The Plant Cell, Vol. 11, 739–750, April 1999, www.plantcell.org © 1999 American Society of Plant Physiologists

Sieve Elements and Companion Cells—Traffic Control Centers of the Phloem

Karl J. Oparka[a,1] and Robert Turgeon[b]

[a] Unit of Cell Biology, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom
[b] Plant Biology, Cornell University, Ithaca, New York 14853

INTRODUCTION

Of all the intricate cell–cell interactions in nature, those between the sieve element (SE) and its companion cell (CC) rank among the most complex and mysterious. Mature SEs are enucleate and retain only a highly degenerate cytoplasm, yet they can remain viable and functional for decades (Parthasarathy and Tomlinson, 1967). Generations of school children have been taught that the CC "keeps the SE alive," an axiom that is only now, with the availability of modern techniques in cell and molecular biology, receiving experimental scrutiny.

We will review recent (and some old) evidence that the SE and CC do indeed interact intimately and that the SE-CC complex plays a central role in trafficking a wide range of endogenous and foreign macromolecules, as well as solutes, throughout the plant. A central tenet is that the SE-CC complex must function in several roles, including phloem loading and unloading, long-distance transport, and as a quality control center for monitoring the nature of substances passing into and out of the phloem. In many ways, the day-to-day problems faced by a SE-CC complex can be likened to a busy international airport through which numerous travelers (and their luggage) pass before embarking on short- and long-distance flights. We develop this analogy further in the following sections.

DESIGN AND CONSTRUCTION

The structure and development of SEs and CCs have been the subjects of numerous reviews (Esau, 1969; Parthasarathy, 1975; Cronshaw, 1981; Behnke and Sjolund, 1990) and are dealt with only briefly here. The concept of the SE-CC complex is an ontogenetic one; the two cells are derived from the unequal division of a "phloem mother cell" (Esau, 1969). The CC is not indispensable; for example, SEs in root protophloem (Esau and Gill, 1973; Eleftheriou, 1996) and thick-walled SEs in grass leaves (Evert et al., 1996) do not have accompanying CCs. In the latter case, the SEs persist throughout the life of the leaf. Although the cytoplasm of the CC, as shown in Figure 1, is usually dense, this is not always the case, and in leaves it is sometimes difficult to distinguish CCs from phloem parenchyma cells.

During differentiation, the SE undergoes a remarkable, although incomplete, autolysis, similar in many ways to programmed cell death (Esau, 1969; Oparka et al., 1981; Sjolund, 1997). This autophagic process is highly selective, degrading several of the SE organelles without affecting others, and the phenomenon has been studied extensively in files of root protophloem SEs. These cells differentiate in vertical files, with the most immature SEs forming close to the apical meristem. Because root protophloem SEs do not have accompanying CCs (Esau and Gill, 1973; Eleftheriou, 1996), the developmental program of the SE is clearly not dependent on the presence of the CC. During autolysis of the SE protoplast, the central vacuole breaks down so that cytoplasmic ribosomes, Golgi bodies, and the nucleus are degraded and eventually eliminated. Filamentous phloem-proteins (P-proteins), however, survive the autophagic process (Cronshaw and Esau, 1967; Esau, 1969). The endoplasmic reticulum is retained in the mature SE in a highly modified form consisting of a smooth anastomosing network of tubules and stacked cisternal aggregates, termed the sieve element reticulum (SER; Wooding, 1967). The SER, along with the plasma membrane, modified mitochondria, P-protein, and plastids, forms part of a functional system of organelles, termed the parietal layer, that is partially evident in Figure 2 (Esau, 1969).

Plasmodesmata connecting SEs across the end walls become greatly modified to form the sieve-plate pores (e.g., Behnke, 1989). The lateral (axial) plasmodesmata that connect the SE and CC are always branched on the CC side of the shared wall only (Figure 2) and have been termed pore-plasmodesma units (PPUs; Figure 2) (van Bel, 1996). In stem SE-CC complexes derived from the cambium, the cambial precursors that eventually give rise to the SE-CC complexes are symplastically connected to surrounding cells but with time become cut off by successive closure of plasmodesmata,

[1] To whom correspondence should be addressed. E-mail kopark@scri.sari.ac.uk; fax 44-1-382-562-426.

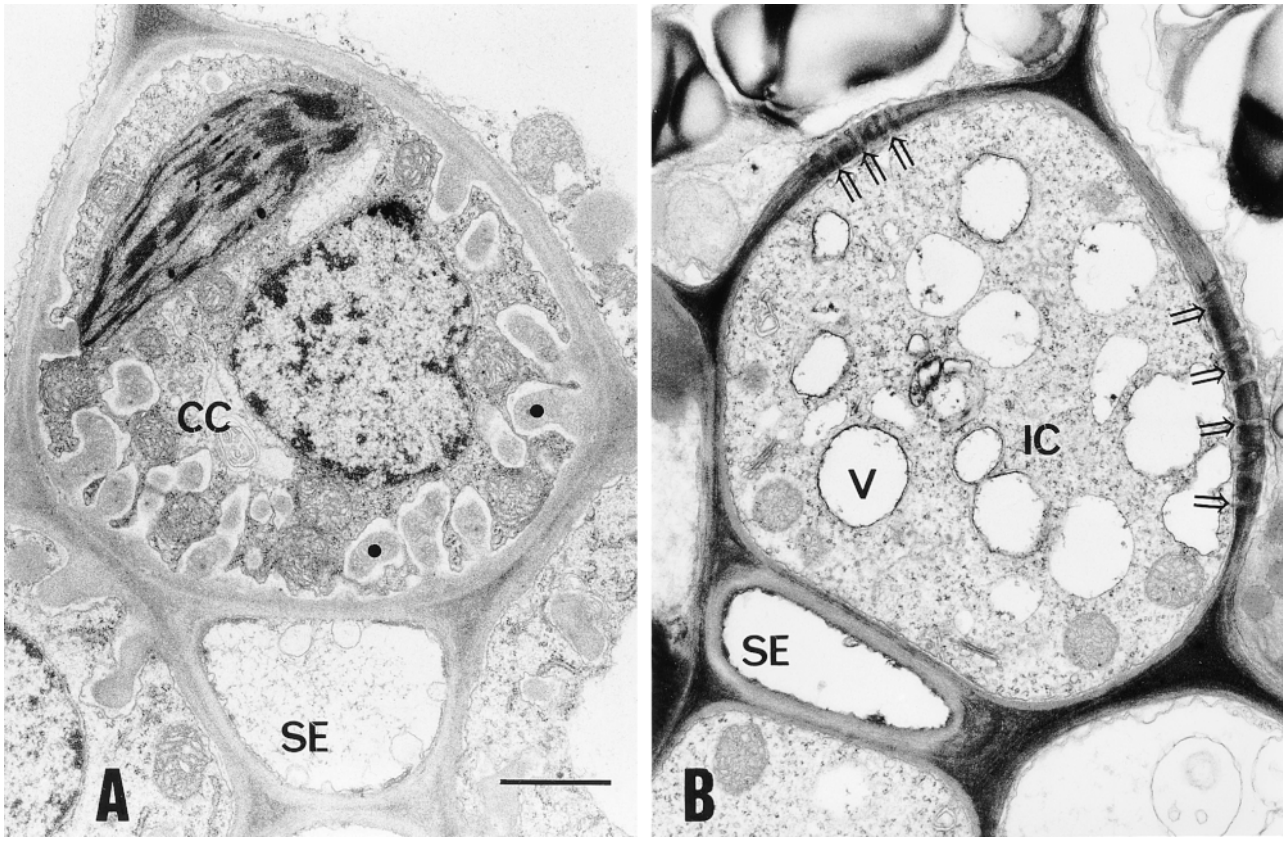

Figure 1. SE-CC Complexes in Minor Veins.

(A) Apoplastic loading configuration in *Zinnia elegans.* The CC possesses numerous wall ingrowths (filled circles) to facilitate apoplastic solute transfer. Bar = 1 μm.

(B) Symplastic loading configuration in *Cucurbita pepo.* The intermediary cell (IC) is connected to the bundle sheath cells by numerous plasmodesmata (arrows). V, vacuole. Same scale as in **(A)**. (Micrographs courtesy of S. Dimitrovska and A.J.E. van Bel).

retaining communication only between SE and CC (van Bel and van Rijen, 1994).

