

Integrative Plant Biology: Role of Phloem Long-Distance Macromolecular Trafficking

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Abstract

Recent studies have revealed the operation of a long-distance communication network operating within the vascular system of higher plants. The evolutionary development of this network reflects the need to communicate environmental inputs, sensed by mature organs, to meristematic regions of the plant. One consequence of such a long-distance signaling system is that newly forming organs can develop properties optimized for the environment into which they will emerge, mature, and function. The phloem translocation stream of the angiosperms contains, in addition to photosynthate and other small molecules, a variety of macromolecules, including mRNA, small RNA, and proteins. This review highlights recent progress in the characterization of phloem-mediated transport of macromolecules as components of an integrated long-distance signaling network. Attention is focused on the role played by these proteins and RNA species in coordination of developmental programs and the plant's response to both environmental cues and pathogen challenge. Finally, the importance of developing phloem transcriptome and proteomic databases is discussed within the context of advances in plant systems biology.

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INTRODUCTION

To understand the context in which long-distance molecules function, whole-plant signaling must be viewed from the perspective of the evolutionary development of organismal complexity. As biological systems advanced from cellular to multicellular, a parallel increase in the sophistication of their signaling

processes had to occur in order to allow for the integration of processes at a multicellular level (94). In this regard, retention of the cell wall by the plant kingdom imposed an interesting challenge to the development of an informational network that could operate at the multicellular/organismal level. The outcome was that, within plant tissues, coordination was achieved by a combination of cell-cell, receptor-ligand signaling systems (33, 140) and cell-to-cell communication through plasmodesmata (PD). Biogenesis, structure, and dynamics of PD have been extensively reviewed elsewhere (34, 38, 59, 92, 112, 168). The important feature of PD is that they establish continuity between the cytoplasm of neighboring cells, thereby forming a symplasmic state that allows for the local exchange of molecules and ions.

Inter-organ communication was achieved through the development of a vascular system. In this regard, it is important to note that significant differences exist between the vascular systems that evolved in the animal and plant kingdoms; however, both conform to Murray's Law of fluid dynamics (100, 105). It is an axiom that, in animals, the arteries and veins circulate blood, carrying nutrients, various signaling molecules, and a range of cell types around the body. In plants, the vascular system is comprised of the xylem and phloem conducting tissues whose mode of formation and function are strikingly different from that of animals. For the purpose of this review, the basic difference is that, except for water and ions, (macro)molecules moving in one direction in the phloem do not circulate back to their sites of origin through the xylem, i.e., the plant vascular system is noncirculatory in nature (**Figure 1**). One important consequence of this system is that, whereas the shoot apical meristem (SAM) can receive input signals from the mature regions of the plant, output signals cannot be translocated back to the lower organs, as both the xylem and phloem operate in the same direction (**Figure 1a**). (Note that hormones, such as auxin, produced in the apex move locally down the stem via a

