The Phloem Sieve Element: A River Runs through It

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OVERVIEW: TRANSPORT IN AN ENUCLEATE SYNCYTIUM

The evolution of a multicellular body plan resulted in the need to transport nutrients from regions where they are acquired or produced, such as the small intestine in higher animals or the leaf mesophyll in higher plants, to dependent cells located at long distances from the nutrient sources. Both higher plants and higher animals solved this problem by evolving vascular tissues, but there are fundamental differences in the physiological and structural details of the two systems. In animals, the movement of the nutrients is powered by pressures produced by the contraction of a muscular pump, the heart. In the phloem tissue of higher plants, however, the pressure required to move the nutrients is developed in the vascular tissue itself. Here, a kind of "solid state" pump creates pressure through the transport of assimilate molecules into a membrane-lined cell, the sieve element, and the subsequent uptake of water into the cell by osmosis.

The uptake of the assimilate molecules by the sieve elements is part of the process known as phloem loading (Van Bel, 1993). The high hydrostatic pressure, which can reach 30 atmospheres (Geiger, 1974), developed within the sieve element is believed to power the movement of the loaded molecules and water to regions of phloem unloading (sinks) (Oparka et al., 1994). Determining the sites of phloem loading and unloading and the roles of plasmodesmata (Lucas et al., 1993) or membrane transport proteins (Sussman, 1994; Tanner and Caspari, 1996) that may be involved is the current focus of research on phloem, a field that only recently has begun to be explored with molecular and cellular approaches (Rentsch and Frommer, 1996).

There is another major difference between nutrient transport in plants and animals, one that is less obvious but nonetheless important. In the vascular systems of both plants and animals, the nutrients move through conducting tubes that are formed by cells, but even in the case of small capillaries, the movement in animals takes place outside of the cells themselves.

During phloem transport in higher plants, however, the movement of nutrients is accomplished inside the phloem cells. Transport occurs interior to the plasma membrane of the sieve tube, which is formed by a series of connected phloem sieve elements. This fundamental difference imposes requirements on the development of the sieve element that are unique to these cells, and the differentiation of sieve elements follows a course that is unparalleled in the biological world.

Essentially, a series of differentiating sieve elements form a syncytium, a single compartment bound by a plasma membrane (Murphy and Aikman, 1989). Within the developing syncytium, the larger cellular organelles, which could impede assimilate flow, are degraded and removed, and the mature sieve tube lacks nuclei, vacuoles, Golgi bodies, or ribosomes. The plasmodesmata in the walls between the individual sieve elements are converted into large openings (sieve pores) that facilitate flow from cell to cell through the sieve tube. Significantly, this differentiation process, including the formation of sieve pores, is accomplished while maintaining the continuity of the plasma membrane around the individual cells and through the pores formed between the cells. The result of this unique differentiation process is the formation of a continuous, membrane-lined compartment, the sieve tube, that provides a pathway for nutrient flow and for signaling activities (Ryals et al., 1996) throughout the plant body.

LIFE IN THE FAST LANE: THE TRANSLOCATION STREAM AND SIEVE ELEMENT STRUCTURE

For more than 30 years, transmission electron microscopy (TEM) has been used to study the development of sieve elements, and a clear picture of how the structure of sieve elements is related to phloem function has emerged. Several reviews of this literature are available (Evert, 1977; Cronshaw, 1981; Behnke, 1989; Behnke and Sjolund, 1990; Eleftheriou, 1995), and only a brief summary is provided here.

Cell Walls and Sieve Area Pores

Because sieve elements develop high hydrostatic pressures, the walls surrounding these cells are modified to keep

them from bursting. Careful studies of developing sieve elements in cotton (Thorsch and Esau, 1982) and wheat (Eleftheriou, 1987b) show numerous microtubules located next to the plasma membrane in early stages of sieve element differentiation. These microtubules, which are oriented at right angles to the long axis of the cell, are degraded as the cell differentiates and are absent in mature sieve elements. The orientation of the densely packed cellulose microfibrils in the inner layer of the sieve element cell wall mimics that of the microtubules (Deshpande, 1976). The microtubule-determined orientation of the microfibrils, like hoops around a barrel, creates a cell wall that does not "bulge" laterally under the hydrostatic pressure, which, as a result, is transmitted lengthwise through the cell.