DEPARTURES: PHLOEM LOADING AT THE SE-CC COMPLEX

The structure of minor veins varies considerably with species, suggesting that the routes and mechanisms of solute transfer into the phloem of leaves are equally diverse (Gamalei, 1989; van Bel, 1993; Grusak et al., 1996; Komor et al., 1996; Turgeon, 1996). The final transport step into the SE-CC complex, either from the apoplast or the symplast, is commonly referred to as loading, a term that indicates use of energy to drive movement of sugar against a concentration gradient (Loomis, 1955). Both SEs and CCs in minor veins have solute potentials much higher than surrounding cell types (Geiger et al., 1973), an observation that provides a functional as well as a structural and ontogenetic basis for regarding the two cell types as a complex. Of course, plasmolysis does not identify solute species; elevated sugar concentrations in the SE-CC complexes of minor veins have only recently been measured directly (Haritatos et al., 1996).

In 1930, Münch formulated the now widely accepted pressure-flow theory of translocation. According to this concept, the sieve tube (a longitudinal series of SEs) functions as an impermeable pipe within which the mass flow of solutes is driven by a pressure gradient from regions of high solute concentration to regions of low concentration. Whereas loading is usually considered to be a universal phenomenon and integral to the pressure-flow mechanism, Münch (1930) did not include this step in his model. The loading concept came later, after a consensus arose that the solute content of the phloem is especially high.

At present, there are thought to be two types of phloem loading, apoplastic and symplastic. In apoplastic loading, sugar transport occurs actively into the SE-CC complex

from the extracellular space and involves membrane transporters located in the plasma membrane of the SE-CC complex. In symplastic loading, sugars are thought to enter the SE-CC directly via plasmodesmata (Turgeon, 1996). Because almost all the plasmodesmata joining the SE-CC complex to bordering cells lead into the CC rather than the SE (Botha and van Bel, 1992), connectivity to the CC provides the structural basis for tentatively assigning a plant to one category or the other (Gamalei, 1989). Species with few such plasmodesmata are thus putative apoplastic loaders, and in some of these plants the CCs are transfer cells (Figure 1A), that is, they have wall ingrowths that facilitate sucrose uptake from the apoplast (Wimmers and Turgeon, 1991). Most herbaceous species of temperate origin, including the majority of crop plants, are putative apoplastic loaders (Gamalei, 1991). Photoassimilate could enter the SE directly from the apoplast in these species; indeed, in corn, thin-walled sieve tubes accumulate radiolabeled photoassimilate without the CCs acting as intermediates (Fritz et al., 1983), and thick-walled sieve tubes have no associated CCs (Evert et al., 1996). Furthermore, the leaf sucrose transporter SUT1 locates to the plasma membrane of SEs but not to that of CCs (Kuhn et al., 1997; see also Lalonde et al., 1999, in this issue). On the other hand, there is strong evidence to suggest that CCs are involved in phloem loading in other systems. When morning glory and soybean leaves are pulse-labeled with $^{14}CO_2$, minor vein CCs are prominently labeled and the kinetics of ^{14}C exchange in CCs matches well with kinetics of ^{14}C-photoassimilate translocation to sinks (Fisher et al., 1978). Also, the SUC2 transporter is

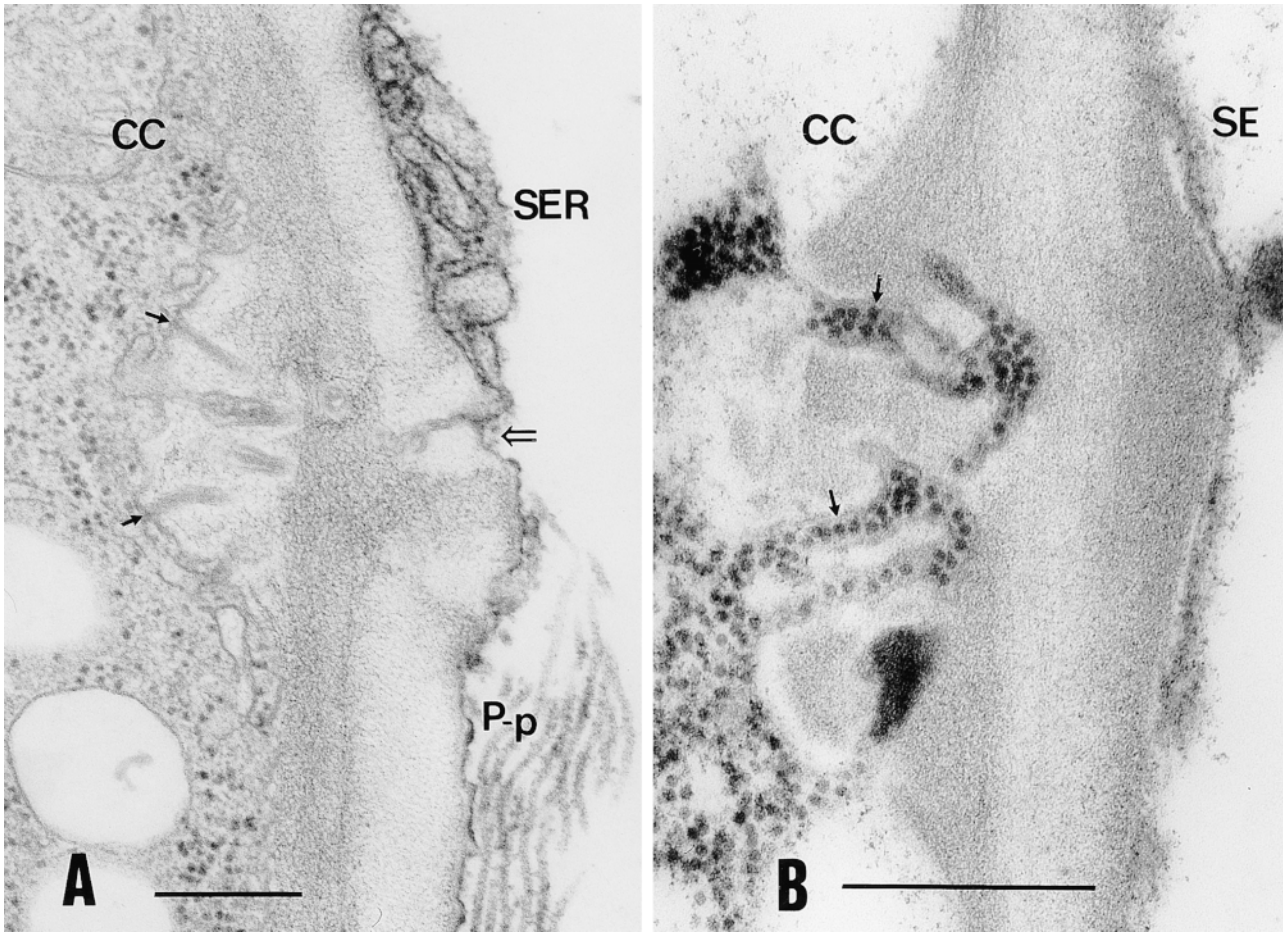

Figure 2. PPUs between CCs and SEs.

(A) The Arabidopsis plasmodesmal complex is characteristically branched in the CC wall (solid arrows), with a single pore opening into the SE lumen (open arrow). See text for details. Part of the SE parietal layer is also shown. P-p, P-protein. Bar = 250 nm.

(B) *Anthriscus cerefolium* infected with carrot red leaf virus. Icosahedral virus particles are present in the plasmodesmal branches of a PPU (arrows). The pore leading into the SE is not shown in this section. Bar = 250 nm. (Micrograph courtesy of I.M. Roberts).

located on the plasma membrane of CCs but not on that of SEs (Stadler et al., 1995), and the plasma membrane proton pump (H^+-ATPase) has been localized to the CC (DeWitt and Sussman, 1995).

In symplastic loaders such as the cucurbits, CCs are clearly involved in phloem loading because they translocate a high proportion of raffinose and stachyose, sugars that are made in specialized CCs known as intermediary cells (ICs; Figure 1B) (Turgeon et al., 1993). ICs are very large in relation to their SE. They have rudimentary plastids without thylakoids or starch, and many small vacuoles. The most distinguishing feature of intermediary cells, however, is the high abundance of plasmodesmata that join them to the bundle sheath (Turgeon, 1995; Figure 1B). These plasmodesmata are secondary in the sense that they are formed across already existing walls, just prior to the sink–source transition (Volk et al., 1996). They are highly branched, and the branches on the intermediary cell side are more numerous and more narrow that those on the bundle sheath side. Loading in all such species examined to date is insensitive to inhibition of the sucrose-proton co-transport carrier (Turgeon, 1996).