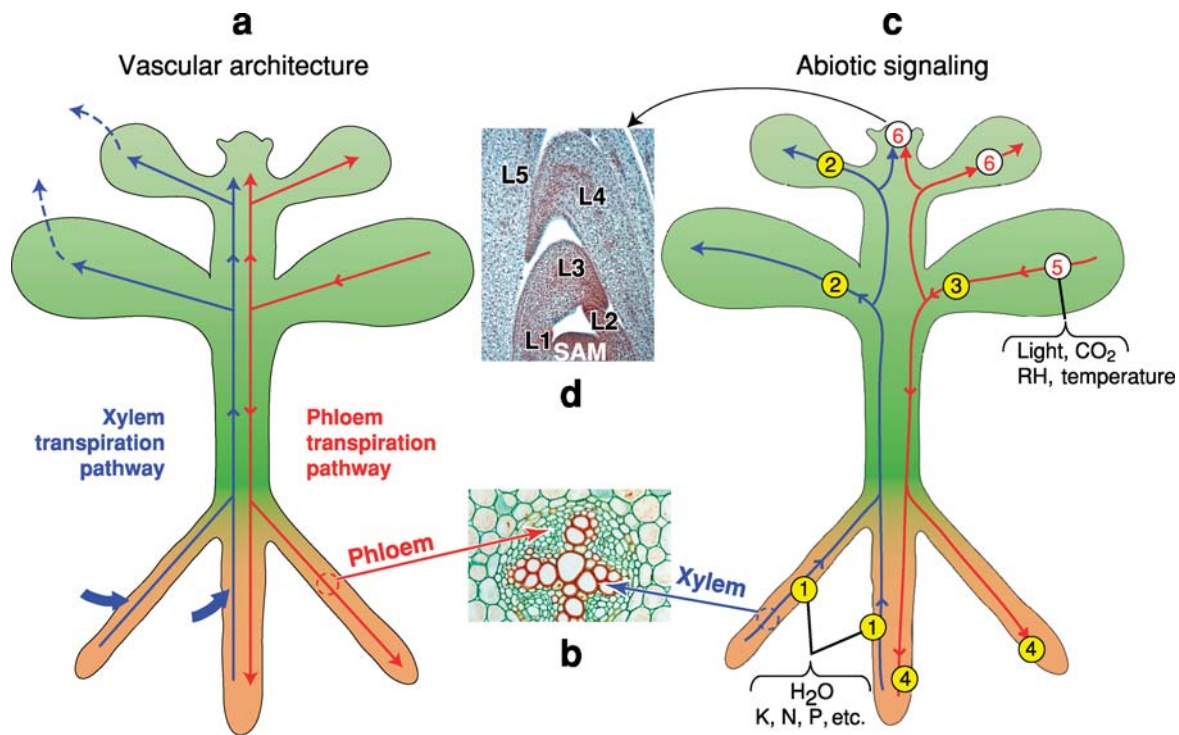


Figure 1

The plant vascular system functions as the conduit for long-distance communication between distantly located organs. (a) Schematic illustration of the xylem and phloem, illustrating the fundamental feature that, in contrast to the animal kingdom, the flow pathway is noncircular in nature. (b) Transverse section of a root demonstrating the physical separation of the phloem and xylem tissues. Xylem conducting elements are dead at maturity, whereas the sieve tube system acts as a specialized symplasmic domain; thus, the plasma membrane of the phloem cells acts as a barrier to exchange between the two streams. (c) Coordination of physiological and developmental programs requires the integration of a spectrum of abiotic input signals. Root-shoot-root relay signaling: (1) input signal for nutrient deficiency produced in the roots is transported, via the xylem, to the shoot; (2) signal recognition activates a pathway located in the symplasm of the leaf; (3) response signal enters the phloem for delivery to the roots; (4) output signal adjusts the genetic program controlling nutrient uptake. Leaf-to-apex signaling: (5) input signal for an environmental parameter enters the phloem for transport to sink organs, including the apex; (6) developmental program is adjusted to optimize the morphological/anatomical/physiological features of the developing organ for the existing conditions. (d) Shoot apical meristem (SAM) functions in a protected environment markedly different from that experienced by mature organs. A median longitudinal section through the vegetative apex illustrates the manner in which the developing leaves (L1–L5) ensheath the SAM. Identity of the proposed long-distance signals remains to be elucidated, but current research indicates the involvement of information macromolecules.

cell-cell transfer process.) This architectural feature most surely imposed novel challenges on the evolution of an effective long-distance communication system in plants.

The primary focus of this review is the analysis of current knowledge on the operation of the phloem as a conduit for long-

distance macromolecular signaling in plants. This topic was last reviewed in this series by Oparka & Santa Cruz (113). Signaling systems, incorporating phytohormones, sugars, jasmonic acid, and similar such low molecular weight compounds have been reviewed elsewhere (31, 39, 41, 49, 90, 133, 137, 147,

Plasmodesmata (PD): unique plasma membrane-lined cytoplasmic channels that establish symplasmic continuity between neighboring plant cells

Shoot apical meristem (SAM): the vegetative apex of a plant is comprised of a group of stem cells that form the shoot apical meristem. These cells give rise to leaf primordia, vascular initials, axillary buds, and the stem

Non-cell-autonomous proteins (NCAPs): molecules that traffic cell to cell through plasmodesmata to exert control over physiological and developmental programs in cells located beyond their site of synthesis

Ribonucleoprotein (RNP) complex: a complex formed between proteins and RNA. In certain cases, a protein can recognize its cognate mRNA molecule by the presence of a sequence and/or structural motif, or zip code, and mediate its delivery to a specific location within the cell

Sieve tube system: the phloem conduit in the angiosperms that is comprised of files of enucleate sieve elements supported by their neighboring companion cells

157, 160). Although a number of studies have shown that transcripts (125), proteins (51), and small RNA (167) are present in the phloem translocation stream, the pressing issue facing this field is the functional significance of these molecules and the roles they play in the integration of whole-plant signaling processes.

Plasmodesmata, Protein Trafficking, and Developmental Domains

In addition to facilitating the cell-to-cell exchange of metabolites and ions, PD provide a pathway for the trafficking of macromolecules, including proteins and RNA. Proteins that move through PD are defined as non-cell-autonomous proteins (NCAPs); RNA is thought to traffic cell to cell as a ribonucleotide-protein complex (RNP). It is now well established that the local movement of NCAPs and RNPs can contribute to the establishment of cell fate and patterning in plant tissues (43, 59, 60, 77, 94, 106, 107, 132).

As this PD property is important to an understanding of how and why macromolecules are trafficked through the phloem, we will first describe an elegant illustration of this concept derived from studies on *Arabidopsis* root development. Endodermal cell fate is controlled by SHR, a putative transcription factor expressed only in the stele (107). Studies on transgenic plants expressing *P_{SHR}:SHR-GFP* revealed the presence of a strong fluorescent signal in the nuclei of endodermal initials, identifying it as an NCAP. As SHR-GFP did not move through PD connecting endodermal and cortical cells, it represents an excellent case of controlled NCAP trafficking, across a single cell boundary that is essential for the orchestration of cell fate (43, 107). An ever-increasing number of NCAPs are currently being shown to participate in tissue/organ development (26, 78, 124). The important question is whether NCAPs or RNPs also function to coordinate and synchronize developmental activities between distantly located organs.

PHLOEM AS A CONDUIT FOR DELIVERY OF MACROMOLECULES

Vascularization and SE-CC Properties

Evolution of the plant vascular system for long-distance delivery of nutrients relieved prior size constraints and opened new opportunities for the colonization of terrestrial habitats. A low-resistance water-conducting conduit, comprised of dead xylem tracheids and vessel elements, provides water and mineral nutrients to aerial parts of the plant. In contrast, photoassimilates (sugars) are transported within nucleate cells of vascular cryptogams and other primitive land plants. The evolutionary trend of these conducting vessels was a progression toward simplification. The nuclei of these conducting cells became reduced in gymnosperms and in angiosperms they are completely degraded, giving rise to enucleate sieve elements (SE) that form a conduit called the sieve tube system. Lacking any apparent capacity for transcription or translation, the angiosperm sieve tube system became dependent on an association with the neighboring companion cells (CCs), which are considered to provide all cellular components required for maintenance and function of the sieve tube system. As such, this obligatory association is best considered as the SE-CC complex (145, 146). A consequence of this operating system is that any macromolecule identified within the sieve tube system was probably delivered through PD from an associated CC (124).

