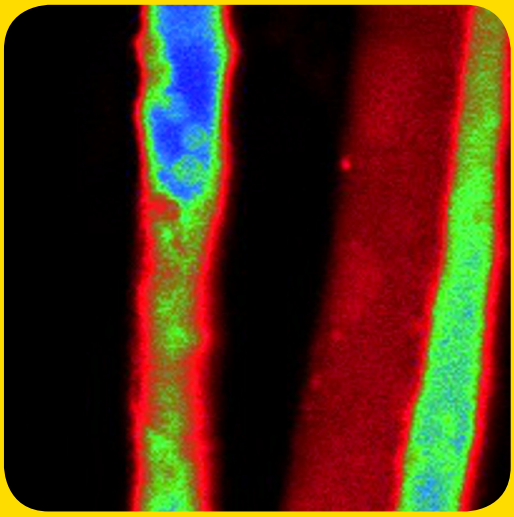
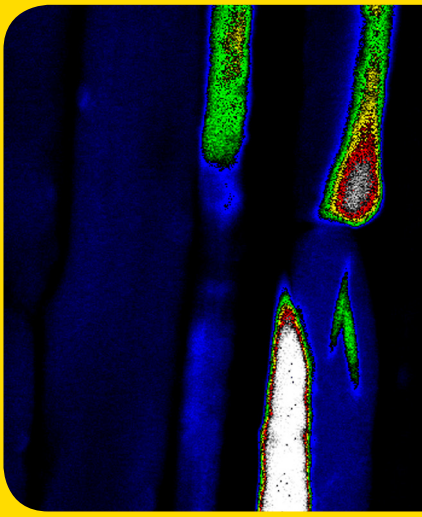
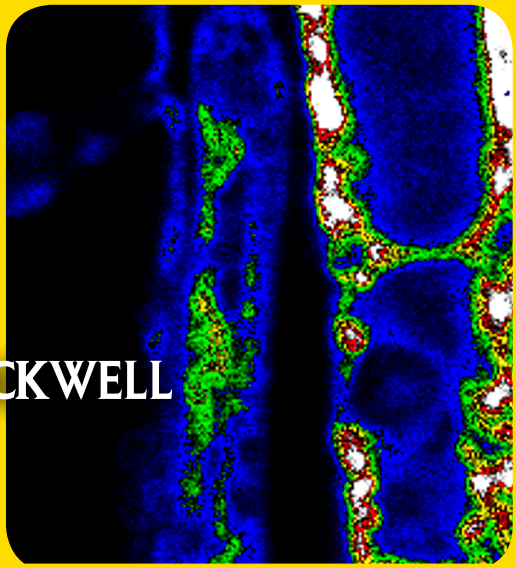
PHLOEM

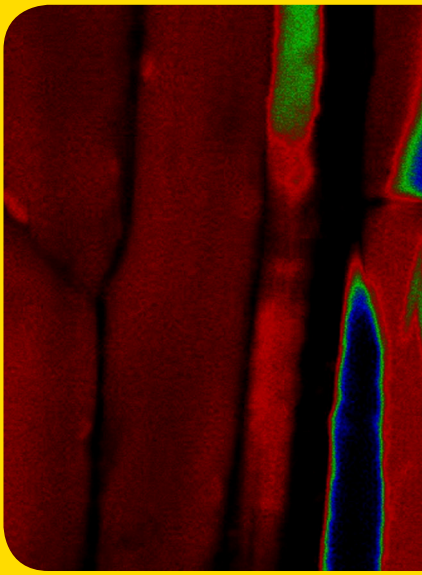
MOLECULAR CELL BIOLOGY, SYSTEMIC COMMUNICATION, BIOTIC INTERACTIONS

Edited by Gary A. Thompson and Aart J.E. van Bel









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Phloem: Molecular Cell Biology, Systemic Communication, Biotic Interactions

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Cover images: False-color confocal laser scanning images of phloem cells in intact plants.
Upper left: CMEDA/CMFDA-stained sieve element (reddish) and two companion cells, Upper right: At the right-hand side of the picture, two consecutive sieve elements (blue) each with a companion cell in a staggered position. The lower sieve element contains an arrowhead-shaped forisome (green colored) near the sieve plate (not visible). The other longitudinal cells are phloem parenchyma cells. Lower left: ER-Tracker Green-stained intact phloem tissue. At the left-hand side of the picture a sieve element (black with blue traces of ER near the sieve plate) and a companion cell (blue and green). At the right-hand side, the ends of two adjacent phloem parenchyma cells with a broad margin of cytoplasm (green and white). Lower right: Same picture as upper right with a different false-color setting.
Courtesy of Dr. Jens B. Hafke
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Section A
Introduction

1 Phloem, the Integrative Avenue

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By the end of the nineteenth century, plant biologists recognized the paramount importance of phloem transport for plant growth. They suspected that plant growth strongly relies on the phloem-mediated supply of photosynthates and other organic compounds. These initial studies culminated in 1930 with the pressure flow hypothesis proposed by Ernst Münch, which offered a solid theoretical and unifying platform to understand the fundamental mechanism of phloem translocation. For decades following the general acceptance of Münch's concept, phloem research predominantly focused on the movement and distribution of photoassimilates. Source supply and sink demand combined with the concepts of donor and receiver organs were seen as key factors in determining plant productivity and, hence, the agricultural yield.

The field of phloem physiology became well established as new tools were developed that allowed researchers to quantifiably measure translocation and to visualize the phloem tissue at high resolution. Many studies of photoassimilate movement throughout the plant were conducted using ^{14}C-labeled carbohydrates. These approaches were widely used in the 1970s and early 1980s to learn about carbohydrate metabolism and sugar carrier activities in source and sink tissues. From the 1960s, transmission electron microscopy provided views unparalleled at the time into the ultrastructure of phloem cells. Great strides were made in detailing the variation and development of sieve element–companion cell complexes and other phloem cell types in different plant taxa. However, the challenges associated with cellular preservation were recognized as limiting factors in obtaining a reliable view of this dynamic tissue.

New tools associated with molecular biology and genomics combined with significant advances in real-time microscopy rejuvenated phloem physiology. Identifying and manipulating the genes encoding phloem-specific proteins were only initial steps leading to comprehensive cataloging of genes, proteins, and metabolic components of the phloem. Advances in cell biology, such as development of molecular makers combined with new fluorescent tagging technologies, micromanipulation, and confocal microscopy, have provided new levels of resolution that continue to contribute to our understanding of this tissue.

Abbreviations: ^{14}C, carbon-14; Ca^{2+}, calcium; CC, companion cell; Cl^{-}, chloride; K^{+}, potassium; miRNA, micro RNA; Na^{+}, sodium; PD, plasmodesmata; PPUs, pore-plasmodesma units; *R*, resistance; rRNA, ribosomal RNA; SE, sieve element; SE-CC, sieve element-companion cell complex; siRNA, short-interfering RNA; smRNA, small RNA; tRNA, transfer RNA

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The discoveries enabled by molecular approaches are now being combined with the tools of physics and chemistry to address the complex physiological questions that have been posed by investigators for many decades. Understanding physical forces such as the thermodynamics of membrane transport and quantification of parameters such as the transporter density and their turnover rates relies on integrated approaches. Unraveling complex signaling and metabolic networks within the phloem during plant development, and as plants interact with the environment, will only be resolved by using all available tools and continually developing new analytical approaches.

This book is intended to showcase the advances in our understanding of phloem biochemistry, molecular biology, physiology, and interactions with other living organisms as we continue in the second decade of the twenty-first century. One notable feature of the book is the considerable and intended overlap that occurs among the chapters, which is further demonstration of the integrated nature of the topics and the work that is ongoing at this point in time.

The text is divided into four sections: the first section is an introductory overview composed of three chapters designed to provide a contextual framework for chapters in the subsequent sections. Chapter 2 (White) focuses on the intimate relationships that occur between sieve elements and companion cells. Sieve element–companion cell (SE–CC) complexes are the modular components of sieve tubes that are symplasmically connected to one another, yet relatively isolated from surrounding cells along most of the transport path. Plasmodesmata (PD) in the nascent sieve plates located between successive SEs transform into sieve pores with large functional diameters, establishing a contiguous, living conducting sieve-tube conduit. Simultaneously, SEs detach fully from the surrounding cells with exception of the companion cells, to which they become linked by pore–plasmodesma units (PPUs). CCs have a reduced connectivity to phloem parenchyma cells by a low number of optically conventional plasmodesmata. This plasmodesmal configuration is thought to create an exclusive trafficking system for a diversity of substances between SEs and CCs. In the relatively short stretches of collection and release phloem, plasmodesmal connectivity between CCs and phloem parenchyma cells strongly varies among species. In collection phloem, the symplasmic connectivity varies by a factor of 1000; in release phloem, there is abundant symplasmic connection between SEs and surrounding cells.