A "polymer trap" model, as schematized in Figure 3, has been put forward to explain phloem loading in species with ICs (Turgeon, 1991, 1996), and it has more recently been invoked to explain the loading of oligofructans in *Agave* (Wang and Nobel, 1998). This model holds that sucrose diffuses into ICs from the bundle sheath down a concentration gradient for use in the synthesis of raffinose family oligosaccharides (RFOs), which accumulate to high concentrations because they are too large to diffuse back to the bundle sheath. Indeed, recent studies have shown that raffinose and stachyose are present in high concentrations in ICs but are almost undetectable in bordering mesophyll. In this way, the sugars themselves act as probes of molecular size, indicating that diffusion of oligosaccharides does not take place through IC plasmodesmata (Haritatos et al., 1996).

It has been suggested that apoplastic phloem loading also takes place in plants with ICs, although direct evidence has been difficult to acquire. Interestingly, in squash tissue cultures, SEs accumulate solute but their associated CCs do not (Lackney and Sjolund, 1991). One interpretation of these data is that the SE-IC complex in squash leaves loads symplastically, via the IC, and apoplastically into the SE, with only the latter function expressed in vitro.

Although much effort has gone into studying the ultrastructure of minor veins and the mechanism(s) of phloem loading, the picture is not yet complete. For example, many plants appear to have "leaky" minor vein phloem, with many plasmodesmata joining CCs to the mesophyll, yet these species do not translocate RFOs or other polymers that could be trapped (Turgeon, 1995). How does loading occur under these circumstances? In willow, it does not; instead, the mesophyll acts as the "source," and sucrose diffuses into the SE-CC complex, just as Münch envisioned (Turgeon and Medville, 1998). It is not clear if this is a common phenomenon. It may be that other species are able to load actively, even though their phloem has extensive symplastic continuity with surrounding cells.

SPECIAL TRAVELERS

Solutes

Phloem sap is complex, containing a wide range of organic and inorganic substances (Ziegler, 1975; Komor et al., 1996) that, with some exceptions (Weiner et al., 1991), are also found in similar concentrations in surrounding cells (Riens et al., 1991; Winter et al., 1992). Many ions and molecules enter the sieve tubes in the loading zone from the xylem (Jeschke et al., 1991). Apparently, bidirectional solute exchange also takes place between CCs and SEs through the PPUs that connect them. Because these units have a large size exclusion limit (SEL), exchange is rapid, and fluorescent dyes flow rapidly into CCs when they are injected into sieve tubes (van Bel and Kempers, 1991; Oparka et al., 1992; Kempers and van Bel, 1997) or when they are translocated along the phloem (Oparka et al., 1994; Knoblauch and van Bel, 1998). It is not known if this solute exchange is regulated.

Proteins

Analysis of phloem exudate from a range of species has demonstrated the presence of numerous proteins with a molecular mass in the 20- to 60-kD range (see Sjolund, 1997). There is little doubt that some proteins synthesized in CCs are transferred via plasmodesmata to SEs (Fisher et al., 1992; Sakuth et al., 1993; Ishiwatari et al., 1995). For example, the mRNAs for two P-proteins in cucurbits are found for the most part, if not entirely, in CCs, whereas these proteins accumulate in the SEs (Clark et al., 1997; Dannenhoffer et al., 1997). The exact mechanism of intercellular protein transfer is unclear. Microinjection experiments have demonstrated that fluorescent dextrans in the range of 3 to 20 kD can pass freely through the PPUs, indicating the possibility of passive (diffusional) trafficking of macromolecules between SE and CC (Kempers et al., 1993; Kempers and van Bel, 1997). An example of such passive trafficking has recently been shown by Imlau et al. (1999). These authors made a transcriptional fusion between the Arabidopsis sucrose transporter promoter (AtSUC2), which is active only in CCs, and the *gfp* gene. The green fluorescent protein (GFP) synthesized in the CCs of source leaves entered the SEs and was translocated to sink regions of the plant. Furthermore, the GFP was unloaded in sink tissues, such as root tips, anthers, and developing leaves, and was transported subsequently through non-phloem tissues. These are watershed findings in that they indicate that plasmodesmata along the

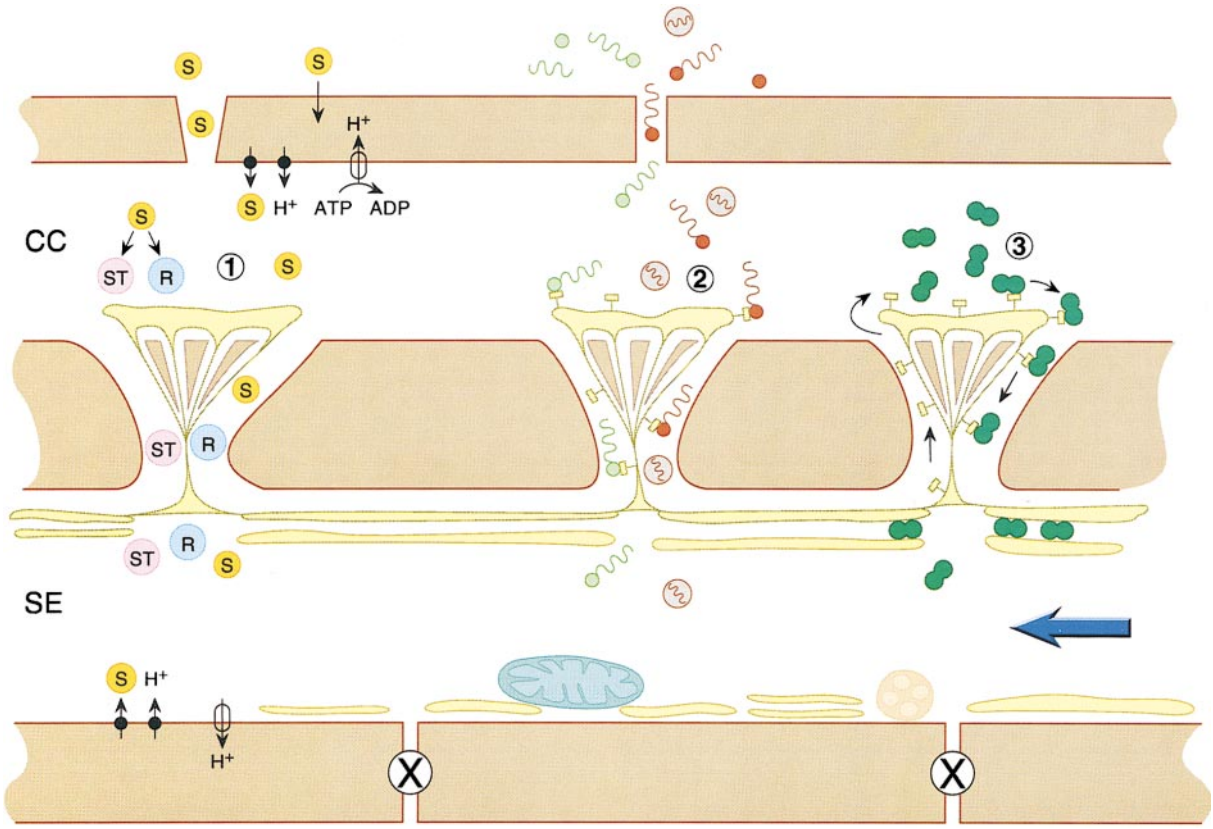

Figure 3. Schematic Model Depicting Molecular Trafficking between the CC and the SE.

(1) Solute loading. In symplastic loading, sucrose (S) enters the CC from the bundle sheath and is converted to raffinose (R) and stachyose (ST). These oligosaccharides are too large to diffuse back out to the bundle sheath, resulting in "polymer trapping," but can diffuse through the PPUs to enter the SE. In apoplastic loaders (to the right of 1), sucrose is actively pumped into the CC by specific carriers located on either the CC membrane (as shown) or the SE membrane. Plasmodesmata connecting the SE-CC complex with surrounding cells (encircled crosses) are held in a closed configuration by an energy-dependent mechanism.