To facilitate the unimpeded flow of the translocation stream, plasmodesmata in the end walls between adjacent sieve elements are converted into sieve pores (Figures 1A and 1D). Plasmodesmata have an average diameter of only ~33 nm (Schulz, 1995; see also McLean et al., 1997, in this issue), much of which is occupied by a desmotubule (Ding et al., 1992). By contrast, sieve pores are usually ~200 to 400 nm in diameter, but they may be up to 1 μm in some cucurbits.

The mechanism of sieve pore development is still somewhat controversial, but in most cases the process appears to involve the deposition of callose (β-1,3-glucan) in the wall around the plasmodesmata (Deshpande, 1975; Lucas et al., 1993). The callose is thought to displace cellulose microfibrils in the wall such that when later the callose is degraded, a large opening is left behind. The controversy surrounding the role of callose in pore formation results from the rapid synthesis of callose at sieve pores after phloem wounding (Figure 1D). The rapid synthesis of callose is part of a wound response mechanism that seals off the pores in damaged phloem to prevent assimilate loss from cut sieve tubes (Kallarackal and Milburn, 1983; Sjolund et al., 1983). Thus, it is not clear whether the callose seen around developing pores functions in pore formation or is a response to phloem injury caused by the processing of samples for TEM.

Plasmodesmata, Transporters, and Models of Phloem Loading

During phloem development, the division of a phloem mother cell leads to the formation of a sieve element and a nucleated sister cell, the companion cell. Plasmodesmata located in the walls that separate companion cells from sieve elements (Figure 1C) are branched on the companion cell side (Lucas et al., 1993). These plasmodesmata are currently the subject of intense study to determine their role in the trafficking of assimilates and other molecules between sieve elements and companion cells (Lucas et al., 1996). Earlier morphological observations demonstrated that the frequency of plasmodesmata, especially between companion cells and neighboring cells, varies widely among different kinds of plants (Gamalei, 1989). In some cases, a well-developed symplastic pathway (Russin and Evert, 1985) from the mesophyll to the phloem is obvious, whereas in other plants, the sieve elements and companion cells are virtually isolated from symplastic connections to the mesophyll (Van Bel et al., 1992).

These differences provide evidence to support the hypothesis that higher plants may have evolved more than one mechanism for phloem transport (Gamalei, 1985; Van Bel and Gamalei, 1992). Indeed, two different models of phloem loading are currently being investigated: symplastic loading, the result of transport through continuous plasmodesmatal connections from the mesophyll to the phloem (Gamalei, 1991; Van Bel et al., 1992); and apoplastic loading, the result of the active transport of assimilate molecules (primarily sucrose) from the apoplast (extracellular space) across the plasma membrane of the sieve element or the companion cell (Komor et al., 1996).

The polymer-trapping model of symplastic loading (Haritatos et al., 1996; Turgeon, 1996) proposes that sucrose or galactinol formed in the mesophyll can diffuse into specialized companion cells, termed intermediary cells, through plasmodesmata. In the intermediary cells, the sucrose and galactinol could be used to synthesize larger molecules, such as stachyose and raffinose. According to this model, the larger molecules are able to diffuse into the sieve elements through the branched plasmodesmata, but they are too large to diffuse back through the narrower plasmodesmata to the mesophyll. By contrast, evidence for active transport and the apoplastic loading of sucrose into the phloem is provided by immunolocalization studies, which demonstrate the presence of both a proton pump (i.e., an H^+-ATPase), termed AHA3 (Dewitt and Sussman, 1995), and a sucrose-H^+ symporter protein, SUC2 (Stadler et al., 1995; Stadler and Sauer, 1996), in the companion cells of Arabidopsis.