Chapter 3 (Patrick) reflects on the diverse structural frameworks in which SE–CCs are embedded and, in which, SE–CC specialization gives rise to different functions in the successive collection, transport, and release phloem sections. In sources (mostly green leaves), the collection phloem accumulates an arsenal of biochemical substances among which carbohydrates predominate. In main veins of stems and roots, balanced accumulation and release by SE–CCs in transport phloem facilitates development of cambial tissues, maintenance of mature cells in transport organs, and exchange of compounds with the surrounding cells. In terminal sinks (e.g., shoot and root apices, flowers, fruits, seeds), release phloem delivers materials to the target organs. This general structure and organization of the phloem is responsible for mass flow and concerted action among the organs.

The second section explores the structural and functional relationships of SE components. Revealing the diversity and nature of associated and integral membrane proteins along with mapping their location has tremendously contributed to the fundamental understanding of phloem physiology. Chapter 4 (Tegeder, Ruan, and Patrick) provides an overview of the progress in understanding membrane transporters. A wealth of carriers facilitates the transfer of sucrose, the primary carbohydrate transported in many plant species, in particular fast-growing herbaceous plants of the temperate zones. Moreover, membrane-bound translocators for hexoses, raffinose-related sugars and sugar alcohols are responsible for distribution of carbohydrates and are part of a plant-wide carbohydrate-controlled communication network. Because sucrose is the principal energy-carrying compound for long-distance transport, the control mechanisms behind sucrose processing could be

more elaborate than those for other compounds. Chapter 5 (Kühn) discusses the machinery behind the membrane transfer of sucrose and the complicated regulatory mechanisms, of which details are beginning to emerge, that appear to be responsible for fine-tuning of sucrose carrier activities.

Plasma membrane ion channels also play a pivotal role in phloem function. Chapter 6 (Hafke and van Bel) shows that a large variety of ion channels are involved in ion uptake and release as well as counterbalancing the electrical consequences of carbohydrate uptake and in propagating electrical signals. Electrical signaling in plants largely diverges from that in animals. In plants, the ions involved are K^+, Cl^-, and Ca^{2+} rather than K^+ and Na^+, and energy for ion exchange is provided by proton pumps rather than Na^+/K^+ pumps. In contrast to animals, moreover, where minor amounts of ions are exchanged along the path to influence targets at the end of the propagation pathway, electrical propagation in plants displaces large amounts of ions along the pathway. In particular, Ca^{2+} ions are presumed to trigger a variety of intracellular cascades.

The cellular basis of sieve pore occlusion and its effect on mass flow is discussed in Chapter 7 (Knoblauch and Mullendore). Mass flow calculations are still not entirely conclusive, in particular for low-concentration solutes. One reason for the inaccuracies could be the exchange of solutes between sieve tubes and adjacent cells. Their exchange rates determine the amount of each individual solute in the solvent flow. Partial occlusion of sieve pores in intact plants as well as the nonuniform diameters of sieve tubes and sieve pores could also invalidate mass flow calculations. Furthermore, inconsistencies in the calculations could be linked to lateral exchange between parallel sieve tubes, possibly via lateral sieve plates, transporting in opposite directions.

The third section of the book focuses on long-distance signaling via the phloem. Work in the past decade revealed the phloem as the key integrator of genetic, developmental, and physiological responses that are conveyed over long distances throughout the plant. Signaling molecules, including proteins and RNAs, transported in the sieve-tube sap appear to be distributed over long distances. Chapter 8 (Dinant and Lucas) presents a comprehensive overview of the soluble proteins identified in sieve tubes and their potential functions. A surprisingly large proteome composed of hundreds of proteins has been identified in sieve-tube exudates. Important classes of proteins appear to assist in PPU-trafficking of both proteins and RNAs and have roles in maintaining protein stability as well as degradation. Proteins impact a variety of putative signaling pathways and regulate the oxidative status of the phloem. Sieve-tube sap appears to be replete with proteins involved in responses to biotic and abiotic stresses. Classical structural phloem proteins are joined by structural components of the translational machinery that perform puzzling functions in the highly modified conducting elements that by all accounts seem to lack ribosomes.

The transformative discovery of RNA in sieve-tube exudates along with putative large protein–RNA complexes that could bind and convey RNA species over long distances emphasize the integrated nature of macromolecules in the phloem. Chapter 9 (Kehr and Buhtz) critically reviews recent developments in the rapidly expanding area of RNA biology within in the solute stream. Several RNA species have been detected, each with a specific spectrum of tasks. Messenger RNAs (mRNA) in sieve-tube sap could intervene in metabolism and protein synthesis in distant cells. Nonprotein coding RNAs including ribosomal RNAs (rRNA) and transfer RNAs (tRNAs) also have been identified in sieve-tube exudates. Regulatory small RNAs (smRNA), including short-interfering RNAs (siRNA) and micro RNAs (miRNA) appear to be common and can have diverse roles in affecting plant development and responses to biotic and abiotic stresses.

The elaborate signaling system, composed of proteins and RNAs translocated from source organs via the phloem, impacts differentiation of growing zones to mediate developmental processes. Several case studies are presented in Chapter 10 (Hannapel) describing how phloem transport of macromolecules affects development in remote meristems. Flower induction has been a prominent

and long-standing example of this mechanism. The identity of the enigmatic floral activator, florigen, was discovered to be a protein expressed by *FLOWERING LOCUS T*. A second case study examines the evidence for a phloem–mobile ribonucleoprotein complex that mobilizes mRNAs, affecting the gibberellic acid signaling pathway. The final case study reveals that mRNA encoding the BEL5 transcription factor is transported from leaves to the tips of stolons to activate the formation of potato tubers.

Chapter 11 (Gaupels and Vlot) provides an in-depth view into the challenging world of unraveling stress responses that are perceived locally, yet enhance systemic resistance in distant tissues by transmitting signaling compounds through the phloem. Topics such as the systemic wound response, systemic acquired resistance, and systemic acquired acclimation are coupled with an analysis of their associated systemic signals in response to biotic or abiotic stresses. Given the enormous diversity of candidates, the quest to identify more than a few stress signaling compounds continues to challenge researchers. Signaling can depend upon cell-specific information cascades operating in parallel or antagonistically that can be intertwined by reciprocal amplification and weakening along the phloem pathway.

The fourth and final section of the book demonstrates that sieve tubes not only provide avenues for integrative signaling but also offer rich resources and a transport system that is often exploited by other living organisms. The spectrum of organisms that successfully interact with plant vascular systems have evolved complex biochemical, structural, and in some cases behavioral mechanisms to exploit this nutrient-rich resource while coping, often unsuccessfully, with plant defense responses. Chapter 12 (Hagel, Onoyovwi, Yeung, and Facchini) sheds light on the secondary metabolism of phloem, which is a largely unexplored yet intriguing field in plant biology. In many plant species, sieve tubes contain repellents or toxic substances to combat animal predators. The cooperation between various cell types in phloem and intercellular trafficking among associated tissues is often required to synthesize these chemical deterrents. Specialized phloem structures such as latex-exuding laticifers and resin ducts provide physicochemical barriers as a significant line of defense against herbivores.

Many of the interactions that occur among phloem cells and associated tissues were initially revealed by studying phloem-mediated virus movement during systemic infections. Chapter 13 (Stewart, Ding, and Falk) focuses on the interrelations of viruses and viroids with the phloem in higher plants. Plant viruses utilize sieve tubes for systemic movement. Viruses that replicate in parenchyma cells encode specialized viral movement proteins that modify plasmodesmata to facilitate their intercellular movement into sieve tubes. In contrast, phloem-limited viruses are injected directly into sieve tubes or companion cells by phloem-feeding insects and multiply exclusively in phloem cells. Why these viruses remain confined to the phloem is not understood but indicates that PPUs and PDs between SE–CC and phloem parenchyma are of a different molecular nature. Specialization of these cellular connections is further demonstrated by viroids that also interact with the phloem but are able to pass this barrier during systemic infections.

Phytoplasms and spiroplasms are two fascinating groups of microbes that were recently discovered in sieve tubes. Chapter 14 (MacLean and Hogenhout) is one of the first reviews on the relationships between these fascinating bacterial organisms and the phloem. These unusual prokaryotes are inserted by phloem-feeding insect vectors directly into sieve tubes where they are transported into sink tissues to establish systemic infections. Several key metabolic pathways are lacking in these organisms and as a consequence, they rely heavily on the assimilate stream in the phloem to provide adequate nutrition. While effector proteins secreted directly into sieve tubes by these miniscule bacteria have significant effects in altering plant development and morphology, host plants appear to have defense mechanisms that can limit the development of the disease.