(2) RNA trafficking. Endogenous RNA molecules originating in the mesophyll (shown in green) are trafficked by specific molecular chaperones (green circles) into the CC. At the PPUs, an interaction is envisaged between the chaperone and a receptor protein (yellow rectangle) located on the desmotubule running through the plasmodesmal pore. In the case of some systemic RNA viruses (shown in red), the viral RNA is trafficked into the CC by a specific viral MP (red circles). As in the case of endogenous RNA chaperones, the MPs interact with the desmotubule to facilitate RNA movement into the SE. Other systemic viruses may traffic across the PPUs as intact virions (gray circles), without the need for disassembly in the CC.

(3) Selective protein trafficking between CC and SE. Proteins (e.g., P-proteins [green circles]) synthesized within the CC and destined for the SE parietal layer are trafficked across the PPUs by an interaction with receptor proteins located on the desmotubule. Such receptors ensure that the proteins are delivered along the SER (yellow tubes) to their target sites without loss to the translocation stream. After delivery of the protein(s), the receptor proteins may be recycled back into the CC to collect further cargo (arrows). Other low molecular weight proteins, destined for export, may enter the SE from the CC by diffusion. Such proteins may not possess "retention signals" for the SE parietal layer and may be translocated and unloaded in sink regions of the plant.

post-phloem pathway in sink tissues may accommodate the transport of relatively large proteins. It is also possible that trafficking of specific proteins may not occur solely by diffusion through enlarged plasmodesmata. If small CC proteins were constantly and nonspecifically lost to the translocation stream, then extensive turnover of these proteins would be required to replenish constant losses to the SE. A second possibility is that many proteins are exchanged between SE and CC by specific molecular chaperones (i.e., supracellular control proteins) that interact with the newly synthesized CC proteins to direct their passage into SEs (Mezitt and Lucas, 1996).

What happens to proteins once inside the SE? Sjolund (1997) has pointed out that the velocity of translocation

within sieve tubes can easily exceed 40 cm/hr and has likened the contents of the SE lumen to a rapidly flowing river. If proteins entering the SE through branched plasmodesmata were simply dumped into the translocation stream, they would be swept away very rapidly to sinks. Some macromolecular signals might well be designed exactly for this fate (see below). However, it seems likely that at least some proteins are anchored. P-proteins are the most prominent proteins in the SEs of many species and have been widely assumed to occupy a parietal position in the cell (Evert, 1990), perhaps anchored by the lectin PP2 (Smith et al., 1987). However, recent evidence from interspecific grafts of *Cucurbita* spp indicates that at least some P-proteins are translocated along with photoassimilates (Golecki et al., 1998).

It is also possible that proteins trafficked across the branched plasmodesmata remain bound to the desmotubule during passage between SE and CC (Sjolund, 1997) and subsequently move in association with the SER (Figure 3). Because the SER may be continuous between adjacent SEs across the sieve-plate pores (Esau, 1969), the possibility exists for selective protein trafficking along individual sieve tubes. Recent evidence has shown that the movement protein (MP) of cucumber mosaic virus (CMV), fused to GFP, was targeted strongly to mesophyll plasmodesmata and also to the PPUs between the CC and SE. Upon entry into the SE, the MP trafficked along a reticular structure within the parietal layer (Blackman et al., 1998; see also Lazarowitz and Beachy, 1999, in this issue). The potential for selective proteins to "creep" along the blanket of the SER would provide a means of anchoring proteins not destined for immediate translocation as well as a mechanism for supplying the SE organelles with those proteins essential for maintenance and repair (Raven, 1991; see Figure 3). The sucrose transporter SUT1 is also transferred from its site of synthesis in CCs to the adjoining SE through plasmodesmata (Kuhn et al., 1997). The successful insertion of this protein into the plasma membrane of the SE clearly requires a targeting mechanism that delivers SUT1 to the plasma membrane without loss to the translocation stream (see Lalonde et al., 1999, in this issue).

RNA

The systemic movement of numerous plant RNA viruses is well documented, and different virus groups appear to employ different strategies for invading the phloem (Nelson and van Bel, 1998). To move systemically, the viral genome must pass first into the SE-CC complex (Carrington et al., 1996). This is generally assumed to occur in minor veins, but larger veins might also be targets. The exact form in which viruses enter the SE-CC complex is unclear. In the mesophyll, some viruses pass through plasmodesmata as a linear RNA complex that is trafficked by the specific viral MP (Gilbertson and Lucas, 1996; Nelson and van Bel, 1998; see also Lazarowitz and Beachy, 1999, in this issue). However, as ribonucleases have been detected in phloem exudates (Eschrich and Heyser, 1975), unprotected viral RNA in the translocation stream would probably be degraded. Viral RNA, therefore, is probably protected in transit by another viral protein, usually assumed to be the coat protein (CP; Gilbertson and Lucas, 1996). In the case of CMV, it appears that the viral RNA is trafficked through the PPUs as a ribonucleoprotein complex, not as a complete virion particle, with subsequent viral assembly occurring within membrane-bound complexes attached to the SER (Blackman et al., 1998). In contrast, intact virus particles of carrot red leaf virus have been found within the PPUs of minor-vein phloem (Murant and Roberts, 1979; Figure 2B). Inasmuch as these virions have a diameter of 30 nm, the SEL of the PPUs can clearly accommodate substantial macromolecular trafficking. A remarkable case of long-distance viral RNA trafficking involves the viroids, a group of pathogenic RNAs that have no protein coding capacity and whose entire genome consists of only 250 to 350 nucleotides (see Ding et al., 1997). Nonetheless, viroids appear to contain sequences or motifs that mediate cell–cell movement (Ding et al., 1997) so as to move systemically via the phloem (Palukaitis, 1987). It is not known whether viroids recruit a host "protection" protein prior to long-distance transport.

Some endogenous RNA molecules may also traffic through mesophyll plasmodesmata by using specific host proteins (see Mezitt and Lucas, 1996), and recent work indicates that endogenous plant RNAs may traffic between CCs and SEs. In the case of the sucrose transporter protein SUT1, antisense inhibition of *SUT1* expression under control of a CC-specific promoter shows that *SUT1* mRNA is synthesized specifically within the CC (Kuhn et al., 1997). Nevertheless, in situ hybridization localizes the *SUT1* mRNA mainly to the parietal layer of SEs, preferentially associated with the SE-CC plasmodesmata. Kuhn et al. (1997) have suggested that the *SUT1* mRNA must be translated in the SE. However, this suggestion contradicts the commonly accepted view that ribosomes are absent from mature SEs (Esau, 1969; Sjolund and Shih, 1983; Fisher et al., 1992). Thus, the reason for *SUT1* mRNA entry into the SE at present remains obscure. One possibility is that the mRNA is trafficked into the SE as a ribonucleoprotein complex destined for long-distance transport (Ishiwatari et al., 1998). There have been a number of recent reports that endogenous mRNAs may traffic over long distances through the phloem (Sasaki et al., 1998; Xoconostle-Cazares et al., 1999). A phloem protein from squash (*Cucurbita maxima*), termed CmPP16, has been found to mediate the transport of RNA into the phloem and is also able to move in the translocation stream (Xoconostle-Cazares et al., 1999). The function of such proteins is intriguing because they may be able to mediate the long-distance transport of mRNAs and conceivably their post-phloem delivery to sink tissues. The reports of RNA-trafficking proteins have coincided with the recent discovery that a virus-encoded protein from the umbravirus, groundnut rosette virus, can facilitate the long-distance movement of heterologous viral RNA

(Ryabov et al., 1999). It remains to be shown whether this viral protein can also traffic host mRNAs over long distances. However, it is possible that some systemic viruses may have "hijacked" one or more of the host's endogenous long-distance transport proteins during the course of viral evolution.