The interconnection of local tissues to the phloem sieve tube system, through PD, established the potential for both local and long-distance macromolecular trafficking. The central question, therefore, is whether the phloem developed solely to deliver photoassimilate to heterotrophic regions of the plant, or did information molecules also become transported, in parallel, as a component of a more sophisticated control system for coordination of plant processes? The latter would

allow plants to operate as unique integrated supracellular organisms.

Whole-Plant Function

Plant growth and development is the execution of genetic programs conditioned by environmental inputs. These inputs include both biotic events, for instance pathogen attack or herbivory, and abiotic, such as seasonal variation in temperature, day length, water status, and nutrient availability, etc. (Figure 1c). This plasticity is an adaptive response to ensure the best possible chance of completing the plant life cycle. The implication, therefore, is that meristematic regions act in concert with environmental inputs. The question then arises as to how the SAM (plus other meristematic tissues) detects these environmental inputs to respond with an optimal pattern of growth and development.

It is important to note that, generally, the SAM is protected within a modified environment formed by ensheathing developing lateral organs (Figure 1d). Such an environment prevents the SAM from directly detecting variation in some inputs, e.g., ambient relative humidity, CO₂ levels, fluctuations in light intensity/wavelength and day length. Plants appear to have resolved this situation by evolving a sophisticated system that perceives environmental inputs in distant tissues and translates them into signals that are then communicated to, and acted upon, by the SAM and other meristematic regions. Ample experimental evidence exists to support this whole-plant signaling/integration hypothesis (15, 21, 41, 56, 115, 141); however, until recently, little information was available as to whether macromolecular constituents were involved in this long-distance signaling network.

ENDOGENOUS MACROMOLECULES ON THE MOVE

Phloem Proteins

Analysis of phloem exudates, principally collected from cucurbits (9, 50, 51), *Ricinus* (128),

and lupin (97), has revealed a complex population of NCAPs present in the phloem of plants. Convenient collection of milliliter quantities of phloem sap makes the cucurbits an excellent model for the analysis of phloem function. The presence of contaminating material both from the cut surface and CC contents can be excluded based on a number of findings. First, grafted plant systems utilizing taxonomically diverse rootstocks (base) and scions (grafted tissue) have been used to examine the translocation of phloem-mobile molecules, including proteins (50, 51). Detection of pumpkin stock-derived phloem sap proteins, following direct analysis of cucumber scion sieve tube contents, confirms that these molecules are mobile across a graft union (50) and, thus, are bona fide constituents of the phloem sap. Additionally, the association between the holoparasite *Cascuta reflexa* and *Nicotiana tabacum* established that macromolecular trafficking also occurs across a naturally formed graft union (57).

The diversity of proteins identified in the phloem translocation stream implicates them in a wide variety of functions. Structural components include phloem protein 1 (PP1) and phloem protein 2 (PP2) (51) that function to block damaged sieve tubes in a calcium-dependent manner (81). A complete antioxidant defense system, including thioredoxin-h, cytosolic Cu/Zn superoxide dismutase, monodehydroascobate, and peroxidase, probably operates as a protective system against reactive oxygen species (66, 155). A functional cytoskeleton system is suggested by the presence of profilin and actin in the translocation stream (128). The extensive array of proteinase inhibitors may well reflect a defense mechanism to counter probing insects (54, 166). These proteinase inhibitors may also function to protect NCAPs against proteolysis during long-distance trafficking to their target tissues, or function to regulate peptide NCAP maturation from protein precursors. The molecular chaperone, CmHsc70, may function to assist delivery of protein "cargo" into the sieve tube system (4). Finally, the

Supracellular organisms: tissues of these complex organisms consist of populations of cells, but most cells are interconnected by plasmodesmata to their nearest neighbors. As the phloem interconnects all symplasmic domains within the body of the plant, such a system of organization works above the level of the cell, hence the term supracellular

Graft union: plant tissues from a given species, termed a scion, can be transferred to the body of a different species, termed the stock, and if they are compatible the vascular tissues will form a functional graft union to allow the exchange of water and photosynthate

CmPP16:Cucurbita maxima
PHLOEM
PROTEIN 16**CmPSRP1:**Cucurbita maxima
PHLOEM SMALL
RNA-BINDING
PROTEIN 1**CC-SE:** companion
cell-sieve element
complex

presence of a number of RNA-binding proteins, including CmPP16-1, CmPP16-2, and CmPSRP1, provide a foundation for protein components functioning within an RNA-based communication network (74, 163, 167).

If the phloem operates as a conduit for long-distance communication of macromolecules, then clearly this system would have evolved mechanisms for controlling NCAP/RNP entry into and exit from the sieve tubes. Evidence for the operation of such a system is afforded by studies on CmPP36 (162). For this phloem-mobile protein, the N terminus contains a predicted plasma membrane-anchoring domain and, following its cleavage, the processed form of the protein enters and moves within the phloem translocation stream. Interestingly, this Δ N-CmPP36 may maintain Fe within the phloem sap in a reduced state, in order to ensure the efficient delivery of this essential mineral nutrient to sink tissues.