Companion cells are implicated in the delivery of assimilate molecules to the sieve elements in both symplastic and apoplastic pathways of phloem loading. However, several models of phloem loading invoke the possibility that the sieve element may also perform some apoplastic loading of sucrose (Komor et al., 1996; Van Bel, 1996), possibly to recover sucrose that diffuses out of sieve tubes along the transport path. These models predict that in addition to sucrose uptake sites in companion cells, a sucrose transport protein may also be located in the sieve element plasma membrane. Evidence that this is the case, at least in some plants, has recently been provided by the demonstration that antibodies formed against the potato sucrose transporter protein SUT1 label the sieve element plasma membrane of potato, tomato, and tobacco (Kühn et al., 1997). Earlier studies of antibodies formed against a 62-kD sucrose binding protein also demonstrated a specificity for the sieve element plasma membrane (Warmbrodt et al., 1991).

The uptake of sucrose by a sucrose–proton symporter located in the sieve element plasma membrane is unlikely to occur in the absence of a proton gradient (Van Bel and
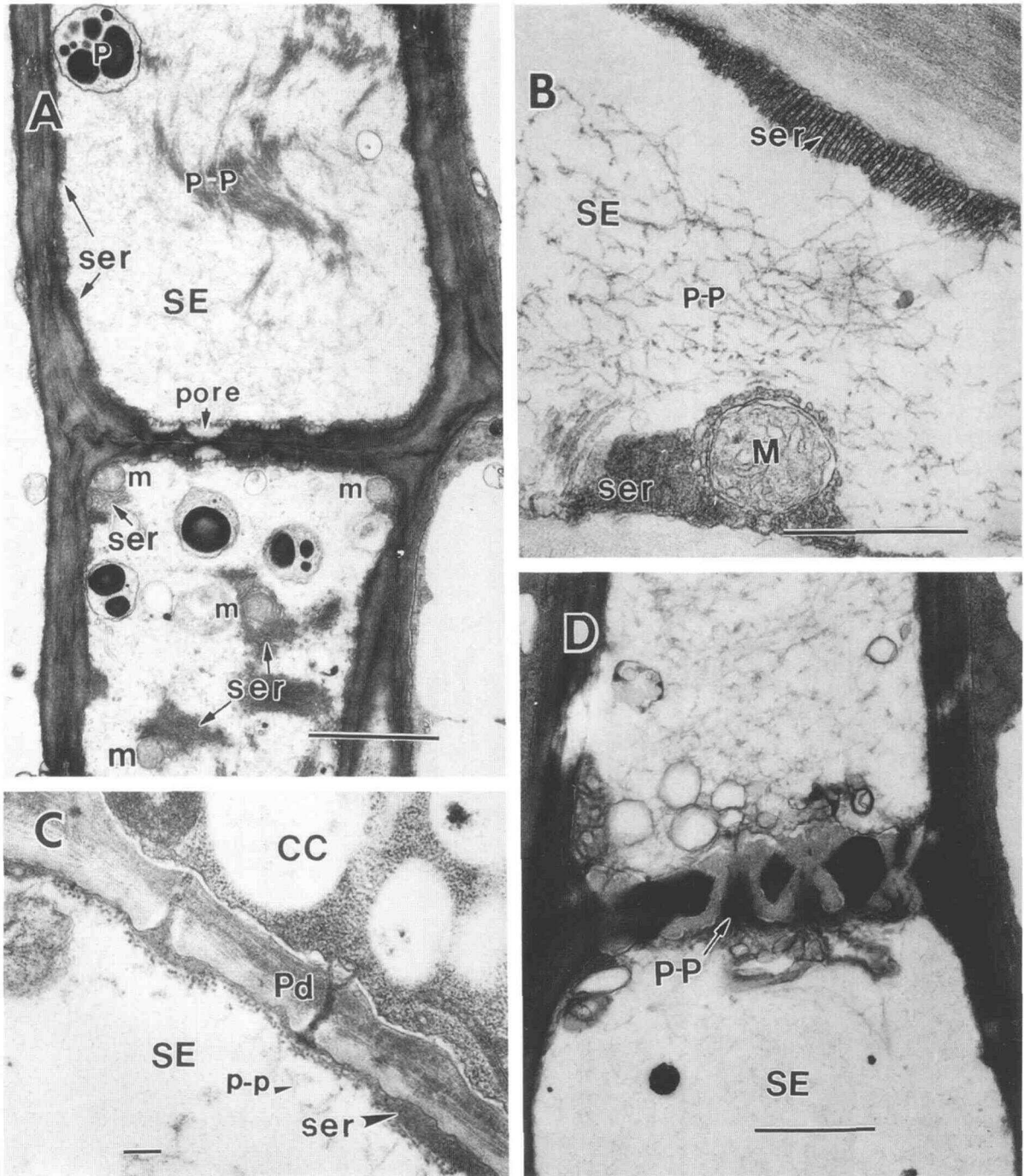
Figure 1. Phloem Sieve Elements and Companion Cells from *Streptanthus tortuosus*.