Experiments on the phenomenon of cosuppression, in which a transgene "silences" not only its own expression but also that of similar endogenous genes (Metzlaff et al., 1997; Palauqui et al., 1997), demonstrate that gene silencing can be transmitted between cells and over long distances in the plant. Grafting experiments, in which a nonsuppressed scion was grafted onto a cosuppressed stock, showed that a signal for cosuppression was transmitted through the graft union over considerable distance to induce cosuppression in the scion (Palauqui et al., 1997). The grafting experiments point to the existence of a gene-specific, mobile signal that travels through the sieve tubes and from these cells to surrounding tissues. Jorgensen et al. (1998) have speculated that a likely candidate for the cosuppression signal is an RNA molecule, possibly copy RNA (cRNA), produced from sense transcripts. Such RNA molecules would have to pass through the SE-CC plasmodesmata, whereby RNA entry into the SE may be mediated by specific host proteins (surveillance translocation proteins; Jorgensen et al., 1998) in the form of ribonucleoprotein complexes (Figure 3). It seems likely that such proteins may also play a role in protection of the RNA during transit. Because plant RNA viruses can be both targets and triggers of cosuppresion, Jorgensen et al. (1998) suggest that a primary function of cosuppression is to destroy viral RNA-associated transcripts when they are expressed at excessive levels. The systemic spread of a cosuppressing signal, via sieve tubes, may be one of a number of evolutionary responses to RNA viruses, allowing the plant to identify, track, and destroy viral RNA in a sequence-specific manner.

Electrical Wound Signals

In situations in which rapid, systemic signaling responses are required, the generation of electrical signals via the SE-CC complex could be potentially effective. Indeed, the lateral symplastic isolation of the SE-CC complexes makes them ideally suited for this purpose (van Bel and van Rijen, 1994). Recently, Rhodes et al. (1996) inserted microelectrodes into a range of cells in tomato petioles and monitored specific cell types with the use of Lucifer Yellow. The wounding of cotyledons, by heat, induced both a traveling electrical signal and systemic proteinase inhibitor activity. Significantly, only SEs and CCs produced large action-potential-like depolarizations upon wounding, providing strong evidence that the SE-CC complex represents a circuit along which electrical signals are propagated. Wound-induced electrical events in plants generally fall into two categories: rapid transients of putative action potentials, and slower transients of variable shape (Julien and Frachisse, 1992). Pickard (1973) has suggested that the rapid transients, as shown to occur within SE-CC complexes, are the true long-distance electrical signals, whereas the slow waves represent the lateral electrical consequences of chemical signals moving in the xylem. The need for electrical signal conduction may have been the evolutionary basis for establishing plasmodesmal links of SE-CC complexes to surrounding cells in minor veins, even in apoplastic loading species, despite the danger that such links pose as a route for systemic invasion by viruses.

TURBULENCE AND DECOMPRESSION: SENSITIVITY OF THE SE-CC COMPLEX TO DAMAGE

The monumental review by Esau (1969) is replete with examples of structural artifacts induced by wounding the phloem. The basis of this sensitivity lies in the enormously high turgor pressures generated within the SE-CC complex. Release of this pressure by cutting the phloem results in rapid displacement of sieve-tube contents toward the cut surface, inducing a range of "surge" artifacts and the deposition of callose over the sieve-plate pores (Eschrich, 1975). Using confocal laser scanning microscopy, Knoblauch and van Bel (1998) examined the transport of the fluorescent dye carboxyfluorescein (CF) through functioning SEs in broad bean petioles. Longitudinal movement through the SEs was accompanied by rapid exchange of the dye with the adjoining CCs but not with other cells, consistent with current models for the isolation of SE-CC complexes in the transport phloem (see van Bel, 1996). Upon controlled wounding with a microelectrode, peripheral P-protein complexes became dispersed, plugging the sieve plates and extending through the pores to form the "slime strands" so carefully drawn by early students of the phloem (see Esau, 1969). Starch grains were concomitantly released from plastids, contributing to the occlusion of the sieve plate. Such wound events result in the preclusion of CF (molecular mass of 356 kD) movement from undamaged to damaged SE. Significantly, it was shown that the cause of disturbance was not impalement per se but rather the release of SE turgor.

In addition to the dislocation of SE contents toward the wound surface, it is conceivable that some solutes and also macromolecules are displaced from the CC into the SE as a result of the rapid pressure drop within the SE. Unlike sieve-plate pores, the PPUs that connect the SE with the CC do not appear to have an effective wound-sealing mechanism (Kempers et al., 1993; Kempers and van Bel, 1997). Given that the SE-CC plasmodesmata appear to have a large SEL, small proteins might be pulled into the SE upon pressure release in much the same way that loose luggage might be sucked through the broken window of a decompressed jumbo jet. It is usually assumed that exudate derived from aphid stylets represents natural sieve-tube sap in transit; however, it should be considered that solute may be drawn

from neighboring cells over a period of time, beginning when pressure is released by insertion of the stylet. These considerations also have important implications for in situ localization studies of proteins and RNA within the SE-CC complex. For example, Lehmann (1973) showed histochemically that dehydrogenase activity moved into the SE when the phloem was severed unless the tissue was first frozen, in which case dehydrogenase activity remained exclusively in the CC. Despite the sophistication of current immunocytochemical techniques, such dislocation artifacts remain a risk when preparing the SE-CC complex for microscopy.

MAKING CONNECTIONS: LATERAL SOLUTE EXCHANGE AT THE SE-CC COMPLEX

In the Münch (1930) pressure-flow model, SEs were regarded as impermeable pipes from which the lateral leakage of solutes could not occur. It is now accepted that the lateral transport of photosynthate from the transport SE-CC complexes is substantial (Hayes et al., 1987; Minchin and Thorpe, 1987) and necessary to supply the growth of sink tissues (e.g., the cambium) around the transport pathway (van Bel, 1996). The SE-CC complexes of the transport phloem must therefore negotiate seemingly antagonistic functions (van Bel, 1993). Specifically, photosynthates and pressure gradients must be retained within the SEs to supply terminal sinks, and at the same time the surrounding cells must be provisioned. The precise control between release and retrieval is probably the function of specific carriers and pumps located on the CC plasma membrane (DeWitt and Sussman, 1995; see also Lalonde et al., 1999, in this issue). Regulated "pump-and-leak" mechanisms would benefit from the symplastic isolation of the transport phloem, provided that the relatively few plasmodesmata around the SE-CC complex are either nonfunctional or held in a closed state. Support for the latter condition comes from a recent study in which metabolic inhibitors were applied to intact Arabidopsis roots that had been allowed to translocate CF (Wright and Oparka, 1997). After application of the inhibitors, the dye symplastically leaked out of the SE-CC complexes into surrounding cells, indicating that the plasmodesmata are normally held shut by an energy-dependent process.

ARRIVALS: UNLOADING AT THE SE-CC COMPLEX

In sink tissue, solutes and proteins need to be processed as they arrive. If unloading from the SE-CC complex into surrounding cells were strictly apoplastic, transfer would have to be membrane-mediated and selective for a wide variety of compounds and ions. Indeed, symplastic phloem unloading seems to be more common (Fisher and Oparka, 1996). For example, in the case of seeds, photoassimilate unloads symplastically into cells of the maternal tissue and travels through several cell layers before entering the seed apoplast (Oparka and Gates, 1981; Patrick and Offler, 1995). Thus, the maternal seed tissue may be a processing center, receiving and metabolizing protein and solute that enter it symplastically from the phloem, and releasing into the apoplast only those specific compounds needed by the growing seed.

Recent studies of viruses tagged with GFP have provided evidence that the initial escape of virus in sink leaves mirrors that of phloem-delivered solutes. Potato virus X (PVX), expressing a CP–GFP fusion, was shown to escape predominantly from the SE-CC complexes of class III veins in importing sink leaves of *Nicotiana benthamiana*, the same sites at which symplastic unloading of the dye CF was demonstrated (Roberts et al., 1997). Further experiments, in which transgenic plants expressing the viral CP of PVX were grafted with nontransgenic scions, showed that the movement of CP-defective viral mutants was rescued on the transgenic rootstock and that the CP was translocated across the graft union into sink leaves of the scion. In class III veins, the translocated CP was able to rescue viral cell–cell movement out of the SE-CC complexes, but only to a limited extent, effectively "freezing" the viral escape pattern (Santa Cruz et al., 1998). Recent experiments, designed to examine the phenomenon of systemic acquired signaling, have shown that gene silencing in *N. tabacum*, seen as chlorosis, is first observed in scion tissues close to class III veins (H. Vaucheret, personal communication). Thus, both plant viruses and endogenous systemic silencing signals appear to follow the translocation stream to sites of phloem unloading.