Reminiscent of motifs directing nuclear targeting (134), proteins may similarly engage an NCAP pathway for efficient entry into the sieve tube system. Support for this hypothesis was obtained by detection of a sequence motif present within the C terminus of the phloem-mobile CmHsc70. Function of this motif was proven in gain-of-movement experiments in which a human Hsp70 acquired the capacity to be translocated through PD (4). Interestingly, an endoplasmic reticulum-bound NCAP pathway protein (NtNCAPP1) has the capacity to interact with some 30% of the pumpkin phloem sap proteins (89). Transgenic plants expressing an engineered dominant negative form of NCAPP1, lacking the N-terminal membrane-anchoring domain, displayed developmental changes probably reflecting selective blockage to the cell-to-cell trafficking of specific NCAPs. Collectively, these experiments support the hypothesis that protein targeting to the sieve tube system occurs by a selective, regulated mechanism (**Figure 2a**).

A selective mode of protein translocation through the sieve tube system is fur-

ther illustrated by the differential movement of the NCAP, CmPP16-1, to the roots (5). Stylectomy-assisted introduction of labeled proteins was performed directly into the rice sieve tube system. Biotin-labeled probes, including the NCAPs, CmPP16-1, and CmPP16-2, were introduced directly into a SE and their subsequent translocation was monitored to distant leaf and root tissues. Relative accumulation of CmPP16-1 and CmPP16-2 was measured using a very sensitive biotin detection method. Based on these studies, it would appear that mass flow could account for the observed translocation of CmPP16-1 and CmPP16-2 to leaf tissues. However, movement to the roots seemed to involve an additional selective process resulting in preferential translocation of CmPP16-1. This property was abolished if CmPP16-1 was chromatographically purified from the other phloem sap proteins prior to its reintroduction into the sieve tube system. Interestingly, reconstitution experiments, involving CmPP16-1 and specific pumpkin phloem sap fractions, restored this selective delivery of CmPP16-1 to the roots. Immunoprecipitation experiments identified the molecular constituents associated with a CmPP16-1 complex and it will be of great interest to learn whether these proteins underlie the observed process of selective translocation to a specific sink organ.

Unregulated, diffusion-based movement across the companion cell-sieve element complex (CC-SE) PD also accounts for a proportion of proteins that enter the sieve tube system. Perhaps the best example illustrating this principle is derived from studies performed on plants in which *GFP* expression was controlled by a CC-specific promoter (65, 114). This small, cylindrical heterologous 27-kDa protein was detected in the SE and was carried in the translocation stream to sink tissues. Similar experiments involving CC expression of GFP-fusion proteins suggested that the diffusion-based molecular size exclusion limit for CC-SE PD was on the order of 50 kDa (114). Aphid stylectomy-assisted introduction

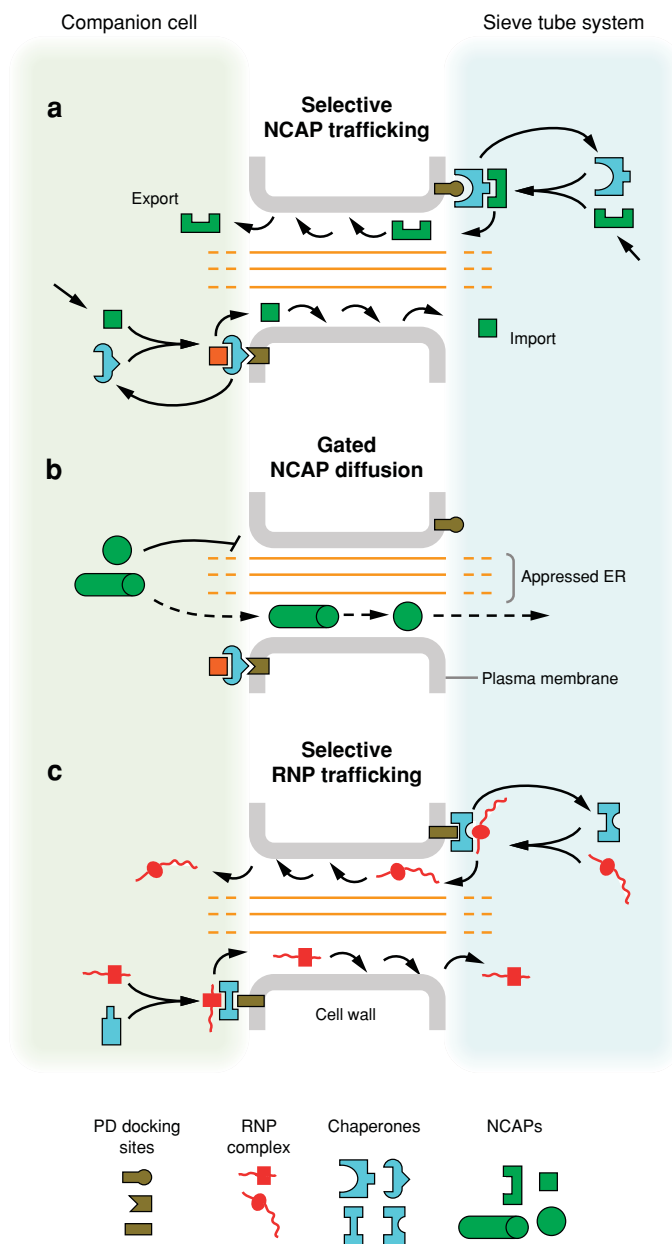
of variously sized fluorescent probes, into wheat grains, provided further support that CC-SE PD permit diffusion-based unloading (40).