(A) Developing sieve elements in a young stem. The plastids have been converted into sieve element plastids. Plasmodesmata in the end wall are surrounded by callose and are forming sieve pores. The P-protein in the upper sieve element cell is not yet fully dispersed. SER membranes line the periphery of the upper cell and surround the mitochondria in the lower cell.

(B) Sieve element from *S. tortuosus* callus culture. The SER membranes are attached to the plasma membrane and surround a peripheral mito-chondrion. The P-protein filaments are dispersed as a network and are anchored to the SER.

(C) Callus culture phloem. A branched plasmodesma between a companion cell and a sieve element spans the cell wall. SER membranes line the periphery of the cell, and P-protein filaments are dispersed in the lumen.

(D) An injured sieve tube from an *S. tortuosus* stem. Sieve pores are plugged with P-protein filaments, and the pores are surrounded by large callose deposits.

CC, companion cell; m and M, mitochondria; P, plastids; Pd, plasmodesmata; P-P, P-protein; SE, sieve element; ser, sieve element reticulum. Bars in **(A)**, **(B)**, and **(D)** = 1 μm; bar in **(C)** = 0.1 μm.

Kempers, 1997), suggesting that an H⁺-ATPase may also be found in these cells. The only phloem-specific H⁺-ATPase identified to date is the Arabidopsis AHA3, which is located on the companion cell plasma membrane. Is there also an unidentified H⁺-ATPase on the sieve element plasma membrane? If so, what is the source of the ATP required for its function? Although significant amounts of ATP and ADP have been detected in phloem exudates (Ohshima et al., 1990), their origin is unknown. One possibility is that the ATP is produced by the companion cells, which have more abundant mitochondria than do mature sieve elements, and is then transferred to sieve elements by means of trafficking through the branched plasmodesmata (Van Bel, 1996). However, sieve elements also contain mitochondria (Figures 1A and 1B), even at maturity (Esau and Cronshaw, 1968), and the possibility that sieve element mitochondria could provide the ATP required by an H⁺-ATPase (Sjolund and Shih, 1983a) remains to be established. Nevertheless, evidence that sieve element mitochondria are metabolically active comes from experiments showing that they take up the dye Janus green B (McGivern, 1957; Lee et al., 1971) and that they accumulate the lipophilic cation Rhodamine 123 (Moniger et al., 1993).

Endoplasmic Reticulum, Vacuoles, P-Protein, Plastids, and the Nucleus

Several major organelles, including the nucleus, vacuole, rough endoplasmic reticulum, and Golgi bodies, are degraded during sieve element differentiation (Esau and Gill, 1971, 1972; Thorsch and Esau, 1981b; Eleftheriou, 1987a), creating an organelle-free path for assimilate transport through the lumen of the cell. The loss of these structures, especially the nucleus and the ribosomes, has profound consequences for the continued life of mature sieve elements.

Although plastids have no known function in sieve elements, they are retained in the mature cell (Figure 1A). However, sieve element plastids lack thylakoid membranes and contain only protein crystals or small grains of starch (Behnke, 1971) that stain red or brown with iodine (Esau, 1965) and consist of highly branched amylopectin molecules (Palevitz and Newcomb, 1970). Because the large, starch-filled amyloplasts that are found in many nutrient-rich storage tissues would severely block transport through sieve pores, and despite the fact that they are located in a cell filled with an abundance of sugars, starch synthesis by sieve element plastids appears to be restricted.