Phloem-feeding insects are a spectacular example of structural, biochemical, and physiological adaptation to parasitize the vascular tissues of plants. Most of these insect taxa utilize their highly modified mouthparts, called stylets, to penetrate through the weak pectin lamellae inside cell walls, puncturing and ultimately feeding from the sieve tubes. Chapter 15 (Will, Carolan, and Wilkinson) discusses the integral role of aphid saliva as the molecular interface between the insect and plant. Two types of saliva are involved aphid probing: gel saliva forms a flexible, lubricating, protective tube around the stylet tip during cell wall penetration, whereas aqueous saliva is secreted after cell puncture. Both saliva types are likely responsible for molecular interactions with host plants. Components of the aqueous saliva are only now becoming fully characterized; some molecules aid in establishing an effective feeding environment, while others could serve as a likely source of molecular effectors that trigger plant resistance.

The coevolution of plants and phloem-feeding insects has resulted in sophisticated biochemical and genetic mechanisms that govern their interactions. Genetic mechanisms that confer resistance to phloem-feeding insects are reviewed in Chapter 16 (Walling and Thompson). Insects inject virulence factors contained within their saliva that overcome the plant's innate immune response to establish a compatible interaction. Plant resistance (*R*) proteins are able to perceive and counteract the virulence factors allowing the perception of the insect and activating defenses that confer resistance to the phloem-feeding insect. Significant advances have been made in understanding *R* gene-mediated resistance against phloem-feeding insects and the deployment of signaling cascades to induce defense molecules.

In conclusion, phloem research has made a quantum leap forward since the publication of the classic phloem textbooks. While some of the questions in phloem physiology have been solved, new challenges continually emerge. Novel developments in research show that the phloem provides a plant-wide communication system that unites the capabilities of nervous, hormonal, and blood systems in animals.

2 Cell Biology of Sieve Element–Companion Cell Complexes

Rosemary G. White

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The distinct structure and central role of the phloem in long-distance transport have intrigued scientists ever since Malpighi (1686) observed continued growth in tissue immediately above a stem girdle and suggested that food transported downward from shoot to roots accumulated above the girdle and stimulated growth there (cited in Esau, 1969). Hartig (1837) was the first to define sieve tubes (Siebröhren) as the transporting cells in phloem (reviewed in Esau, 1939, 1969), and a century later, electron microscopy revealed their intricate ultrastructural details (Esau, 1969; Evert, 1990; Figure 2.1). Subsequent studies also revealed the diversity of phloem types, their interconnections, and their integration with surrounding tissues in a number of species (Esau, 1969; Gamalei, 1989; 1991; Kempers et al., 1998; Haritatos et al., 2000). Phloem ultrastructural and functional analysis slowed until recently (Thompson, 2006; Mullendore et al., 2010; Barratt et al., 2011), when advances in light and fluorescence microscopy combined with molecular approaches to study phloem function led to a renaissance in phloem cell biology (Martens et al., 2006; Thompson and Wolniak, 2008; Truernit et al., 2008; Fitzgibbon et al., 2010; Barratt et al., 2011; Xie et al., 2011). The study of phloem dynamics has been particularly challenging because this deeply buried tissue is predisposed to shut down transport with any perturbation (Knoblauch and van Bel, 1998; Imlau et al., 1999; Knoblauch et al., 2001; van Bel et al., 2002; Lalonde et al., 2003; Stadler et al., 2005; Knoblauch and Peters, 2010). This chapter reviews the fundamentals of phloem development and structure with particular focus on their intercellular connections.

Abbreviations: ATP, adenosine triphosphate; BS, bundle sheath; C, callose; CALS7, CALLOSE SYNTHASE 7; Ch, chloroplast; CC, companion cell; CLSM, confocal laser scanning microscopy/microscope; CW, cell wall; D, dictyosome; DT, desmotubule; DW, desmotubule wall; ER, endoplasmic reticulum; GFP, green fluorescent protein; GSL7, GLUCAN SYNTHASE-LIKE 7; IPM, plasma membrane inner leaflet; kDa, kiloDaltons; *knotted1*, mutant/nonfunctional knotted1; M, mitochondrion (pl. mitochondria); ML, middle lamella; MP, movement protein; mRNA, messenger RNA; PD, plasmodesma (pl. plasmodesmata); PL, plastid; PM, plasma membrane; PP, phloem parenchyma; PPC, phloem parenchyma cell; PPU, pore–plasmodesma unit; RNA, ribonucleic acid; RPP13-1, rice phloem protein 13-1; SE, sieve element; SEL, size exclusion limit; SER, sieve element reticulum; SP, sieve plate; SUC2, sucrose/proton transporter 2; SUT1, sucrose/proton transporter 1; TEM, transmission electron microscopy/microscope; UDP, uridine diphosphate; V, vacuole; VP, vascular parenchyma; XP, xylem parenchyma

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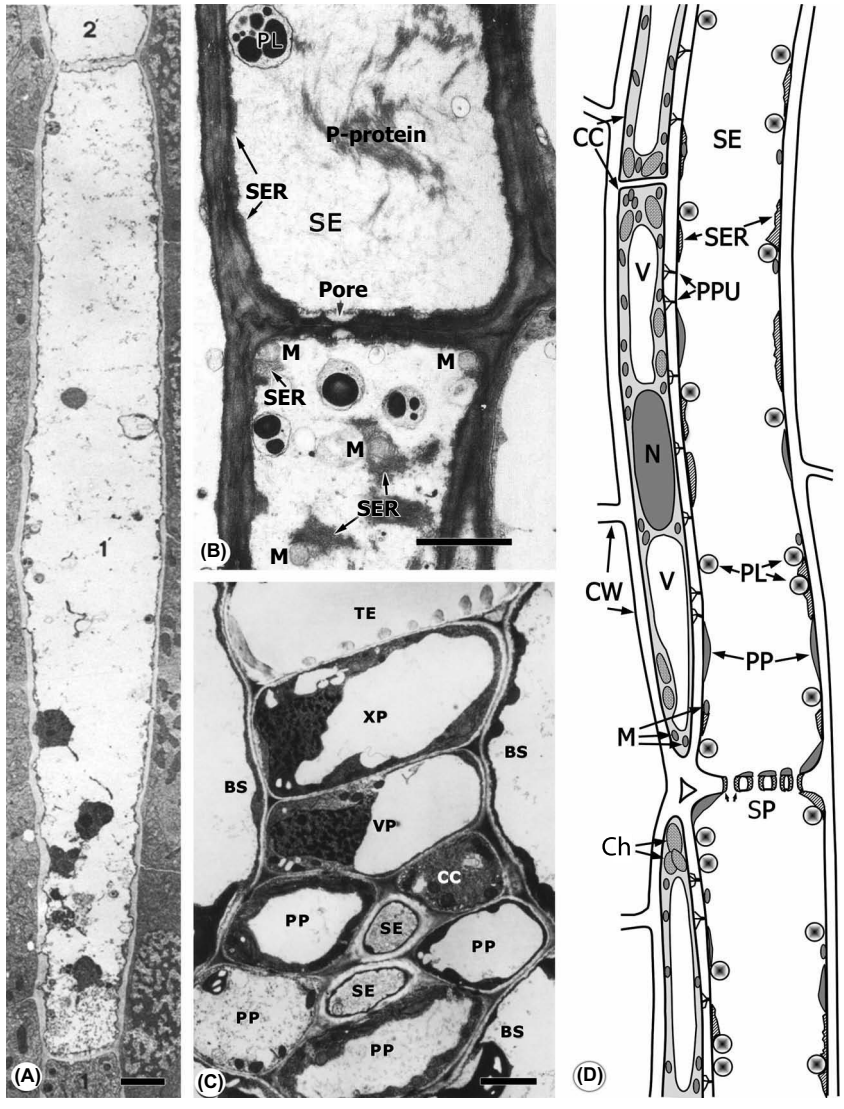

Figure 2.1 Ultrastructure of developing phloem sieve elements (SEs) and their organelles as seen in transmission electron microscopy (TEM). (A) Longitudinal section through an immature protophloem SE approximately 0.5 mm from the root tip of goatgrass (*Aegilops comosa* var. *thessalica*). Most of the cell contents have been degraded, with the exception of dark remnants of the nucleus near the base of the cell. Bar = 2 μm (Eleftheriou and Tsekos, 1982). (B) Longitudinal section through maturing SEs in a young stem of shieldleaf (*Streptanthus tortuosus*) showing remaining plastids (PL), sieve element reticulum (SER), and phloem protein (P-protein) inside the SE. A maturing sieve pore and remnant mitochondria (M) are visible in the lower SE. Bar = 1 μm (Sjölund, 1997). (C) Cross section of a minor vein from nonimporting leaf tissue of tobacco (*Nicotiana tabacum*) showing the arrangement of SE and companion cell (CC) surrounded by phloem parenchyma (PP) and vascular parenchyma (VP). Above the phloem is a tracheary element (TE) of the xylem and adjacent xylem parenchyma (XP) cell, all enclosed in bundle sheath (BS) cells. Bar = 2 μm (Ding et al., 1988). (D) Diagram of a longitudinal section of phloem CC and SE showing their relationship and typical components, including SER, pore–plasmodesma units (PPUs), PL, parietal phloem protein (P-protein), a few M, and sieve plate (SP) lined with callose (small arrows) in the SE. The CCs are shown with vacuoles (V), a nucleus (N), chloroplasts (Ch) and many more M in their cytoplasm. (Adapted from Knoblauch and van Bel, 1998.)