Post-phloem diffusion of GFP has been used to examine properties of the symplasmic pathway for the movement of nutrients and macromolecules. As GFP is not of plant origin it is unlikely to enter a selective translocation pathway. It is important to note that similar experiments based on CC-mediated expression of GFP indicate post-phloem transport and symplasmic domain establishment in the root apical meristem (136) and in cortical tissues destined to develop as root nodules (28). Collectively, these studies support the notion that some proteins can codiffuse through CC-SE PD that are actively engaged in selective NCAP trafficking. In this case, protein diffusion is probably made possible by the increase in PD size exclusion limit(s) that takes place during selective NCAP trafficking. A molecular mechanism may also operate to gate the CC-SE PD for

the sole purpose of facilitating protein exchange by simple diffusion (94). It will be interesting to learn the relative importance of these two pathways on overall protein exchange within the phloem long-distance communication pathway.

Figure 2

Potential mechanisms for the cell-to-cell trafficking of macromolecules through plasmodesmata. (a) Selective delivery of non-cell-autonomous proteins (NCAPs) to specific plasmodesmata (PD) docking sites is mediated by chaperones. Docking of the NCAP-chaperone complex induces dilation in a PD microchannel that allows NCAP translocation along the length of the PD. Mutations within the NCAP can prevent formation of the NCAP-chaperone complex, thereby inhibiting delivery to and/or trafficking through the PD. The model illustrates NCAP import into and export from the sieve tube system. (b) Dilated PD microchannels can act as a size-exclusion limit barrier to NCAP diffusion. Mutations within NCAPs that move in this manner are not expected to affect their capacity for movement. (c) In a process analogous to (a), cell-to-cell trafficking of RNA molecules into and out of the sieve tube system is mediated by RNA binding proteins in conjunction with specific chaperones. Mutations in the RNA may affect delivery to and/or translocation through PD.



miRNA: microRNA

siRNA: small interfering RNA

Phloem mRNA and small RNA

The certainty that RNA transcripts and small RNA are present, mobile in the phloem, and likely function as information macromolecules is one of the most exciting new prospects in plant biology. Building on the foundation that mRNA encoding the plant transcription factor, *KNOTTED-1*, and many plant viruses can move between cells through PD (20, 46, 91), RNA localization experiments revealed that *SUCROSE TRANSPORTER-1 (SUT1)* RNA was present within the SE-CC complex (85). Numerous other transcripts, including transcriptional regulators, genes controlling cell fate, the cell cycle, phytohormone response, and metabolic genes were subsequently identified in the pumpkin and tomato sieve tube systems (60, 77, 125). Confirmation that these representative transcripts were phloem mobile was gained from grafting studies and RT-PCR analysis, using gene-specific primers. Additional studies based on rice (127) and barley (36) indicate that these results may well have general applicability to higher plants, at least for the angiosperms. Indeed, this mobile population of mRNA species may be quite extensive with >1500 transcripts already identified from the phloem of cucurbits (W.J. Lucas & T.J. Lough, unpublished data). The presence of these transcripts supports the notion that RNA-based signaling networks may function to control many plant processes (27, 59, 73, 74, 95, 161).

Selective translocation of RNA, via the sieve tube system, is indicated by differential localization of RNA transcripts in apical tissues and the presence of RNA-binding proteins in the phloem. Heterografted plants were once again employed to reveal that pumpkin stock-derived *CmNACP* transcripts were present in the cucumber scion meristem (125). Selectivity was confirmed by differential detection of other phloem-derived transcripts in apical tissues of the cucumber scion. A population of RNA-binding proteins, present in the phloem, suggests formation

and regulated movement of RNP complexes. The pumpkin *CmPP16-1*, immunologically related to movement proteins of viruses, binds mRNA, enters the sieve tube system, and translocates long distance (163). Identification of both RNA-binding proteins and cognate RNA in the phloem (52, 167) attests to the existence of numerous RNPs most likely functioning as information molecules within an RNA-based network (**Figure 2c**).

Micro (mi)RNA-mediated regulation of gene expression has been identified in both plants and animals and is currently an area of intense research activity (12, 30). A diverse and dynamic population of small RNA (19–25 nucleotides), present in phloem sap of cucurbits, *Ricinus*, *Yucca*, and lupin, suggests a potential long-distance signaling role for small RNA (167). This small RNA population includes known miRNA species, siRNA reflecting virus infection, transposon challenge, and other classes of small RNA of undefined function. The potential role played by these molecules, in response to pathogen attack, is considered in a later section.

EXOGENOUS MACROMOLECULES: HITCHING A RIDE

Viroids

A phloem-based RNA signaling network presupposes that mechanisms evolved to regulate the entry and exit of specific transcripts. Insight into the operation of such a system has been gained through studies on exogenous RNA molecules, acting as pathogenic RNAs, including both viroids and RNA viruses. Each viral RNA system recognizes the host's machinery to support replication, cell-to-cell movement, and systemic spread via the phloem (153). As viroids do not encode any proteins, they must rely on host cellular factors for all required functions of replication and movement. *Potato spindle tuber viroid (PSTVd)* has been utilized to examine the regulation of RNA trafficking in

the phloem (121, 169, 170). In this study, tobacco was used for the serial maintenance of a poorly accumulating strain, PSTVd^{NT}. After a number of passages, these workers isolated a new strain, PSTVd^{NB}, that could accumulate to a much higher level when reinoculated onto tobacco. Equivalent replication levels in protoplasts suggested that relative accumulation levels might well reflect differences in the capacity of these two viroid strains to invade specific tissues.