The characteristic changes that plastids undergo is one of the earliest markers of sieve element differentiation in many plants (Eleftheriou, 1996). These changes are clearly demonstrated in the development of wound phloem, which forms when vascular bundles of Coleus or Pisum stems are severed and the surrounding parenchyma cells are induced to differentiate into sieve elements around the cut sites. The

wound phloem functions to bypass the injured phloem and restores phloem transport through the stems. One of the first changes noted in the parenchyma cells that differentiate into wound sieve elements is the loss of amyloplast starch in their plastids. This is followed by the conversion of the existing plastids into the characteristic sieve element form (Behnke and Schulz, 1983). A monoclonal antibody, RS5, which was raised against sieve elements isolated from tissue cultures of Streptanthus tortuosus, recognizes a sieve element–specific form of the enzyme β-amylase (Wang et al., 1995). Although this β-amylase may play a role in sieve element starch degradation, it is unclear whether the enzyme is targeted to sieve element plastids.

An elaborate system of membranes, the sieve element reticulum (SER) (Srivastava and O'Brien, 1966; Sjolund and Shih, 1983a), is a prominent feature of the mature sieve element in virtually all plants studied to date (Figures 1A and 1B). The SER lacks ribosomes and is often described on the basis of its morphology as stacked, anastomosing, or parietal endoplasmic reticulum. The origin of the SER during differentiation has not been established, but in some cases it is associated with the envelope of degenerating sieve element nuclei (Esau and Gill, 1971; Eleftheriou, 1987a). Although the function of the SER is unknown, there is evidence that it may sequester calcium (Arsanto, 1986; Sjolund, 1990b), a role that may be particularly important in the mature sieve element after the vacuole has been degraded.

A unique filamentous protein termed P-protein (Cronshaw and Esau, 1967, 1968a, 1968b; Kollmann et al., 1970) is formed in the sieve elements of many (but not all) plants during differentiation. Filaments of P-protein span the sieve element lumen (Figures 1A and 1B) and are anchored to the periphery of the mature cell (Smith et al., 1987). After injury to the phloem, P-protein is released from its anchoring sites, and the filaments are swept downstream. Thereafter, the filaments accumulate at and effectively seal the sieve pores (Figure 1D) to prevent assimilate loss at the injury site (Kallarackal and Milburn, 1983).

The Sieve Element Lumen and Peripheral Membranes

In the normal in vivo state, the plasma membrane, membranes of the SER, mitochondria, and plastids are all restricted to a thin, parietal layer on the inner surface of the sieve element. This layer of membranes and organelles is not part of the assimilate stream that flows through the lumen, which makes up most of the cell's volume. Although TEM produces images of the phloem that are static, models of phloem function, especially the movement of molecules such as ATP into and within the sieve element, must take into account the flow rate of assimilates passing through the cell's lumen. A consideration of the mature sieve element as two compartments, one fixed and one flowing, is important for gaining a full understanding of how the sieve element is

organized. The interface between the fixed, peripheral membranes of the sieve element and the translocation stream flowing through the lumen of the cell may be the site of significant shear forces.

Sieve tube transport is amazingly rapid. The rate of phloem translocation has been estimated to be 40 cm/hr (Fisher, 1990), a flow rate that is ~110 μm/sec at the cellular scale. If a sieve element were 250 μm long, the transport stream could traverse the cell in ~2 sec. The diameter of sieve elements in most dicot plants is 5 to 20 μm, although it can vary widely (Parthasarathy, 1975). Thus, even in a large (20 μm) sieve element, the assimilate flow rate is equal to approximately five times the cell diameter per second.

Although it is difficult to appreciate the rate of phloem transport from the perspective of cell and organelle dimensions, it is possible to imagine the comparable "human scale" task of moving materials to the opposite side of a 20-m-wide river that is flowing at a comparable (five times the diameter per second) rate of 110 m/sec. The river would flow past its banks at 396 km/hr, traveling faster than the record pace (386 km/hr) set by a Porsche 917 on the Le Mans race track! This relationship between cell diameter and flow rate has implications for the movement and compartmentalization of nontransported molecules in the sieve element.

The river analogy highlights two problems unique to the sieve element: the organelles that persist in the mature cell must be anchored to the cell periphery, and the trafficking of molecules to the membranes of the sieve element must be channeled within the peripheral membrane complex. Further studies are needed to determine the relationship between SER membranes in sieve elements and the endoplasmic reticulum that is associated with the branched plasmodesmata of companion cells. The SER may play a role in the directed trafficking of ATP or other molecules from the companion cells to sites on the sieve element plasma membrane (Thorsch and Esau, 1981a; Sjolund and Shih, 1983a).