Development of the Sieve Element–Companion Cell Complex

One of the most intriguing aspects of the phloem is the connection between the living sieve tubes formed from interconnected sieve elements (SEs) and the sieve-tube control system provided by the intimately associated companion cells (CCs). In angiosperms, these two tightly linked but very different cell types derive from an unequal division of a fusiform mother cell (Esau, 1969; Behnke and Sjölund, 1990). One daughter cell develops into one or several metabolically active CCs possessing a dense cytoplasm and numerous mitochondria with well-developed cristae (Esau, 1969; van der Schoot and van Bel, 1989; van Bel, 1993; Sjölund, 1997; van Bel and Knoblauch, 2000; Evert, 2006; Figure 2.1). The other daughter cell goes through a controlled loss of most cellular components but survives as a living cell, sometimes for many years, in a dependent relationship with the CC (Esau, 1969; Wergin et al., 1975; Behnke, 1981; Evert, 1990; Raven, 1991; van Bel, 2003a, 2003b).

During SE maturation, the SE nucleus swells before gradually disintegrating, while the vacuolar membrane together with cytoskeletal elements, ribosomes, and Golgi bodies becomes reduced in number and eventually disappears (Esau, 1969; Behnke and Sjölund, 1990). At maturity, a highly modified cell remains that is composed of the plasma membrane (PM) and a thin layer of parietal cytoplasm with a few often dilated mitochondria (Evert, 1990), phloem-specific plastids (Behnke, 1991a), and, in all species except the grasses, phloem proteins (P-proteins; Cronshaw and Sabnis, 1990; Evert, 1990; Behnke, 1991b; Iqbal, 1995; van Bel 2003a). Depending on the species, the SE plastids contain protein filaments or crystals or amylopectin-rich starch grains (Behnke, 1991a). In angiosperms, the only structures within the lumen of a SE are the phloem-specific proteins arranged as filaments, tubules, or crystalline bodies. The major component of the remaining cytoplasm is the sieve element reticulum (SER); an elaborate peripheral meshwork of stacked or fenestrated endoplasmic reticulum (ER) that is free of ribosomes (Thorsch and Esau, 1981a,1981b; Sjölund and Shih, 1983).

Symplastic Connections to and within the Phloem

Early in development, the SE and CC have similar plasmodesmal contacts at the various interfaces with adjoining meristematic cells (Esau, 1939, 1969). During differentiation, all plasmodesmata (PDs) shut off in the developing SE–CC complexes (SE–CCs; van Bel and van Rijen, 1994; Ehlers and van Bel, 2010), which appears to be common in differentiating meristematic cells (Ehlers et al., 1999; Ruan et al., 2001, 2004; Kim 2007) as a prerequisite for autonomous development without interference by neighboring cells (Pfluger and Zambryski 2001; Kim et al., 2005). At maturity, the SE–CCs are linked by highly specialized PD connections to surrounding tissues, recognizable by their different ultrastructural modifications, detailed in Section “Connections between SE–CCs and Surrounding Cells”.

Origins and Development of Plasmodesmata

Plasmodesmata, the symplastic connections between plant cells, are inserted into the new cell wall that forms between daughter nuclei during the final stage of cell division. Nascent PDs are initiated when strands of ER become trapped among the coalescing vesicles of the forming cell plate (Hepler, 1982; Overall et al., 1982). As the new cell wall thickens and solidifies, each captured ER strand becomes compressed into a tight tube known as the desmotubule that links the ER networks of the two adjacent cells (Figures 2.2A and B). Each desmotubule is surrounded by a thin layer of cytoplasm,

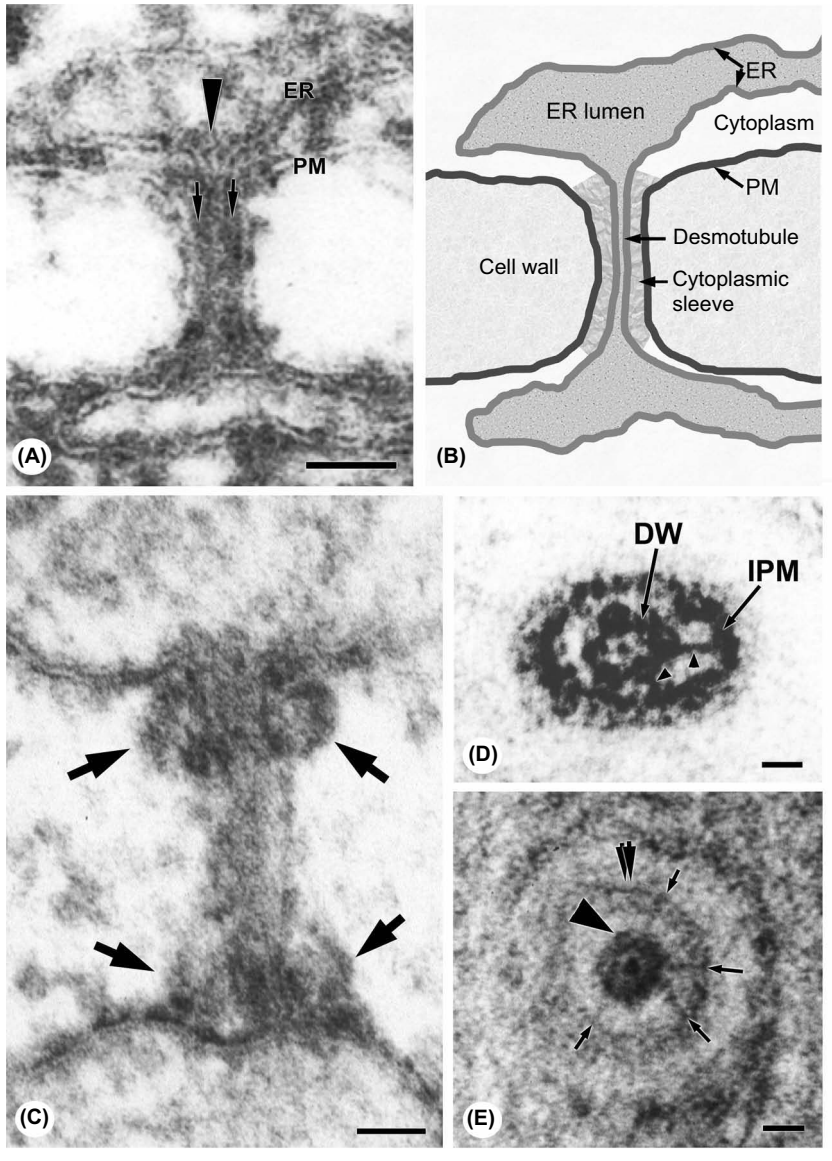

Figure 2.2 Ultrastructure of plant plasmodesmata (PDs) revealed by transmission electron microscopy (TEM) analysis of intact tissues. (A) Longitudinal section of a single mosquito fern (*Azolla pinnata*) PD, showing cytoplasmic endoplasmic reticulum (ER) narrowing into the desmotubule (DT; large arrowhead), which links the ER of two adjacent cells. The plasma membrane (PM) and cytoplasmic sleeve (small arrows) are also continuous from cell to cell. Bar = 50 nm (Overall et al., 1982). (B) Diagram highlighting PD components, showing how the ER narrows into the DT within the cytoplasmic sleeve of the PD, all enclosed in the PM. (Adapted from White and Barton, 2011.) (C) Longitudinal section showing a young onion (*Allium cepa*) root PD with electron-dense material (arrows) in the cell wall around the neck region. Bar = 50 nm (Radford et al., 1998). (D) Transverse section of tobacco leaf PD, showing particles possibly attached to or embedded in both desmotubule wall (DW) and PM (IPM, inner plasma membrane) and connected by strands (arrowheads) across the cytoplasmic sleeve. Bar = 10 nm (Ding et al., 1992). (E) Transverse section of a PD in Brazilian waterweed (*Egeria densa*) in which particles (e.g., large arrowhead) are mainly associated with the DT, and strands (arrows) appear to link the DT and PM (double arrow). The dark central core of the DT is prominent. Bar = 10 nm (Overall and Blackman, 1996). (*Continued*)