Specific RNA sequence motifs, or zip codes, can direct the interaction with cellular factors to regulate targeted delivery of RNA (71). Evidence that such a system acts at the level of the PD has been gained through experiments in which a CC-specific promoter was used to express infectious PSTVd strains in transgenic tobacco lines. Detailed in situ RNA localization experiments performed on these lines demonstrated that the poorly accumulating strain, PSTVd^{NT}, lacked the capacity to move beyond phloem tissues. In contrast, the new mutant strain, PSTVd^{NB}, was detected in phloem, bundle sheath, and mesophyll cells. Clearly, the nucleotide changes present in the PSTVd^{NB} genome permit its viroid RNA to pass through the bundle sheath to enter the mesophyll tissues (121).

Additional insight into the presence of an endogenous mechanism aimed at regulating cell-to-cell trafficking of RNA was also provided by experiments in which both viroid strains were mechanically inoculated onto mature (source) tobacco leaves. Quite unexpectedly, both strains were capable of replicating in and moving cell-to-cell through mesophyll, bundle sheath, and phloem cells (121). This finding provided strong support for the notion that bundle sheath-mesophyll PD function as a rectifier to regulate viroid RNA movement. Mutagenesis experiments defined a bipartite motif, comprising 4 separate nucleotide changes, that was necessary and sufficient to regulate unidirectional trafficking of PSTVd. This elegant study identified what may be the first zip code essential for

RNA delivery to and/or movement through PD.

Examination of the tissue-specific patterns of viroid RNA accumulation in sink organs indicated that developmental programs could influence cell-to-cell movement of macromolecules. The bundle sheath-mesophyll PD of younger (sink) but not older (source) leaves acted as a barrier for RNA trafficking (121). In addition, PSTVd was able to enter some, but not all, sink tissues (169), suggesting that simple mass flow alone does not control systemic movement. Rather, some form of selectivity appears to dictate the delivery of viroid RNA into certain sink tissues/organs. These features of viroid RNA trafficking are therefore distinct, and studies of this nature provide important insights into the dynamic capacity of plants to regulate the movement of RNA out of the phloem into surrounding tissues.

RNA Viruses

In contrast to viroids, RNA viruses encode proteins required for replication and movement. These so-called movement proteins (MPs) (93, 153) function in a manner analogous to NCAPs (89, 94), and interact with host components of the cell-to-cell translocation pathway (84). Numerous studies have demonstrated that MPs bind viral RNA, potentiate dilation of PD, and facilitate translocation of the viral RNA-MP complex to the adjacent cells (59). Transported forms of viral RNA include both virions and RNPs (46). Support for translocation of viral RNPs, both cell-to-cell and long-distance, via the phloem, is derived from analysis of plant RNA viruses that lack a functional coat protein gene (88). In these instances, viral RNA movement must be in the form of an RNP complex. An immunological relationship between CmPP16 and the MP of *Red clover necrotic mosaic virus* suggests not only functional but also distant evolutionary origins (163). This observation reinforces the premise that plant viruses can be used as powerful genetic tools to further

MP: movement protein

Florigen: a graft-transmissible substance that mediates in the transition of the vegetative apex into an inflorescence meristem

explore the mechanisms controlling endogenous RNA-based signaling systems.

It is generally accepted that the majority of plant viral MPs function in cell-to-cell movement of the infectious nucleic acids in the absence of a requirement for cognate viral sequence recognition (44). This assertion is based on experiments including the functional rescue of RNA movement when the MP was expressed, *in cis*, in the context of a hybrid viral genome (32, 45), or *in trans*, using transgenic plants or cobombardment experiments (29, 101). However, reminiscent of regulated unidirectional translocation of viroid RNA, a number of viral systems are restricted in their movement by a defined cellular boundary. Many phloem-limited viruses cannot traffic from phloem parenchyma into the bundle sheath, whereas other viruses that are unable to enter the phloem generally cannot move from the bundle sheath into the phloem parenchyma. This phloem parenchyma-bundle sheath PD boundary, which is distinct from that operating to control viroid translocation, acts as a checkpoint for entry into and exit from the SE-CC. Hence, the possibility exists that this cellular boundary, within the host plant, may require some degree of additional cognate protein-RNA specificity to permit cell-to-cell transport of RNA. Indeed, such a requirement may explain why for some viruses, including *Tobacco mosaic virus* and *Red clover necrotic mosaic virus*, entry into the plant vascular system requires both the MP and capsid protein (CP) (35, 126, 144).

Biochemical, genetic, and yeast two hybrid-based studies have identified a number of plant genes that play a role in virus long-distance movement (23–25, 96, 143, 158, 159). The pectin methylesterase (PME) and cadmium-ion-induced glycine-rich protein (cdiGRP) are of special interest, as they have been suggested to function in virus exit from the phloem (23, 24, 143). As both PME and cdiGRP are expressed in phloem tissues and appear to be localized to the cell walls, the manner in which these proteins might influ-

ence viral RNA trafficking remains obscure. However, because cdiGRP increased callose deposits in the phloem (143), and callose is deposited around PD (109), its mode of action might be to limit the capacity of PD to undergo an increase in SEL; this could block the movement of viral RNA from the phloem into the surrounding tissues, thereby preventing systemic spread (153). The challenge ahead will be to discover why cdiGRP only caused a virus-specific block to systemic movement in tobacco.

