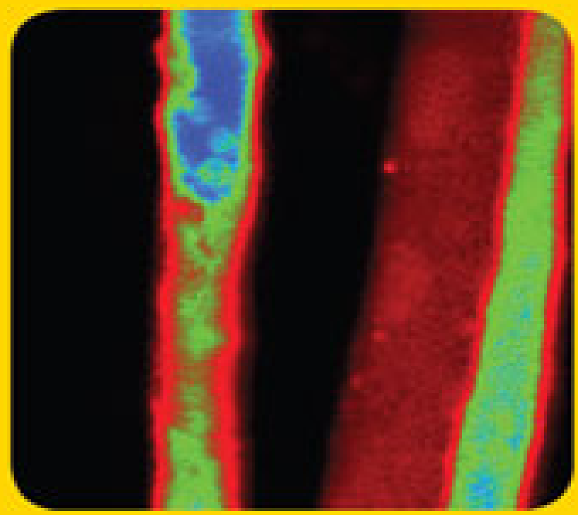
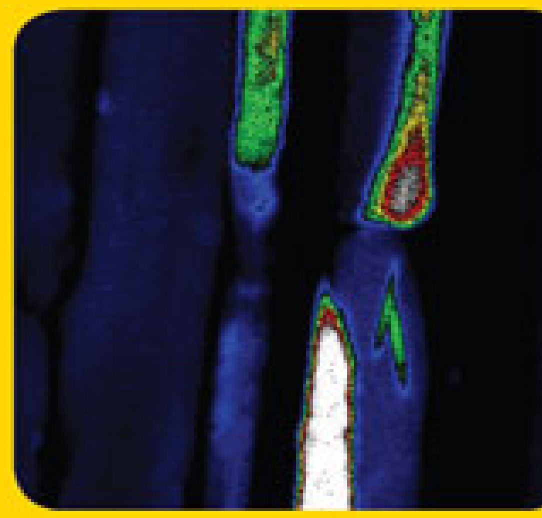
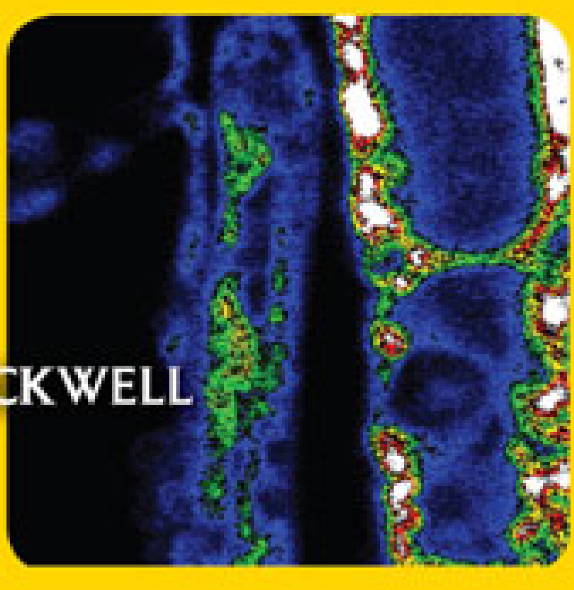
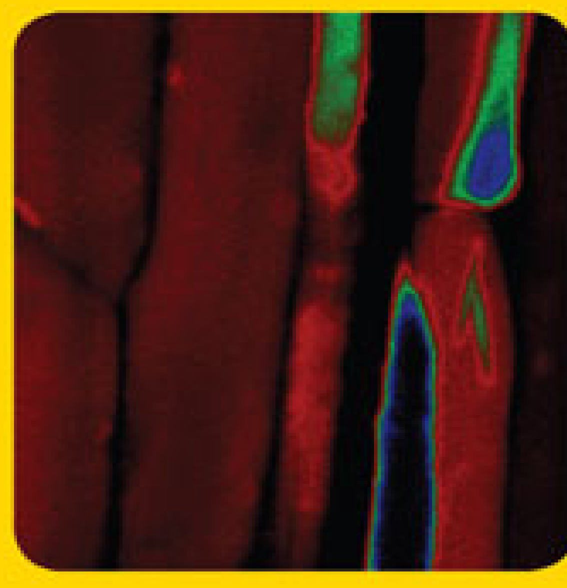
PHLOEM

MOLECULAR CELL BIOLOGY, SYSTEMIC COMMUNICATION, BIOTIC INTERACTIONS

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

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Contents

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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

Aart J.E. van Bel[1] and Gary A. Thompson[2]

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By the end of the nineteenth[1] 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.