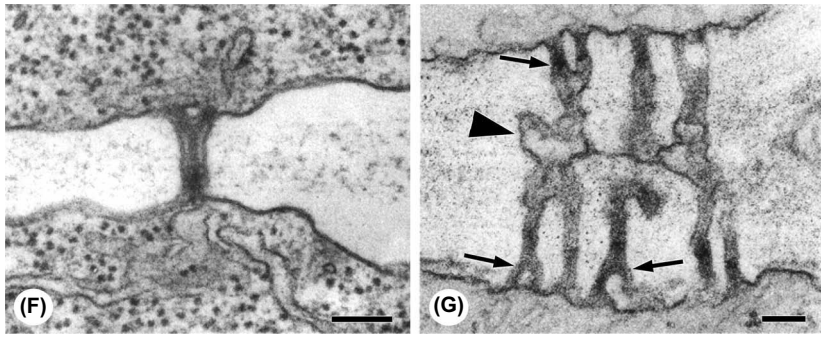

Figure 2.2 (*Continued*) (F) A branched PD from black nightshade (*Solanum nigrum*) showing branching in the younger layers of the plant cell wall. Bar = 100 nm (Ehlers and Kollmann, 1996). (G) A complex branched PD from broad bean (*Vicia faba*) pith cells with several plasmodesmal strands, some showing recent branching (arrows), which merge in a median branching plane, which is dilated to form a central cavity (large arrowhead). Bar = 100 nm (Ehlers and Kollmann, 2001).

and the desmotubule and its cytoplasmic sleeve are enclosed in PM (Hepler, 1982; Overall et al., 1982; reviewed in Maule, 2008), which is derived from the phragmoplast vesicle membranes. In this way, the ER, cytoplasm, and PM are continuous from cell to cell. In young PDs, the membranes form straight concentric cylinders, and either at this stage or later in development extracellular proteinaceous structures may be detected at each end of the PD, termed the "neck," where the PD cytoplasmic sleeve joins the cytoplasm of the connected cells (Robards, 1976; Mollenhauer and Morré, 1987; Olesen and Robards, 1990; Tilney et al., 1991; Badelt et al., 1994; Waigmann et al., 1997; Overall, 1999; Heinlein and Epel, 2004; Figure 2.2C). As PDs mature, they usually gain an extracellular collar of callose, a β-1,3-linked glucan polymer. Older PDs often become branched to varying degrees and may develop a median cavity within the interconnecting cell wall, in which the desmotubule and cytoplasmic sleeve are enlarged (Lucas et al., 1993; Oparka et al., 1999; Roberts et al., 2001; Figures 2.2F and G).

These ultrastructural changes usually coincide with changes in the size and type of molecules that will readily traffic from cell to cell (Oparka et al., 1999; Overall, 1999). Young PDs may allow passage of proteins up to 54 kDa (kilo-Daltons) in size (Kim et al., 2005), and the youngest phloem elements in *Arabidopsis* roots certainly allow efflux of green fluorescent protein (GFP) (27 kDa) into surrounding tissues (Imlau et al., 1999; Stadler et al., 2005). As they mature and become more complex in structure, PDs generally close to allow only fairly small molecules, approximately 0.6–1.0 kDa in size, free passage from cell to cell (reviewed in Lucas et al., 2009). Some signaling molecules, such as transcription factors (e.g., KNOTTED1) (Jackson et al., 1994 or SHORTROOT, Nakajima et al., 2001), move across one or more cell boundaries in more mature tissue, and certain cell boundaries allow one-way traffic of proteins as large as GFP (Christensen et al., 2009). The largest molecule that can move through PDs defines their size exclusion limit (SEL), and although molecular weight does not correlate exactly with molecular size, this is usually how PD SEL is characterized.

Ultrastructural analyses show that either or both of the PM and ER of PDs may be lined with electron-dense structures, interpreted to be macromolecular components (Overall et al., 1982; Ding et al., 1992; Overall, 1999; Oparka, 2004; Roberts, 2005; Benitez-Alfonso et al., 2010; Faulkner and Maule, 2011; Radford and White, 2011; White and Barton, 2011; Figures 2.2D and E). Although none of these structures have been unequivocally resolved, they are likely to comprise one or more

cytoskeletal proteins, adenosine triphosphatases (ATPases) and ribonucleic acid (RNA) unwinding proteins, plus a range of PD-specific proteins that are slowly being identified (reviewed in Faulkner and Maule, 2011). As yet unidentified PD proteins are proposed to actively regulate PD permeability and may be involved in loading and unloading cargo at each end of the PD. The PDs of phloem CCs and SEs undergo specific structural modifications and changes in their SEL that are likely to involve insertion and/or deletion of regulatory proteins, depending on the cell types they are connecting.

Connections between SE–CCs and Surrounding Cells

Symplastic connections between the SE–CCs and surrounding phloem parenchyma cells (PPCs) are generally absent or very restricted (Fisher, 1986; van Bel and Kempers, 1991; Wimmers and Turgeon, 1991; Botha, 1992; Botha and van Bel, 1992; van Bel and van Rijen, 1994; Botha et al., 2000; van Bel, 2003a). Isolation of the SE–CC in transport phloem presumably limits symplastic leakage of solutes into surrounding tissue, which is reflected in changes in PD structure, distribution, and SEL (Kempers et al., 1998; Ehlers and van Bel, 2010). PDs are very rare at the SE–PPC interface and tend to be single and unbranched, while PDs at the CC–PPC interface are sparse and occasionally branch at the CC side (Botha and van Bel, 1992; Kempers et al., 1998).

Even those PDs present appear less permeable than other, ultrastructurally similar PDs connecting, for example, mesophyll tissues (Kempers et al., 1998). Certain proteins normally found exclusively within the phloem, such as P-proteins (Balachandran et al., 1997; Clark et al., 1997) and thioredoxin h (Ishiwatari et al., 1995, 1998), can increase the SEL of PDs between tobacco (*Nicotiana tabacum*) mesophyll cells and themselves move from cell to cell. However, they appear unable to either increase the SEL or move through PDs connecting CCs to surrounding cells, except to their associated SEs. This suggests that the PDs around the SE–CC may not be modified readily by endogenous movement factors, preventing protein escape from the SE–CC, and this may also prevent, or at least restrict, invasion of the phloem by viruses in the mesophyll (Leisner and Turgeon, 1993; Oparka and Turgeon, 1999).

Sieve Pores—Connecting Sieve Elements into Sieve Tubes

SEs in primary phloem are usually in vertical files with mostly transverse connecting walls, whereas SEs in later-formed secondary phloem are often more fusiform with oblique abutting walls that provide a larger contact area (Esau, 1939, 1969). PDs in the transverse walls connecting two SEs enlarge to form pores of up to 1 μm in diameter, and this porous wall is termed a sieve plate (Figures 2.3A, B, C, and D; see Chapter 7). The SE PM is continuous through the sieve pores with a net-like ER residing at the pore margins (Esau, 1969; Evert, 1990). Pore width must accommodate the rapid mass transit of photosynthates through SE files as well as numerous large and small proteins, ribonucleoprotein (RNP) complexes, RNAs, and other compounds that have been identified in sieve-tube sap (see Chapters 8 and 9). The wide pores in sieve plates facilitate rapid transport, but their very interconnectivity presents a potential point of vulnerability to the vascular system. Elaborate protective mechanisms have evolved to occlude the sieve pores to prevent the loss of energy expensive metabolites when sieve tubes are damaged (see Chapter 7).

One such mechanism is callose deposition, which plays a central part in sealing of damaged SEs (for details and information on the role of P-proteins, see Chapter 7). Callose is especially prominent at the sieve plates of wounded sieve tubes where it forms callose collars around the