PASSPORT CONTROL: PLASMODESMATA AROUND THE SE-CC COMPLEX

The efflux of large proteins, RNA, or ribonucleoprotein complexes via the CC is likely to involve modification of the plasmodesmata that connect the SE-CC with surrounding parenchyma elements. Whether all macromolecules that function as long-distance signals leave the SE-CC complex remains to be demonstrated. Mezitt and Lucas (1996) have suggested that flowering signals, translocated through the SE-CC complexes, may act directly on CCs to elicit the production of a second short-distance signaling system outside the phloem. This latter system may involve a ribonucleoprotein complex that propagates a secondary signal that travels to the central zone of the meristem.

Curiously, a number of the proteins that have been isolated from sieve tube exudates such as thioredoxin h (Ishiwatari et al., 1998) and P-proteins (Balachandran et al., 1998) have been shown to possess the ability to traffic between tobacco mesophyll cells and to increase the SEL of these cells to ~20 kD. These proteins are normally located

exclusively within the SE-CC complexes (Smith et al., 1987; Ishiwatari et al., 1995; Clark et al., 1997); thus, their ability to traffic between mesophyll cells at first appears strange. One possibility is that when injected into the mesophyll, these proteins re-create their normal behavior when trafficking across the PPUs that connect CC and SE. It appears, however, that they cannot modify the plasmodesmata that connect the SE-CC complex with surrounding cells; otherwise, they would constantly escape from the phloem and presumably mediate the release of other solutes and proteins held within the SE-CC complex. This raises the interesting prospect that the plasmodesmata that surround the SE-CC complex may not be modified readily by endogenous movement factors, thus preventing the escape of key proteins from the SE-CC complex to the mesophyll. Looked at from a different point of view, these plasmodesmata may also function to prevent the invasion of the phloem by viruses that are present in the mesophyll (Leisner and Turgeon, 1993). To use a further airport analogy, the same "passports" (MPs or other endogenous chaperones) that allow passage through mesophyll plasmodesmata may not be valid on those that surround the SE-CC complex. Only those viruses or systemic macromolecules with a "security pass" (MP or chaperone able to modify both mesophyll and SE-CC plasmodesmata) will be able to enter the SE-CC complex. From the plant's point of view, it is absolutely essential to have a stringent security control around the SE-CC complex, both to prevent "ground staff" from leaving and to prevent "illegal aliens" from entering.

During the course of evolution, it seems likely that the phloem of many species had to face a trade-off between maintaining essential intercellular (electrical?) communication with the mesophyll, while at the same time providing an effective means of loading solutes and retaining them within the SE-CC complex. In species with an apoplastic loading configuration, this dilemma appears to have been resolved by reducing the numbers of plasmodesmata around the SE-CC complex and, in parallel, by imposing regulatory control over the plasmodesmata that remain. In the case of viruses that evolved MPs for intercellular passage through plasmodesmata, these plasmodesmata represent the Achilles heel of the SE-CC complex. To ensure essential intercellular communication between the mesophyll and SE-CC complex, a family of key chaperones may have evolved to carry several unique proteins and signals across this crucial boundary. Recent data on the pattern of infection of minor veins of apoplastic loaders (Fabaceae) by various viruses suggest that some viruses exploit the plasmodesmata between SE and vascular parenchyma elements to gain access to the phloem, rather than entering the CCs directly (Ding et al., 1998). In the case of species with a symplastic loading configuration, in which many plasmodesmata connect the IC with the bundle sheath (see Turgeon, 1996), the SE-CC complex appears to be potentially exposed to symplastic attack by pathogens. To prevent this from occurring, emphasis appears to have been placed on the tight regulation of these plasmodesmata rather than on reduction of plasmodesmal numbers.

PERSPECTIVES

A clear challenge for the future is to identify those macromolecules that are capable of crossing the plasmodesmata around the SE-CC complex to gain access to the plant's long-distance translocation pathway and, in particular, to identify the host chaperone proteins that are responsible for mediating their passage across these plasmodesmata. The use of CC-specific promoters, such as that of the *SUC2* gene (Imlau et al., 1999), to generate the expression GFP-tagged proteins within the phloem may provide an important experimental tool with which to follow the fate of different macromolecules along the translocation stream. Such studies could resolve many of the long-standing puzzles concerning macromolecular signaling via the phloem.

ACKNOWLEDGMENTS

We are grateful to Aart van Bel, Don Fisher, Simon Santa Cruz, Peter Palukaitis, and Edith Haritatos for helpful comments during the preparation of the manuscript. We also thank S. Dimitrovska and A.J.E. van Bel for supplying Figure 1 and I.M. Roberts for supplying Figure 2B.

REFERENCES

Balachandran, S., Xiang, Y., Schobert, C., Thomson, G.A., and Lucas, W.J. (1998). Phloem sap proteins from *Cucurbita maxima* and *Ricinus communis* have the capacity to traffic cell to cell through plasmodesmata. Proc. Natl. Acad. Sci. USA **94,** 14150–14155.

Behnke, H.-D. (1989). Structure of the phloem. In Transport of Photoassimilates, D.A. Baker and J.A. Milburn, eds (Harlow, UK: Longman), pp. 79–137.

Behnke, H.-D., and Sjolund, R.D. (1990). Sieve Elements: Comparative Structure, Induction and Development. (Berlin: Springer-Verlag).

Blackman, L.M., Boevink, P., Santa Cruz, S., Palukaitis, P., and Oparka, K.J. (1998). The movement protein of cucumber mosaic virus traffics into sieve elements in minor veins of *Nicotiana clevelandii.* Plant Cell **10,** 525–537.

Botha, C.E.J., and van Bel, A.J.E. (1992). Quantification of symplastic continuity as visualized by plasmodesmograms. Diagnostic value for phloem-loading pathways. Planta **187,** 359–366.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K., and Schaad, M.C. (1996). Cell-to-cell and long-distance transport of viruses in plants. Plant Cell **8,** 1669–1681.

Clark, A.M., Jacobsen, K.R., Bostwick, D.E., Dannenhoffer, J.M., Skaggs, M.I., and Thompson, G.A. (1997). Molecular characterization of a phloem-specific gene encoding the filament protein, phloem protein 1 (PP1), from *Cucurbita maxima.* Plant J. **12,** 49–61.

Cronshaw, J. (1981). Phloem structure and function. Annu. Rev. Plant Physiol. **32,** 465–484.

Cronshaw, J., and Esau, K. (1967). Tubular and fibrillar components of mature and differentiating sieve elements. J. Ultrastruct. Res. **34,** 244–259.

Dannenhoffer, J.M., Schulz, A., Skaggs, M.I., Bostwick, D.E., and Thompson, G.A. (1997). Expression of the phloem lectin is developmentally linked to vascular differentiation in cucurbits. Planta **201,** 405–414.

DeWitt, N.D., and Sussman, M.R. (1995). Immunocytological localization of an epitope-tagged plasma membrane proton pump (H^+-ATPase) in phloem companion cells. Plant Cell **7,** 2053–2067.

Ding, B., Kwon, M.-O., Hammond, R., and Owens, R. (1997). Cell-to-cell movement of potato spindle tuber viroid. Plant J. **12,** 931–936.

Ding, X.S., Carter, C., Deom, M.C., and Nelson, R.S. (1998). Tobamovirus and potyvirus accumulation in minor veins of inoculated leaves from representatives of the Solanaceae and Fabaceae. Plant Physiol. **116,** 125–136.

Eleftheriou, E.P. (1996). Developmental features of protophloem sieve elements in roots of wheat (*Triticum aestivum* L). Protoplasma **193,** 204–212.

Esau, K. (1969). The Phloem. Handbuch der Pflanzenanatomie, Vol. 5, no. 2, W. Zimmermann, P. Ozenda, and H.D. Wulff, eds (Berlin: Gebruder Borntraeger).

Esau, K., and Gill, R.H. (1973). Correlations in differentiation of protophloem sieve elements of *Allium cepa* root. J. Ultrastruct. Res. **44,** 310–328.

Eschrich, W. (1975). Sealing systems in phloem. In Transport in Plants. I. Phloem Transport, Encyclopedia of Plant Physiology, M.H. Zimmermann and J.A. Milburn, eds (Berlin: Springer-Verlag).