WHOLE-PLANT SIGNALING

Flowering & Florigen

The holy grail of plant biology is the identification of the phloem-mobile stimulus involved in floral induction. This vegetative-to-floral transition is induced by environmental cues, with day length considered the most important. This cue is perceived in the mature leaves and a signal is transported in the phloem translocation stream to the shoot apex. Pioneering work by Chailakhyan defined this substance(s) as florigen, on the basis that a graft-transmissible substance could induce flowering in a scion being maintained under a noninductive photoperiod (21). Symplasmic tracers have shown that the onset of the floral transition is highly correlated with a transitory decrease in symplasmic connectivity between the apex and the terminal phloem, consistent with a major change in the selectivity of communication between the body of the plant and the vegetative meristem (47, 48). Numerous substances, including gibberellins (79), sucrose (14), cytokinin (70), and peptides (64), have been advanced as the florigenic stimulus, but to date formal proof is lacking.

Molecular genetic approaches have revealed four different pathways involved in controlling flowering time in *Arabidopsis* (13, 102). Two pathways are responsive to environmental cues, namely photoperiod and vernalization; there is also an autonomous pathway that promotes flowering under all conditions

and, finally, a gibberellin pathway that is required to promote flowering under short-day conditions (102). Key elements involved in integrating the input signal(s) from the photoperiodic pathway are the zinc-finger protein, CONSTANS (CO) (120), and the raf-1 kinase inhibitor (RKIP)-like protein, FLOWERING LOCUS T (FT) (75, 82). Promoter GUS analyses have revealed vascular- rather than meristem-specific expression patterns for both genes, findings consistent with signaling roles for both CO and FT in floral induction (3, 138).

A signaling role for CO was confirmed using tissue-specific promoters in which CO expression, within CCs located in source leaves, promoted flowering in the *co* mutant background (3). Grafting studies confirmed that CO controls the production of a phloem-borne substance essential for floral induction, i.e., the ever elusive “florigen.” Assignment of CO, per se, as florigen could be discounted as CC-specific expression of CO fused to GFP did not reveal a capacity of CO to traffic beyond the cells in which it was transcribed (3, 7). However, this CO-GFP fusion protein was still able to rescue flowering in the *co* mutant background.

A high probability exists that FT and/or FT mRNA are components of the phloem-mobile florigenic signal. Parallel experiments using tissue-specific promoters revealed a capacity for FT to promote flowering, not only through expression in CCs, but also in numerous other tissues, including those of the meristem. As activation of FT expression is, in itself, controlled by CO and is required to promote flowering, these results once again underscore the importance of long-distance signaling through the phloem. The capacity to induce flowering and its relatively small size make FT a good candidate as a component of the phloem-mobile stimulus.

Support for such a model is provided by the capacity of FT to induce flowering when present in cell types beyond the vasculature (3, 138). Protein-protein interactions involving (non-) phosphorylated forms of the hu-

man FT ortholog, RKIP, modulate outputs from the MAPK, GRK-2, and NF κ B signaling pathways (110, 164, 165). Yeast two-hybrid studies utilizing a tomato FT homolog, *SELF PRUNING* (SP), have identified interacting proteins including a kinase (SPAK) and a G-box transcription factor (117). Taken together, these data are consistent with a model in which FT/FT mRNA controls floral induction by manipulating kinase signaling cascades or transcriptional events in cells associated with the phloem.

The demonstration that FT/FT mRNA has the capacity to traffic cell to cell, and move long-distance via the phloem, has been keenly anticipated. Indirect evidence that FT mRNA can move from mature (source) leaves to the SAM is provided by studies on transgenic Arabidopsis lines expressing FT driven by a heat shock promoter (64a). Application of a heat shock stimulus to a single source leaf induced accumulation of the transgene FT mRNA in both the source leaf and the shoot apex. The time course of FT mRNA accumulation was highly correlated with the triggering of floral induction in the apex. Importantly, delivery of the “florigenic” signal into the shoot apex causes endogenous FT mRNA to increase, suggestive of a positive autoregulatory loop in the “florigen” signaling pathway whereby FT, directly or indirectly, controls its own expression. It remains to be established whether FT also moves to the apex through the phloem (64a). It could well be that de novo synthesis of FT in the terminal protophloem, from the imported mRNA, initiates the signaling relay that propagates from this region of the apex to the SAM (**Figure 3**). How FT/FT mRNA navigates its way through the intervening tissues to enter a specific subset of cells which then give rise to the floral meristem remains a challenge for the future.

Tuberization and Tuberigen

Short-day photoperiodic induction and graft transmissibility of a signal leading to potato tuber formation indicate that parallels exist