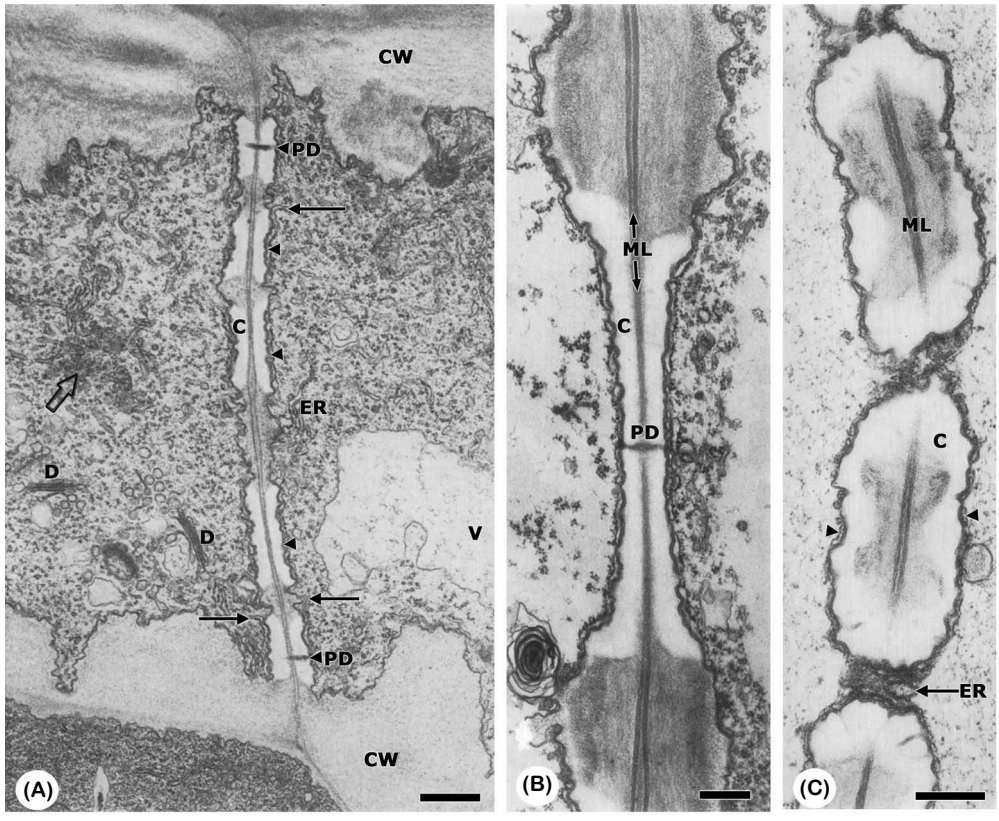

Figure 2.3 Developing and mature sieve plates in wild-type and mutant phloem, showing the importance of callose (C) in sieve pore development, and a mature pore–plasmodesma unit (PPU), seen in transmission electron microscopy (TEM) (A, B, C, D, G, and J) and confocal laser scanning microscopy (CLSM). (A) Longitudinal section of portions of two immature sieve elements (SEs) of pumpkin (*Cucurbita maxima*) with a differentiating sieve plate between them. Future sieve pores contain abundant C in bright sieve-plate regions and are traversed by plasmodesmata (PDs). The endoplasmatic reticulum (ER) is associated with ribosomes (ER), and also localized along the plasma membrane (PM; arrowheads) and leads away from the PM at or near noncallosic sites (long arrows). Dictyosomes (D) are seen in cross section and ER cisternae (open arrow) in glancing section in these still active cells, in which vacuoles (V) are beginning to develop. CW = longitudinal wall of the SE. Bar = 1 μm (Esau and Cronshaw, 1968). (B) A section through a young pumpkin sieve plate shows a single sieve-plate pore site with C deposits and a central plasmodesma (PD), which is somewhat enlarged in the middle lamella (ML) region. Bar = 0.5 μm (Esau and Cronshaw, 1968). (C) Sieve pores in a mature sieve plate of pumpkin have a C lining, with ER close to the PM (arrowheads) and continuity of ER through one of the pores (arrow). ML = middle lamella. Bar = 0.5 μm (Esau and Cronshaw, 1968). (*Continued*)

pores, which become plugged with P-proteins (Esau and Cheadle, 1961; Evert and Derr, 1964; Eschrich, 1975; Furch et al., 2007; Mullendore et al., 2010). Early experiments showed that phloem transport is reduced by callose-inducing treatments (Scott et al., 1967; McNairn and Currier, 1968), and SE damage can block phloem transport within seconds (Currier, 1957; Eschrich, 1965). More recent evidence shows that callose synthesis is sufficiently fast for full occlusion of the sieve plates almost instantly (Nakashima et al., 2003) or within minutes (Furch et al., 2010; Mullendore et al., 2010). Furthermore, phloem transport can resume within less than 1 hour with rapid degradation of wound-induced callose (Currier and Webster, 1964; Meier et al., 1981; Iglesias and Meins, 2000; Bucher et al., 2001; Nakashima et al., 2003; Furch et al., 2007).

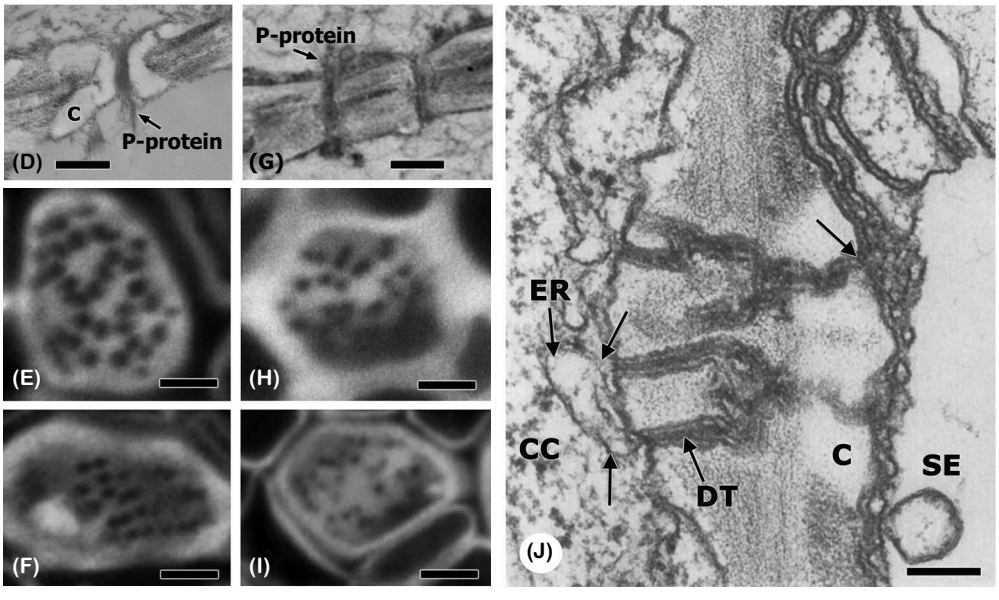

Figure 2.3 (*Continued*) (D) Single pore in a sieve plate of *Arabidopsis* phloem, with C lining and P-protein trapped within. Bar = 0.5 μm (Barratt et al., 2011). (E), (F) In two representative sieve plates from *Arabidopsis*, the pores can be seen by observing cellulose fluorescence using a CLSM. Bars = 2 μm (Barratt et al., 2011). (G) Two pores in a sieve plate from *Arabidopsis* lacking a phloem-specific callose synthase (GSL7) showing absence of C but containing P-protein. Bar = 0.5 μm (Barratt et al., 2011). (H), (I) Two representative sieve plates from the *gsl7* mutant of *Arabidopsis* showing smaller sieve pore diameters, detected by cellulose fluorescence on a CLSM. Bars = 2 μm (Barratt et al., 2011). (J) Highly modified PDs form the PPUs between a companion cell (CC) and mature SE in pumpkin. Arrows indicate association of ER membranes with the desmotubule (DT) within each branch of the PPU. C is deposited around the much less-branched SE side of the PPU strands. Bar = 0.2 μm (Esau and Cronshaw, 1968).

The synthesis and degradation of callose also plays a prominent role in sieve pore development, but its precise function and the details of its appearance in SE maturation are not well understood. Callose in young SEs is deposited on the nascent sieve plates (Esau, 1969; Evert, 1990) and accumulates along the PM in the wall around the PDs (Figures 2.3A, B, and C). Some researchers speculate that callose deposition replaces cellulose around the developing pore to facilitate pore maturation through subsequent callose degradation (Esau and Thorsch, 1985; Thorsch and Esau, 1988). An alternate view is that pore formation requires degradation of the original cellulosic cell wall together with some of the nascent callose. In this case, callose deposition could prevent further deposition of the cellulosic wall around the pore (Evert et al., 1966; Deshpande, 1974, 1975).

Recent analyses of a mutant *Arabidopsis* line lacking the phloem-specific CALLOSE SYNTHASE 7 (CALS7; Xie et al., 2011) (or GLUCAN SYNTHASE-LIKE 7, GSL7; Barratt et al., 2011) have convincingly demonstrated that callose synthesis is essential for correct pore formation. The transport channels in *cals7* mutant and wild-type sieve plates appear similar in dimension, but the mutants have cellulosic material around the channels rather than the usual callose lining (Barratt et al., 2011; Xie et al., 2011; Figures 2.3D and G). Phloem transport in the *cals7* mutants is reduced and plants are dwarfed (Barratt et al., 2011; Xie et al., 2011), and fluorescence staining for cellulose showed fewer and narrower sieve pores in the *cals7* mutant (Figures 2.3E, F, H, and I). This suggests that the cellulose-lined, callose-deficient pores in the *cals7* mutants cannot be modified sufficiently to accommodate normal phloem flow rates. Moreover, the *Arabidopsis cals7* mutants lacking sieve

pore callose also show very reduced callose production in response to phloem wounding (Barratt et al., 2011; Xie et al., 2011). *Arabidopsis* mutants lacking the phloem-specific sucrose synthases SUS5 and SUS6, which are tightly coexpressed with CALS7 (Barratt et al., 2009), also have reduced callose linings around sieve pores, although double mutants show no phenotypic effects (Bieniawska et al., 2007; Barratt et al., 2009).

Mature sieve pores usually have a callose lining (Bouck and Cronshaw, 1965; Deshpande, 1974, 1975; Thorsch and Esau, 1988; Eleftheriou, 1990; Sjölund, 1997; Ehlers et al., 2000; van Bel et al., 2002; Figures 2.3C and D), and the *cals7* mutant phenotype suggests that as in other PDs (Iglesias and Meins, 2000; Bucher et al., 2001; Nakashima et al., 2003) the thickness of this callose lining could regulate sieve pore permeability. It is clear that the size of sieve plate pores and the effects on phloem transport of pore narrowing by callose have a major impact on plant growth and development, although callose regulation *in vivo* is still not well understood.

