: 418–423.
- Turgeon R (1991) Symplastic phloem loading and the sink-source transition in leaves. A model. In: Bonnemain JL, Delrot S, Lucas WJ and Dainty J (eds) *Recent Advances in Phloem Transport and Assimilate Compartmentation*, pp. 18–22. Nantes: Ouest Editions.
- Weise A, Barker L, Kühn C, *et al.* (2000) A new subfamily of sucrose transporters SUT4 with affinity/high capacity localised in enucleate sieve elements of plants. *Plant Cell* **12**: 1345–1355.
- Behnke HD and Sjolund RD (1990) *Sieve Elements: Comparative Structure Induction and Development*. Berlin: Springer-Verlag.
- Gunning BES and Pate JS (1972) Transfer cells. *Annual Review of Plant Physiology and Plant Molecular Biology* **23**: 173–196.
- Grusak MA, Beebe DU and Turgeon R (1996) Phloem loading. In: Zamski E and Scheaffer AA (eds) *Photoassimilate Distribution in Plants and Crop. Source-Sink Relationship*, pp. 209–227. New York: Marcel Dekker.
- Patrick JW (1997) Phloem unloading: sieve element unloading and post-sieve element transport. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 191–222.
- Schulz A (1998) Phloem. Structure related to function. *Progress in Botany* **59**: 429–475.
- Van Bel A (1993) Strategies of phloem loading. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**: 253–281.

## Further Reading

- Behnke HD (1989) Structure of the phloem. In: Baker DA and Milburn JA (eds) *Transport of Photoassimilates*, pp. 79–137. Harlow: Longman Scientific.