The Companion Cell–Sieve Element Complex: Phloem Loading and Phloem Protein Synthesis

Although sieve elements are enucleate and lack ribosomes at maturity, they remain alive, often for many months or even for many years, as in the case of palms (Parthasarathy, 1975). The synthesis of replacement proteins that are required by a mature enucleate sieve element is thought to take place in the associated companion cell. That companion cells are necessary for continued sieve element function is suggested by studies of protophloem elements in developing leaves and stems, which often lack associated companion cells (Eleftheriou and Tsekos, 1982). Although these protophloem sieve elements differentiate normally, they live for only a short time and are replaced by later-formed metaphloem sieve elements. These latter elements are associated with companion cells and live much longer.

Studies of P-protein synthesis, primarily in cucurbits, provide evidence that the companion cell is the site of P-protein synthesis and that the P-protein is trafficked to the mature, enucleate sieve elements through branched plasmodesmata (Figure 1C). Studies of Cucurbita maxima using ^{14}C-labeled amino acids showed that P-proteins were synthesized continuously, probably in the companion cells (Nuske and Eschrich, 1976). Cucurbits have two P-proteins, PP1, the 96-kD filamentous P-protein responsible for sieve pore sealing, and PP2, a 48-kD lectin (Smith et al., 1987) that may serve to immobilize bacteria and fungi at wound sites (Read and Northcote, 1983). Antibodies to PP2 label both the companion cells and the sieve elements of cucurbits (Smith et al., 1987), but in situ hybridization studies demonstrate that PP2 mRNA is expressed only in companion cells (Bostwick et al., 1992; Dannenhoffer et al., 1997). A similar case for the expression of mRNA for the filamentous protein PP1 in companion cells of cucurbits has also been reported (Clark et al., 1997). These results support the role of the companion cell as the site of P-protein synthesis and the need to traffic the protein to the enucleate sieve element.

Similar data have been obtained in the analysis of SUT1. The SUT1 protein has been immunolocalized to the plasma membrane of sieve elements in tobacco, potato, and tomato, but the corresponding mRNA is apparently synthesized in the neighboring companion cells. In situ hybridization studies suggest that SUT1 mRNA may be trafficked through the plasmodesmata from companion cells to the sieve elements, although the mechanism for translation of the mRNA transcripts in the mature sieve elements is unknown (Kühn et al., 1997).

Additional evidence that sieve tube proteins are synthesized in neighboring companion cells is provided by the demonstration that phloem exudates obtained from severed aphid stylets inserted into sieve tubes (Fisher et al., 1992; Ishiwatari et al., 1995) or from exudates obtained at the ends of cut stems contain hundreds of different kinds of proteins, mostly <25 kD in mass. The collective term "sieve tube exudate proteins," or STEPs, has been used to describe these unknown proteins (Sakuth et al., 1993), and labeling studies of STEPs obtained from Ricinus communis seedlings indicate that the proteins are synthesized continually. Because the exudates are obtained from mature enucleate sieve tubes, it seems likely that the proteins are synthesized in neighboring nucleated companion cells.

Based on these studies of phloem function, it appears that there may be two important companion cell functions, both of which require transport through plasmodesmata to sieve elements: the phloem loading of assimilates and the synthesis of proteins targeted to enucleate sieve tubes. These two functions may require separate mechanisms for molecular trafficking through the branched plasmodesmata that connect companion cells to the sieve elements. Loaded

assimilate molecules should be delivered into the rapidly moving assimilate stream in the lumen of the sieve element cell, whereas proteins or ATP targeted to the sieve element plasma membrane are probably channeled within the peripheral membrane system.