Modified Plasmodesmata between Sieve Elements and Companion Cells: The Pore–Plasmodesma Units

Molecules enter and exit sieve tubes through the highly specialized PDs that join SEs and CCs. During differentiation and maturation of the SE–CC, the simple PD connections between CCs and SEs become modified to form pore–plasmodesma units (PPUs) (van Bel and Kempers, 1997; van Bel, 2003a; Figure 2.3J). The PDs become elaborated on the CC side with up to 100 branches linking to a central lacuna (Esau, 1969; Evert, 1990), while on the SE side, the PDs widen to form a single or, at most, a few simple pores (Esau, 1969; Kempers et al., 1998). Tubules of ER appear to traverse the individual branches of PPUs (Ding et al., 1993), but details of the central cavity have not been resolved (van Bel and Kempers, 1997; van Bel et al., 2011a).

It is assumed that the PPU branches are similar to other PDs in their basic structure, but how the initially symmetric PDs develop into the highly asymmetric PPUs is unknown. The CC-side elaboration may be similar to the PD "twinning" seen at the base of trichomes as PDs are added to enlarging cell walls (Faulkner et al., 2008) and in cambial tissues (Ehlers and van Bel, 2010) or to the increased branching and central cavity formation seen during elaboration of other types of secondary PDs (Oparka et al., 1999; Ehlers and Kollmann, 2001; Burch-Smith et al., 2011).

The wider pore on the SE side might arise from a process similar to the final pore widening in the sieve plate, with a thicker callose sleeve in the wall and slightly enlarged central strand of ER. It would be interesting to determine whether the PPUs are widened as usual on the SE side in the *cals7* mutants; if phloem loading is symplastic, the narrower PPU pores may further restrict phloem transport capacity. In any event, the highly asymmetric ultrastructure of PPUs presumably reflects a compositional and functional asymmetry in molecular exchange between CCs and SEs, whose implications are considered in detail in Section "Transport between Sieve Element and Companion Cell."

Other Specialized Features of Sieve Elements

The Sieve Element Reticulum

The SER is a specialized system of smooth ER in mature SEs (Hébant, 1977; Oparka and Johnson, 1978; Thorsch and Esau, 1981a, 1981b; Behnke, 1983; Evert, 1984; Figures 2.4A, B, and C). Confocal laser scanning microscope images (Knoblauch and van Bel, 1998; Thompson and Wolniak,

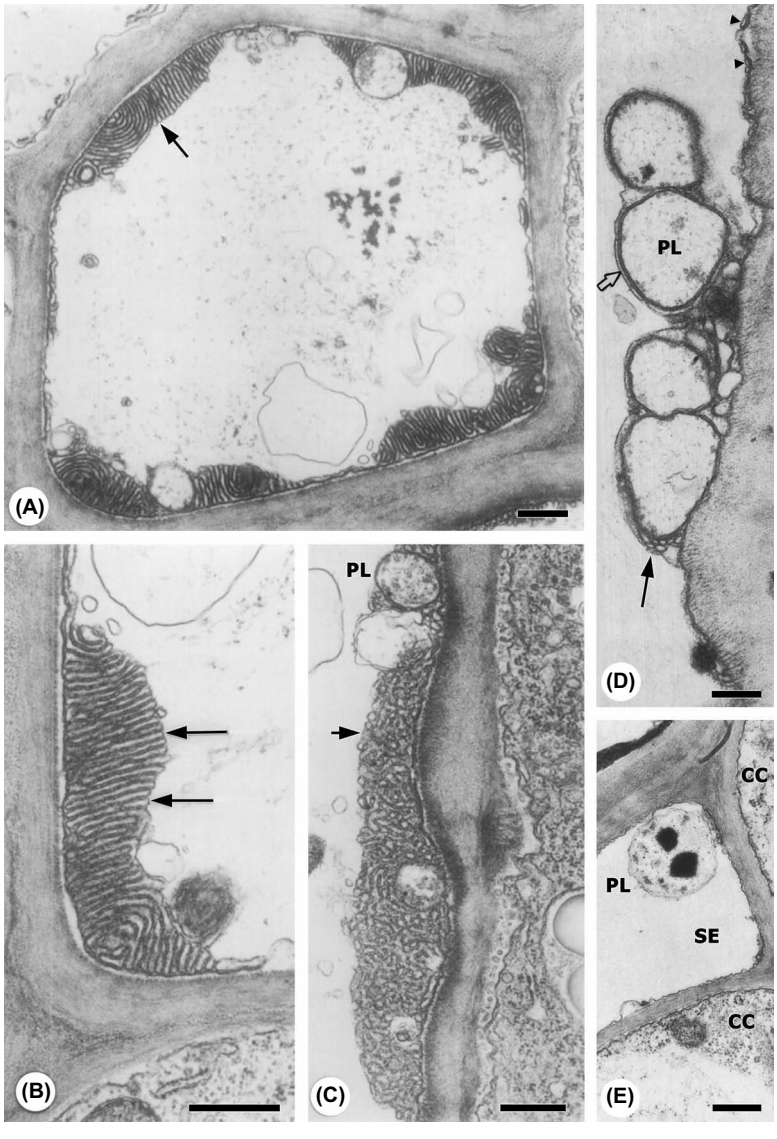

Figure 2.4 Sieve element reticulum (SER) and plastids (PL) in mature phloem, examined by TEM. (A) A mature sieve element (SE) of cotton (*Gossypium hirsutum*) in cross section showing stacked SER oriented perpendicular to the cell wall (arrow). The SER covers much of the PM. Bar = 0.5 μm (Thorsch and Esau, 1981a). (B) Higher magnification of a portion of the SE shown in (A). Arrows indicate individual cisternae of the SER. Bar = 0.5 μm (Thorsch and Esau, 1981a). (C) Convoluted SER (arrow) in a longitudinal section of a mature SE of cotton. The innermost cell wall layer has the dense fibrillar appearance characteristic of nearly mature and mature SE. Bar = 0.5 μm (Thorsch and Esau, 1981a). (D) PL next to the plasma membrane of a mature SE of pumpkin (*Cucurbita maxima*). They may be enclosed by a membrane (open arrow) and have adjacent SER (long arrow). Bar = 0.5 μm (Esau and Cronshaw, 1968). (E) A PL with crystalline inclusions in a mature SE of goatgrass (*Aegilops comosa* var. *thessalica*). CC = companion cell. Bar = 0.5 μm (Eleftheriou and Tsekos, 1982).

2008; Fitzgibbon et al., 2010) and electron micrographs (Esau, 1969; Evert, 1990) show a strictly parietal position of the SE components. In translocating SEs, the SER appears to be held in place by 7-nm-long macromolecular extensions that also anchor mitochondria and plastids (Figures 2.4D and E) to each other and to the PM (Ehlers et al., 2000).

Within mature SEs, the SER forms a layer of cisternae that covers almost the entire PM with deep stacks of ER (Esau and Gill, 1971; Thorsch and Esau, 1981a; Sjölund and Shih, 1983) or circular openings (fenestrations) of approximately 80 nm in diameter (Esau and Cronshaw, 1968; Sjölund and Shih, 1983). The space between the PM and the SER may allow retention of molecules that would otherwise be carried along in mass flow. Most of the stacked SER is closely associated with SE plastids and mitochondria, some of which are completely enclosed by the ER (Thorsch and Esau, 1981b; Ehlers et al., 2000). Unusual SER is seen in waterlily (*Nymphaea* species), which have ER-derived tubules with diameters of 100–200 nm in mature SEs (Behnke 1996). Other unusual ER-derived structures in mature SEs include short curved tubules in weeping fig (*Ficus benjamina*) (Behnke, 1989) and short straight tubules of 35–40 nm diameter in various palms (Parthasarathy, 1974a, 1974b). The ER is a remarkably dynamic and adaptable membrane system, with different conformations achieved by rather subtle modifications in membrane protein or lipid composition and surface charge (Borgese et al., 2006; Shibata et al., 2006), and similar modifications may underlie SER conformation.

Although some investigators have considered the SER to be inactive (Wooding 1967), its retention in mature SEs and elaborate structure speak to some essential function in sieve tubes. In some species, the SER has acid phosphatase activity, suggesting a role in the final autolysis of the SE cytoplasm (Zee, 1969; Esau and Charvat, 1975; Bentwood and Cronshaw, 1976; Oparka et al., 1981). In other species, nucleoside phosphatase activity (Bentwood and Cronshaw, 1978; Cronshaw, 1980; Sjölund and Shih, 1983; Evert, 1984; Arsanto, 1986) has led some investigators to suggest a role in phloem loading. Other suggested functions include calcium storage (Sjölund, 1990) and regulation of SE Ca^{2+} concentration, since very high concentrations of calcium can be maintained near the SE PM compared with calcium levels in the SE lumen (Furch et al., 2009; van Bel et al., 2011b). Calcium release may play an important role in wound signaling, and is known to induce, and be essential for, synthesis of wound-induced callose in SE (King and Zeevart, 1974), and around PD in general (Botha et al., 2000; Iglesias and Meins, 2000; Roberts and Oparka, 2003) (further details in Chapters 6 and 7).