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.

1. *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

2

Cell Biology of Sieve Element–Companion Cell Complexes

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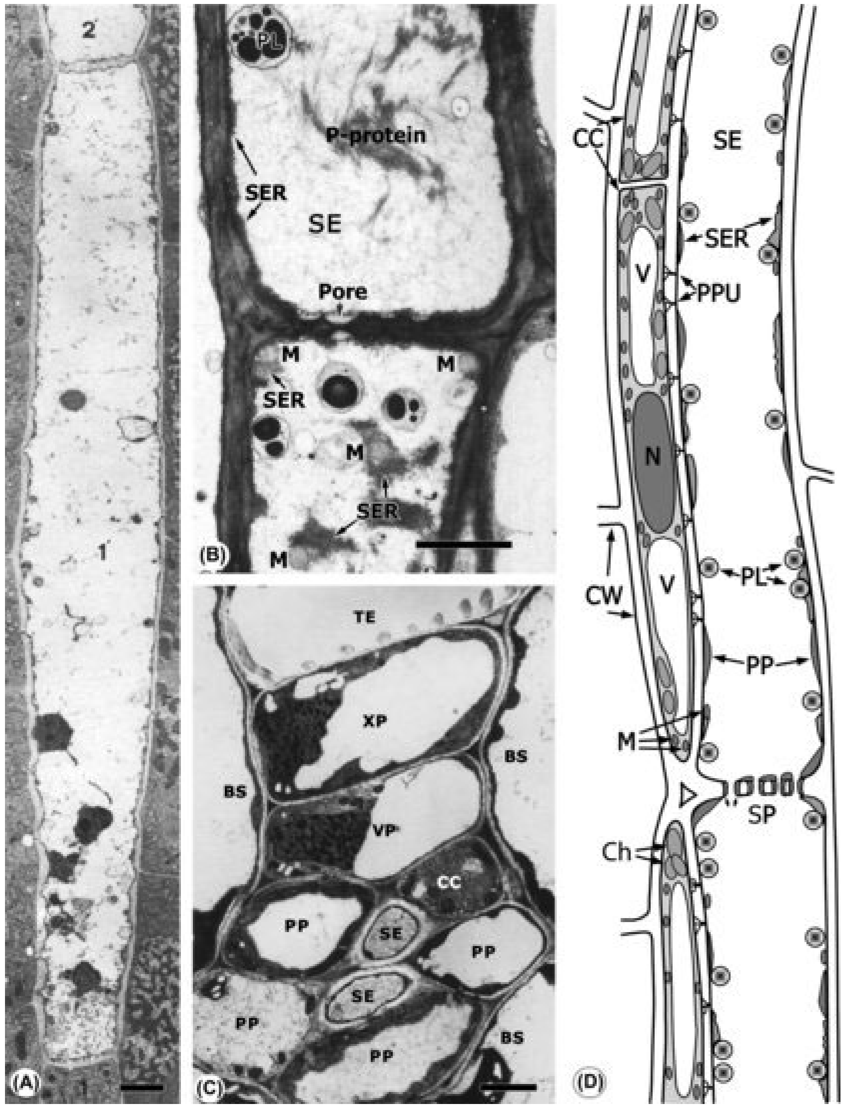
The distinct structure and central[1] role of the phloem in long-distance transport have intrigued scientists ever since Malpighi (1686) observed continued growth in tissue immediately above a stem girdle and suggested that food transported downward from shoot to roots accumulated above the girdle and 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.

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