Another question that remains unanswered in phloem differentiation is the mechanism by which some sieve element organelles, such as the Golgi bodies, vacuoles, nuclei, ribosomes, and microtubules, are targeted for degradation, whereas others, including the plasma membrane, mitochondria, plastids, P-proteins, and the SER, are allowed to survive. Although proteolysis in higher plants is beginning to be understood (Callis, 1995; Vierstra, 1996), no data are available to suggest how this selective degradation of phloem organelles and proteins is accomplished. Nevertheless, the breakdown of the vacuole may release proteolytic enzymes into the cytoplasm at a late stage of sieve element differentiation. The pyncnotic degeneration of the sieve element nucleus has been described (Thorsch and Esau, 1981c; Eleftheriou, 1986), and this pattern of nuclear degeneration may be related to the process of programmed cell death recently described for plants (Wang et al., 1996; see Pennell and Lamb, 1997, in this issue).

Information obtained from the study of proteolysis during xylogenesis, especially in the Zinnia cell culture system (Beers and Freeman, 1997; see also Fukuda, 1997, in this issue), may provide some clues to the process of proteolysis in sieve elements, but the degradation of membranes and organelles in developing xylem elements is total and nonselective. By contrast, proteolysis in differentiating sieve elements is amazingly selective and is almost "surgical" in its precision. Although ubiquitin has been detected in STEPs from R. communis (Schobert et al., 1995), and polyubiquitin was encoded by clones obtained from a cDNA library developed from the phloem of pine (Alosi-Carter et al., 1995), the role of ubiquitin in sieve element differentiation remains to be established.

Although protein synthesis and trafficking from companion cells are important, it is also important to recognize that sieve element differentiation takes place when the cell is nucleated and while it still has ribosomes. In the protophloem, sieve element differentiation apparently can be performed in the absence of an associated companion cell. It seems likely, then, that many sieve element–specific proteins are synthesized in the young sieve elements themselves, while the cells are still capable of transcription and translation. Some of these early sieve element proteins may function only during the process of sieve element differentiation. Other proteins may be initially synthesized by the young nucleated sieve element, but their continued presence in mature sieve elements may require the trafficking of replacement proteins from companion cells.

Ahead lies the challenging prospect of investigating the spatial and temporal regulation of phloem-specific gene expression in differentiating sieve elements and companion cells, a study made more difficult by the selective proteolysis and degradation of many sieve element structures occurring simultaneously during development.

SITES OF SIEVE ELEMENT DIFFERENTIATION: FINDING THE NEEDLE IN THE HAYSTACK

One obstacle to the study of phloem sieve element differentiation is the inaccessibility of the cells in the plant body. Another serious problem is that sieve elements, despite their critical role in nutrient transport, occupy a very small percentage of plant organs, perhaps as low as 0.4% of the total volume in leaves (Orlich and Komor, 1992). Unfortunately, however, the study of sieve element differentiation is even more difficult than the low percentage of phloem may indicate. Almost all of the sieve elements present in the plant body are mature and enucleate. The actual sites of phloem differentiation, the places where it would be possible to investigate sieve element–specific gene expression, proteolysis, or the interactions between nucleated sieve elements and nucleated companion cells, are rare. Only a few cells at the tips of roots or stems, in very young leaves, or in the vascular cambium are engaged in phloem formation. A thorough investigation of protophloem differentiation in wheat roots (Eleftheriou, 1985, 1990, 1996) demonstrated that only seven to nine cells at the end of a file of sieve elements are involved in the process of differentiation and that the entire process of sieve element differentiation lasts only 16 to 21 hr. In wheat roots, the first recognizable protophloem elements are found <300 μm from the root apex (Eleftheriou, 1989), and mature sieve elements are located ~550 μm behind the apex. Thus, although the "cross-section volume" occupied by sieve elements in many plant organs may be only ~0.5%, only a small part of that volume, probably <10% (0.05% of total plant volume), is formed by nucleated, differentiating sieve elements.

These analyses have important implications for the study of "phloem-specific" gene expression, especially for the construction of cDNA libraries. Nucleated sieve elements and therefore sieve element–specific mRNA molecules are rare in the plant body, whereas nucleated companion cells and companion cell mRNAs are comparatively plentiful. Even cDNA libraries made from RNA isolated from vascular bundles may contain few sieve element–specific mRNAs unless care is taken to select for the young sieve elements located at the growing ends of the vascular bundles.