The SER organization as a highly layered or fenestrated membrane system appressed to the PM suggests retention of sufficient surface area for physiological activities, similar to layered Golgi membranes or stacks of thylakoid membranes in chloroplast grana. Clearly, the physiological functions of the SER system remain to be fully elucidated.

Cytoskeletal Proteins in Mature Sieve Elements

Differentiating SEs do contain filamentous structures resembling actin-like filaments or filament bundles (Cronshaw and Esau, 1967; Evert, 1990; Parthasarathy and Pesacreta, 1980; Pesacreta and Parthasarathy, 1984; Figures 2.1B and D), and at one time, it was suggested that these filaments may actively participate in long-distance transport (Thaine, 1969; MacRobbie, 1971), but this was subsequently shown not to be the case (Williamson, 1972). In the widely accepted mass-flow theory of phloem transport, outlined in Chapters 1 and 7, there is no direct role for the cytoskeleton, although recent evidence points to some role in regulating transport. Both actin and myosin were detected at the sieve pores in phloem of hybrid aspen (*Populus tremula* × *Populus tremuloides*),

horse chestnut (*Aesculus hippocastanum*) and stone pine (*Pinus pinea*), and suggested to participate in gating of the pores (Chaffey and Barlow, 2002). Application of actin or microtubule disrupters to the isolated root vascular cylinder (Guo et al., 1998) or to isolated phloem strands (Yang et al., 2007) reduced the flux of radiolabeled sucrose into pea roots (Guo et al., 1998) or radish roots (Yang et al., 2007). It is unclear whether the SEs themselves or the function of the CCs was affected, but these results suggest that an intact cytoskeleton is essential for phloem function.

Immediately after division, both young CCs and SEs contain a full complement of cytoskeletal proteins, and even at maturity, sieve-tube exudate from several species (wheat, *Triticum aestivum*; rice, *Oryza sativa*; Adam's needle, *Yucca filamentosa*; castor bean, *Ricinus communis*; pumpkin, *Cucurbita maxima*; black locust, *Robinia pseudoacacia*; large-leaved linden, *Tilia platyphyllos*) contains readily detectable actin and actin-binding proteins (Schobert et al., 1998; Kulikova and Puryaseva, 2002; Lin et al., 2009). In some cases, filamentous components of exudate have been shown to bind to myosin (Kulikova and Puryaseva, 2002), suggesting the possibility of active actin–myosin motility, but in other cases, this was not observed (Williamson, 1972).

The actin-sequestering monomer, profilin, has also been identified in sieve-tube exudates from the same species listed previously (Schobert et al., 1998; 2000). Profilin could regulate actin dynamics in sieve tubes and perhaps attenuate wound-induced actin polymerization (La Claire, 1989; Foissner et al., 1996; Foissner and Wasteneys, 1997). The absence of readily identified actin microfilaments in transmission electron microscopy (TEM) or fluorescence micrographs of mature SEs could be a result of unconventional assemblage similar to what has been observed in the nuclear cytoskeleton, which is now known to include actin, profilin, and myosin (Cruz and Moreno Díaz de la Espina, 2009). There have been few immunological studies of phloem to identify cytoskeletal elements, so this remains an avenue for future analysis.

Microtubules are generally absent from mature SEs (Toth et al., 1994), but can be associated with the SER stacks in a highly ordered fashion even at later stages of SE maturation (Esau and Hoefert, 1980; Thorsch and Esau, 1981b) and have been observed in mature SEs of horse chestnut (Chaffey et al., 2000). Transcriptomic analyses of isolated celery (*Apium graveolens*) phloem strands (Vilaine et al., 2003), phloem exudates from castor bean (Doering-Saad et al., 2006) and pumpkin (Ruiz-Medrano et al., 2011), and phloem plugs from several ash (*Fraxinus*) species (Bai et al., 2011) have also identified transcripts encoding microtubule-associated proteins, α- and β-tubulins, myosin, and the actin-binding protein actin depolymerizing factor. Further work is required to confirm the presence of intact cytoskeletal elements within SEs and to determine their function, particularly any role in cell-to-cell transport.

Transport between Sieve Element and Companion Cell

The entry of larger molecules into SEs occurs mainly via the PPUs from the CCs. This exchange, and the very active membrane-based loading and unloading of solutes, discussed in Chapters 3, 4, and 5, requires energy to drive the membrane transporters as well as to provide sustained proton export for energizing of carrier-mediated uptake and retrieval of metabolites. Blocking sugar catabolism in CCs inhibits sugar uptake or retrieval by the SE–CC, greatly impairing phloem loading and causing considerable assimilate loss along the translocation pathway (Lerchl et al., 1995; Geigenberger et al., 1996). Also essential is a constant supply of substrates for callose synthesis and turnover. Sucrose synthase specifically located to CCs is required to provide sufficient uridine diphosphate (UDP)-glucose for callose biosynthesis (Martin et al., 1993; Nolte and Koch, 1993). The mode of energy supply is less clear. Since the interface between SEs and CCs may be only

25% of the SE surface in transport phloem, and sugars may escape from areas not covered by CCs, ATP produced by CCs may supply the SEs to support membrane ATPases (Lehmann, 1979; van Bel, 2003a).

Long-term maintenance of the differentiated SE after it has lost the capacity for protein synthesis requires that essential macromolecules, especially proteins, be continually supplied from the CC. Turnover of phloem-specific proteins was demonstrated in CCs using ^{35}S-methionine labeling (Fisher et al., 1992; Sakuth et al., 1993), and many P-proteins are produced in the CCs that accumulate in SEs (Bostwick et al., 1992; Dannenhoffer et al., 1997; Thompson, 1999), indicating that the PPUs allow passage of larger molecules than are generally trafficked through mature PD in other tissues. More recently, most of the components essential for both protein assembly and breakdown have been detected in sieve-tube exudates, suggesting that the SEs are not simply passive carriers of photosynthates and signals, but play an active role in protein turnover to regulate their protein complement (Lin et al., 2009).

Of further interest is the asymmetry of protein components that could regulate transport between the CC and SE. For example, the presence of cytoskeletal proteins such as actin and myosin in PDs (reviewed in White and Barton, 2011) raises interesting questions. If the PPUs contain cytoskeletal elements within the tubules on the CC side, does the actin terminate in the central cavity or does it continue through to the SE? If cytoskeletal elements are present throughout the PPU, does the cytoskeleton then play a role in regulating transport between CC and SE?

Certain SE- and CC-specific proteins do show asymmetries in distribution through the PPU that could reflect function. The leaf sucrose transporter sucrose/proton transporter 1 (SUT1) is a high-turnover protein that is essential for phloem loading and long-distance assimilate transport and is found primarily in the SE PM of tobacco (*N. tabacum*), potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*). In contrast, SUT1 messenger RNA (mRNA) synthesized in CCs is detected in both SEs and CCs and is most abundant at the orifices of the PPUs between the two cell types (Kühn et al., 1997; Kühn, 2003; see also Chapters 5 and 8).

Cytosolic Transport through PPUs

Most discussions of trafficking through PPUs focus on macromolecules with the assumption that small solutes are exchanged by diffusion through the cytoplasmic channels and then transported indiscriminately in the translocation stream. However, an analysis of several endogenous and exotic solutes synthesized in CCs of minor veins showed that while small metabolites enter SEs by diffusion, their selective loss via carrier-mediated membrane transport together with nonselective diffusion out of the SE could either retain a compound for long-distance transport or rapidly clear it from the translocation stream (Ayre et al., 2003; van Bel et al., 2011a).

As well as photosynthates, many of the proteins synthesized in the CCs appear able to move into SEs by passive diffusion even though some are larger than 100 kDa, and they can be specifically targeted to the SE (Bostwick et al., 1992; Fisher et al., 1992; Sakuth et al., 1993; Clark et al., 1997; Dannenhoffer et al., 1997; Kempers and van Bel, 1997; Imlau et al., 1999; Oparka et al., 1999; Fisher and Cash-Clark, 2000; Lin et al., 2009; see also Chapters 8 and 9). Indeed, considering the size of macromolecules exchanged from CC to SE, PPUs were predicted to have a large diameter (Fisher et al., 1992). In living phloem, fluorescently tagged macromolecules in the order of 20–30 kDa were seen to transfer across the PPUs (Balachandran et al., 1997; Kempers and van Bel, 1997), and the 27 kDa GFP, expressed in CCs of tobacco and *Arabidopsis*, could move into the SEs and migrate along the sieve tubes (Imlau et al., 1999). Furthermore, when expressed under the CC-specific