Eschrich, W., and Heyser, W. (1975). Biochemistry of phloem constituents. In Transport in Plants. I. Phloem Transport, Encyclopedia of Plant Physiology, M.H. Zimmermann and J.A. Milburn, eds (Berlin: Springer-Verlag).

Evert, R.F. (1990). Dicotyledons. In Sieve Elements, Comparative Structure, Induction and Development, H.-D. Behnke and R.D. Sjolund, eds (Berlin: Springer-Verlag), pp. 103–137.

Evert, R.F., Russin, W.A., and Botha, C.E.J. (1996). Distribution and frequency of plasmodesmata in relation to photoassimilate pathways and phloem loading in the barley leaf. Planta **198,** 572–579.

Fisher, D.B., and Oparka, K.J. (1996). Post-phloem transport: Principles and problems. J. Exp. Bot. **47,** 1141–1154.

Fisher, D.B., Housley, T.L., and Christy, A.L. (1978). Source pool kinetics for ^{14}C-photosynthate translocation in morning glory and soybean. Plant Physiol. **61,** 291–295.

Fisher, D.B., Wu, Y., and Ku, M.S.B. (1992). Turnover of soluble proteins in the wheat sieve tube. Plant Physiol. **100,** 1433–1441.

Fritz, E., Evert, R.F., and Heyser, W. (1983). Microautoradiographic studies of phloem loading and transport in the leaf of *Zea mays* L. Planta **159,** 193–206.

Gamalei, Y. (1989). Structure and function of leaf minor veins in trees and herbs. A taxonomic review. Trees **3,** 96–110.

Gamalei, Y. (1991). Phloem loading and its development related to plant evolution from trees to herbs. Trees **5,** 50–64.

Geiger, D.R., Giaquinta, R.T., Sovonick, S.A., and Fellows, R.J. (1973). Solute distribution in sugar beet leaves in relation to phloem loading and translocation. Plant Physiol. **52,** 585–589.

Gilbertson, R.L., and Lucas, W.J. (1996). How do viruses traffic on the 'vascular highway'? Trends Plant Sci. **1,** 260–267.

Golecki, B., Schulz, A., Carstens-Behrens, U., and Kollmann, R. (1998). Evidence for graft transmission of structural phloem proteins or their precursors in heterographs of Cucurbitaceae. Planta **206,** 630–640.

Grusak, M.A., Beebe, D.U., and Turgeon, R. (1996). Phloem loading. In Photoassimilate Distribution in Plants and Crops: Source–Sink Relationships, E. Zamski and A.A. Schaffer, eds (New York: Marcel Dekker), pp. 209–227.

Haritatos, E., Keller, F., and Turgeon, R. (1996). Raffinose oligosaccharide concentrations measured in individual cell and tissue types in *Cucumis melo* L. leaves: Implications for phloem loading. Planta **198,** 614–622.

Hayes, P.M., Patrick, J.W., and Offler, C.E. (1987). The cellular pathway of radial transfer of photosynthates in stems of *Phaseolus vulgaris* L.: Effects of cellular plasmolysis and *p*-chloromercuribenzenesulphonic acid. Ann. Bot. **59,** 635–642.

Imlau, A., Truernit, E., and Sauer, N. (1999). Cell-to-cell and long-distance trafficking of the green fluorescent protein in the phloem and symplastic unloading of the protein into sink tissues. Plant Cell **11,** 309–322.

Ishiwatari, Y., Honda, C., Kawashima, I., Nakamura, S., Hirano, H., Mori, S., Fujiwara, T., Hayashi, H., and Chino, M. (1995). Thioredoxin h is one of the major proteins in rice phloem sap. Planta **195,** 456–463.

Ishiwatari, Y., Fujiwara, T., McFarland, K.C., Nemoto, K., Hayashi, H., Chino, M., and Lucas, W.J. (1998). Rice phloem thioredoxin h has the capacity to mediate its own cell-to-cell transport through plasmodesmata. Planta **205,** 12–22.

Jeschke, W.D., Wolf, O., and Pate, J.S. (1991). Solute exchanges from xylem to phloem in the leaf and from the phloem to the xylem in the root. In Recent Advances in Phloem Transport and Assimilate Compartmentation, J.-L. Bonnemain, S. Delrot, W.J. Lucas, and J. Dainty, eds (Nantes, France: Ouest Editions), pp. 96–105.

Jorgensen, R.A., Atkinson, R.G., Forster, R.L., and Lucas, W.J. (1998). An RNA-based information superhighway in plants. Science **279,** 1486–1487.

Julien, J.L., and Frachisse, J.M. (1992). Involvement of the proton pump and proton conductance change in the wave of depolarization induced by wounding in *Bidens pilosa.* Can. J. Bot. **70,** 1451–1458.

Kempers, R., and van Bel, A.J.E. (1997). Symplasmic connections between sieve element and companion cell in the stem phloem of *Vicia faba* L. have a molecular exclusion limit of at least 10 kD. Planta **201,** 195–201.

Kempers, R., Prior, D.A.M., van Bel, A.J.E., and Oparka, K.J. (1993). Plasmodesmata between sieve elements and companion cells of extrafascicular stem phloem of *Cucurbita maxima* permit passage of 3 kD fluorescent probes. Plant J. **4,** 567–575.

Knoblauch, M., and van Bel, A.J.E. (1998). Sieve tubes in action. Plant Cell **10,** 35–50.

Komor, E., Orlich, G., Weig, A., and Kockenberger, W. (1996). Phloem loading—Not metaphysical, only complex: Toward a unified model of phloem loading. J. Exp. Bot. **47,** 1115–1164.

Kuhn, C., Franceschi, V.R., Schulz, A., Lemoine, R., and Frommer, W.B. (1997). Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleated sieve elements. Science **275,** 1298–1300.

Lackney, V.K., and Sjolund, R.D. (1991). Solute concentrations of the phloem and parenchyma cells present in squash callus. Plant Cell Environ. **14,** 213–220.

Lalonde, S., Boles, E., Hellmann, H., Barker, L., Patrick, J.W., Frommer, W.B., and Ward, J.M. (1999). The dual function of sugar carriers: Transport and sugar sensing. Plant Cell **11,** 707–726.

Lazarowitz, S.G., and Beachy, R.N. (1999). Viral movement proteins as probes for intracellular and intercellular trafficking in plants. Plant Cell **11,** 535–548.

Lehmann, J. (1973). Zur lokalisation von deydrogenasen des energiestoffwechsels im phloem von *Cucurbita maxima* L. Planta **111,** 187–198.

Leisner, S.M., and Turgeon, R. (1993). Movement of virus and photoassimilate in the phloem: A comparative analysis. Bioessays **15,** 741–748.

Loomis, W.E. (1955). Resistance of plants to herbicides. In Origin of Resistance to Toxic Agents, M.G. Sevag, R.D. Reid, and O.E. Reynolds, eds (New York: Academic Press), pp. 99–121.

Metzlaff, M., O'Dell, M., Cluster, P.D., and Flavell, R.B. (1997). RNA-mediated RNA degradation and chalcone synthase A silencing in *Petunia.* Cell **88,** 845–854.

Mezitt, L.A., and Lucas, W.J. (1996). Plasmodesmal cell-to-cell transport of proteins and nucleic acids. Plant Mol. Biol. **32,** 251–273.

Minchin, P.E.H., and Thorpe, M.R. (1987). Measurement of unloading and reloading of photo-assimilate within the stem of bean. J. Exp. Bot. **38,** 211–220.

Münch, E. (1930). Die Stoffbewegungen in der Pflanze. (Jena, Germany: G. Fischer).

Murant, A.F., and Roberts, I.M. (1979). Virus-like particles in phloem tissue of chervil (*Anthriscus cerefolium*) infected with carrot red leaf virus. Ann. Appl. Biol. **92,** 343–346.

Nelson, R.S., and van Bel, A.J.E. (1998). The mystery of virus trafficking into, through and out of the vascular tissue. Prog. Bot. **59,** 476–533.

Oparka, K.J., and Gates, P. (1981). Transport of assimilates in the developing caryopsis of rice (*Oryza sativa* L). The pathways of water and assimilated carbon. Planta **152,** 388–396.