The study of xylogenesis in higher plants is also hampered by the same spatial and temporal limitations that make studies of sieve element differentiation difficult. Both xylem elements and sieve elements are only nucleated during early differentiation. The study of xylogenesis, however, has benefited greatly from the development of the Zinnia cell culture system (Fukuda and Komamine, 1980; Demura and Fukuda, 1994; see also Fukuda, 1997, in this issue), which has facilitated the study of tracheary element differentiation.

The development of phloem has also been demonstrated in plant cell cultures (Sjölund, 1996), and the hormonal regulation of phloem formation in callus tissue has been described (Wetmore and Rier, 1963; Aloni, 1980). Plant cell cultures have not been widely used for the study of phloem, however, probably because most research on phloem has been directed toward the study of long-distance phloem transport. Because sieve elements in callus cultures are organized into separate islands of phloem cells rather than lengthy sieve tubes, they do not participate in long-distance transport within the callus (Hanson and Edelman, 1970). The lack of translocation by sieve elements in callus has been interpreted as evidence that phloem in plant cell cultures is "nonfunctional" (Spanner, 1978). The concern that phloem formed in vitro is not "suitable" for research on phloem (Spanner, 1978) still limits the use of cell cultures in phloem research. This attitude needs to be revised.

Sieve element differentiation in callus cultures is very similar to the differentiation of wound phloem from parenchyma cells around cut vascular bundles in plant organs (Schulz, 1986), and cultured callus sieve elements are identical to the sieve elements formed in the callus that develops at graft unions. Significantly, both the sieve elements formed in callus at graft unions (Kollmann and Glockmann, 1990) and the phloem formed from parenchyma cells after wounding do function in transport when they become connected to the existing vascular bundles (Schulz and Gersani, 1990; Wang and Kollmann, 1996). These studies suggest, as abundant TEM studies have shown (Wooding, 1968; Anderson and Cronshaw, 1970; Cronshaw and Anderson, 1971; Sjolund and Shih, 1983a, 1983b; Sjolund et al., 1983), that callus sieve elements formed in culture, even though not part of a translocation stream in vitro, are differentiated in a completely normal manner.

Cellular and molecular approaches to phloem research may benefit from the use of plant cell cultures for several reasons. The population of sieve elements in callus cultures of some plants may constitute 5 to 10% of the total cell population, a significant increase over the levels found in plant organs. Moreover, because sieve elements are differentiated continuously in culture, a higher percentage of nucleated sieve elements can be obtained from callus tissue than from intact plants. Hormonal regulation of phloem development in cell cultures is possible, and hormone levels can be manipulated such that some cultures differentiate sieve elements (i.e., phloem [+] cultures) and others (i.e., phloem [−] cultures) fail to do so. These hormonally regulated cultures could be useful for molecular approaches, such as subtractive hybridization (Cook and Sequeira, 1991), differential display (Liang and Pardee, 1992), or suppression subtractive hybridization (Diatchenko et al., 1996), that take advantage of the differences in gene expression between cell populations.

The discrete nature of phloem islands in callus tissue, although precluding long-distance transport, presents an ideal situation for the separation of the phloem cells from the surrounding callus parenchyma cells. Sieve elements have been enzymatically isolated from callus cultures (Sjolund, 1990a), and the cells have been used as antigens to raise phloem-specific monoclonal antibodies (Köhler and Milstein, 1975; Sjölund, 1995). As expected, the monoclonal antibodies recognize phloem-specific antigens not only in callus sieve elements but also in the sieve elements of intact plants. The antigens, including P-protein and a sieve element–specific form of β-amylase, are detected on immunoblots of total proteins from phloem (+) cultures but are not detected on immunoblots of proteins from phloem (−) cultures (Tóth and Sjölund, 1994; Tóth et al., 1994; Wang et al., 1995), demonstrating the differential expression of phloem-specific genes in this culture system.

There is reason to believe that improved yields of sieve element differentiation in vitro are possible. Investigations of wound phloem development in Coleus blumei (Behnke and Schulz, 1980) suggest that several rounds of cell division are required for sieve element differentiation, but studies of roots treated with colchicine (Eleftheriou, 1993) demonstrate that under some circumstances, sieve element differentiation may proceed without prior cell division. These results suggest that the direct differentiation of sieve elements from parenchyma or mesophyll cells in culture, similar to tracheary element differentiation from mesophyll cells of Zinnia, may be possible.

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