Oparka, K.J., Johnson, R.P.C., and Bowen, I.D. (1981). Sites of acid phosphatase in the differentiating root protophloem of *Nymphoides peltata* (S.G. Gmel.) O. Kuntze: Support for the role of stacked ER in sieve-element autolysis. Plant Cell Environ. **4,** 27–36.

Oparka, K.J., Viola, R., Wright, K.M., and Prior, D.A.M. (1992). Sugar transport and metabolism in the potato tuber. In Carbon Partitioning within and between Organisms, C.J. Pollock, J.F. Farrar, and A.J. Gordon, eds (Oxford, UK: BIOS), pp. 91–114.

Oparka, K.J., Duckett, C.M., Prior, D.A.M., and Fisher, D.B. (1994). Real-time imaging of phloem unloading in the root tip of *Arabidopsis.* Plant J. **6,** 759–766.

Palauqui, J.-C., Elmayan, T., Pollien, J.-M., and Vaucheret, H. (1997). Systemic acquired silencing: Transgene-specific post-transcriptional is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J. **16,** 4738–4745.

Palukaitis, P. (1987). Potato spindle tuber viroid: Investigation of the long-distance, intra-plant transport route. Virology **158,** 239–241.

Parthasarathy, M.V. (1975). Sieve element structure. In Transport in Plants. I. Phloem Transport, M.H. Zimmermann and J.A. Milburn, eds (Heidelberg, Germany: Springer-Verlag).

Parthasarathy, M.V., and Tomlinson, P.B. (1967). Anatomical features of metaphloem in stems of *Sabal*, *Cocus*, and two other palms. Am. J. Bot. **54,** 1143–1151.

Patrick, J.W., and Offler, C.R. (1995). Post–sieve element transport of sucrose in developing seeds. Aust. J. Plant Physiol. **22,** 681–702.

Pickard, B.G. (1973). Action potentials in higher plants. Bot. Rev. **39,** 172–201.

Raven, J.A. (1991). Long-term functioning of enucleate sieve elements—Possible mechanisms of damage avoidance and damage repair. Plant Cell Environ. **14,** 139–146.

Rhodes, J.D., Thain, J.F., and Wildon, D.C. (1996). The pathway for systemic electrical signal conduction in the wounded tomato plant. Planta **200,** 50–57.

Roberts, A.G., Santa Cruz, S., Roberts, I.M., Prior, D.A.M., Turgeon, R., and Oparka, K.J. (1997). Phloem unloading in sink leaves of *Nicotiana benthamiana*: Comparison of a fluorescent solute with a fluorescent virus. Plant Cell **9,** 1381–1396.

Riens, B., Lohaus, G., Heineke, D., and Heldt, H.W. (1991). Amino acid and sucrose content determined in the cytosolic, chloroplastic, and vacuolar compartments and in the phloem sap of spinach leaves. Plant Physiol. **97,** 227–233.

Ryabov, E.V., Robinson, D.J., and Taliansky, M. (1999). A plant-virus encoded protein facilitates long-distance movement of heterologous viral RNA. Proc. Natl. Acad. Sci. USA **96,** 1212–1217.

Sakuth, T., Schobert, C., Pecsvaradi, A., Eichholz, A., and Komor, E. (1993). Specific proteins in the sieve-tube exudate of *Ricinus communis* L. seedlings: Separation, characterisation, and in-vivo labeling. Planta **191,** 207–213.

Santa Cruz, S., Roberts, A.G., Prior, D.A.M., Chapman, S., and Oparka, K.J. (1998). Cell-to-cell and phloem-mediated transport of potato virus X: The role of virions. Plant Cell **10,** 495–510.

Sasaki, T., Chino, M., Hayashi, H., and Fujiwara, T. (1998). Detection of several mRNA species in rice phloem sap. Plant Cell Physiol. **39,** 895–897.

Sjolund, R.D. (1997). The phloem sieve element: A river runs through it. Plant Cell **9,** 1137–1146.

Sjolund, R.D., and Shih, C.Y. (1983). Freeze fracture analysis of phloem structure in plant tissue cultures. I. The sieve element reticulum. J. Ultrastruct. Res. **82,** 111–121.

Smith, A.U., Sabnis, L.M., and Johnson, R.P.C. (1987). Immunolocalization of phloem lectin from *Cucurbita maxima* using peroxidase and colloidal gold labels. Planta **170,** 461–470.

Stadler, R., Brandner, J., Schulz, A., Gahrtz, M., and Sauer, N. (1995). Phloem loading by the PmSUC2 sucrose carrier from

Plantago major occurs into companion cells. Plant Cell **7,** 1545–1554.

Turgeon, R. (1991). Symplastic phloem loading and the sink–source transition in leaves: A model. In Recent Advances in Phloem Transport and Assimilate Compartmentation, J.-L. Bonnemain, S. Delrot, W.J. Lucas, and J. Dainty, eds (Nantes, France: Ouest Editions), pp. 18–22.

Turgeon, R. (1995). The selection of raffinose oligosaccharides as translocates in higher plants. In Carbon Partitioning and Source–Sink Interactions in Plants, M. Madore and W.J. Lucas, eds (Rockville, MD: American Society of Plant Physiologists), pp. 195–203.

Turgeon, R. (1996). Phloem loading and plasmodesmata. Trends Plant Sci. **1,** 403–441.

Turgeon, R., and Medville, R. (1998). The absence of phloem loading in willow leaves. Proc. Natl. Acad. Sci. USA **95,** 12055–12060.

Turgeon, R., Beebe, D.U., and Gowan, E. (1993). The intermediary cell: Minor-vein anatomy and raffinose oligosaccharide synthesis in the Scrophulariaceae. Planta **191,** 446–456.

van Bel, A.J.E. (1993). Strategies of phloem loading. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44,** 253–281.

van Bel, A.J.E. (1996). Interaction between sieve element and companion cell and the consequences for photoassimilate distribution. Two structural hardware frames with associated physiological software packages in dicotyledons. J. Exp. Bot. **47,** 1129–1140.

van Bel, A.J.E., and Kempers, R. (1991). Symplastic isolation of the sieve element/companion cell complex in the phloem of *Ricinus communis* and *Salix alba* stems. Planta **183,** 69–76.

van Bel, A.J.E., and van Rijen, H.V.M. (1994). Microelectrode-recorded development of the symplastic autonomy of the sieve element/companion cell complex in the stem phloem of *Lupinus luteus* L. Planta **186,** 165–175.

Volk, G.M., Turgeon, R., and Beebe, D.U. (1996). Secondary plasmodesmata formation in the minor-vein phloem of *Cucumis melo* L. and *Cucurbita pepo* L. Planta **199,** 425–432.

Wang, N., and Nobel, P.S. (1998). Phloem transport of fructans in the crassulacean acid metabolism species *Agave deserti.* Plant Physiol. **116,** 709–714.

Weiner, H., Blechschmidt-Schneider, S., Mohme, E., Eschrich, W., and Heldt, H.W. (1991). Phloem transport of amino acids. Comparison of amino acid contents of maize leaves and of the sieve tube exudate. Plant Physiol. Biochem. **29,** 19–24.

Wimmers, L.E., and Turgeon, R. (1991). Transfer cells and solute uptake in minor veins of *Pisum sativum.* Planta **186,** 2–12.

Winter, H., Lohaus, G., and Heldt, H.W. (1992). Phloem transport of amino acids in relation to their cytosolic levels in barley leaves. Plant Physiol. **99,** 996–1004.

Wooding, F.B.P. (1967). Endoplasmic reticulum aggregates of ordered structure. Planta **69,** 203–243.

Wright, K.M., and Oparka, K.J. (1997). Metabolic inhibitors induce symplastic movement of solutes from the transport phloem of *Arabidopsis* roots. J. Exp. Bot. **48,** 1807–1814.

Xoconostle-Cazares, B., Xiang, Y., Ruiz-Medrano, R., Wang, H.-L., Monzer, J., Yoo, B.-C., McFarland, K.C., Franceschi, V.R., and Lucas, W.J. (1999). Plant paralog to viral movement protein potentiates transport of mRNA into the phloem. Science **283,** 94–98.

Ziegler, H. (1975). Nature of transported substances in the phloem. In Encyclopedia of Plant Physiology, Vol. 1, Transport in Plants: Phloem Transport, M.H. Zimmermann and J.A. Milburn, eds (Berlin: Springer-Verlag), pp. 59–100.