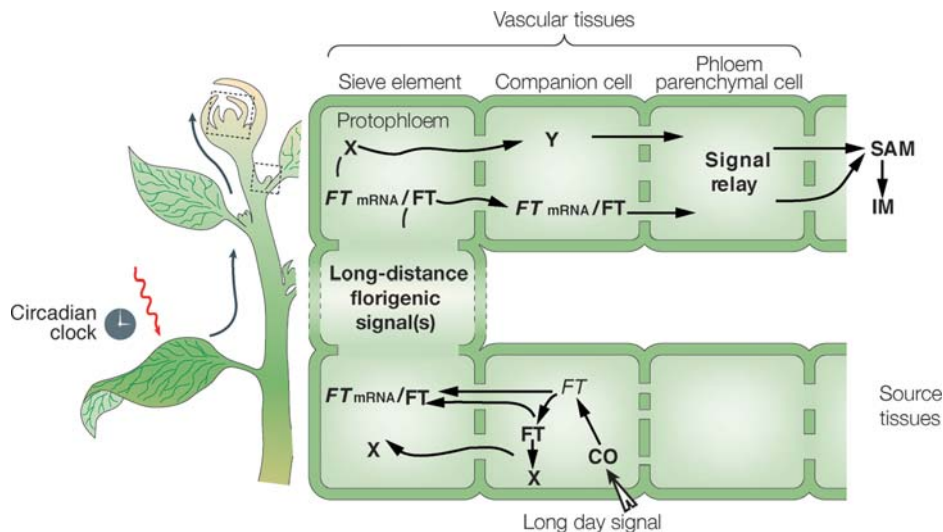


Figure 3

Photoperiodic control over floral induction requires phloem-mediated delivery of florigenic signal(s). *CONSTANS (CO)* expression is under photoperiodic control and occurs in the companion cells (CCs) of source leaves where it activates *FLOWERING LOCUS T (FT)* expression (3, 7, 138). FT and/or FT mRNA may serve as the florigenic stimulus that controls the shoot apical meristem (SAM) to inflorescence meristem (IM) transition (64a). Also, FT may exert control over floral induction through a kinase signaling cascade and/or a transcriptional event within the CCs that regulates entry into the phloem of an as yet unidentified molecule (X). Changes in the population of miRNA molecules, which move in the phloem (167), may serve as one component of the X → Y signal relay. One species, miR159, moves through the cucurbit phloem (167) and exerts an effect on flowering time in *Arabidopsis* (1). Alternatively, FT may serve as the signal relay through upregulation of its own expression. Arrival of FT into specific target cells of the SAM allows an interaction with a bZIP protein, FD, that triggers IM formation and initiates organ development by transcriptional activation of *APETALAI*, a floral meristem identity gene (1a, 159a).

between tuber and floral initiation (68, 118). (Note that the tuber forms at the tip of each stolon, a specialized underground stem.) Downregulation of *PhyB* impairs photoperiod perception leading to constitutive tuber formation under noninductive long-day conditions; this finding is consistent with the involvement of the photoperiodic pathway (69). Furthermore, transgenic potato plants expressing *CO* exhibit strong suppression of photoperiodic tuber induction (98). This finding is similar to the situation in the short-day plant, rice, where high levels of Hd1 (CO homolog) also suppress floral induction (58).

As with floral induction, grafting studies have shown that the tuberizing signal, termed “tuberigen,” is derived from the leaves and is

transported within the long-distance translocation stream (98). Experiments in which transgenic *CO*-expressing potato scions were grafted onto wild-type potato stocks resulted in a significant delay in tuber initiation in the stocks, even when plants were grown under short-day conditions. (Note that, in these studies, the scion functioned as the source of photosynthate being translocated down the plant axis to the underground stolons.) These studies confirmed that *CO* acts in the vegetative tissues and is upstream of tuberigen production. That florigen and tuberigen are components of the same signal is highlighted by tuber induction in potato-stock tissue following grafting onto photoperiodically induced tobacco scions (68). In view of the recent

progress made in our understanding of the role played by the plant vasculature system in photoperiodic control of flowering, it is highly likely that ectopic expression of *CO*, under a CC-specific promoter, will result in the suppression of tuber initiation. Results of this nature would further confirm the central role of the phloem in long-distance signaling to exert photoperiodic control over tuber induction.

Resource Allocation

Do plants measure environmental inputs, allocate resources, and respond as a whole-plant system? Validation that such a signaling network operates in plants was provided by the action of mature leaves sensing environmental inputs that then influenced the characteristics of newly forming leaves (76, 87, 139). Exposing mature *Arabidopsis* leaves to either elevated CO₂ or shade conditions, while allowing newly formed leaves to develop in ambient conditions, resulted in a decrease in stomatal density compared to control plants. Reciprocal experiments confirmed the existence of systemic signals influencing leaf characteristics, such as stomatal pore length, density, epidermal cell shape, and developing leaf size (87, 139). The existence of such signals has appeal, as this offers an explanation as to how the SAM, ensheathed by antecedent leaf primordia, and therefore unable to monitor accurately ambient light and CO₂ levels, is able to produce leaves with optimal characteristics for the environment into which they will eventually emerge.

Acclimation of mature leaves to environmental conditions also appears to involve the operation of a signaling network that integrates carbon resources at the whole-plant level. Clear evidence for such a system was provided by experiments conducted on soybean plants in which individual, attached leaves were exposed to elevated CO₂ conditions (135). Rubisco levels and rates of CO₂ fixation within these leaves reflected those observed for the rest of the leaves that were exposed to ambient CO₂ levels. In the reverse

situation, where individual leaves were provided ambient CO₂ levels while the rest of the soybean plant was exposed to elevated CO₂ conditions, the photosynthetic properties of the individual leaves were the same as those measured for leaves exposed to high CO₂ conditions! Although these findings implicate long-distance signaling through the phloem, the nature of the signaling molecules remains to be elucidated.

Manipulation of PD and the long-distance signaling pathway can influence carbon partitioning in plants. Expression of the TMV MP in tobacco source leaves leads to elevated levels of starch and sugars and a decrease in sugar export during the photoperiod compared to control plants (111). At the whole-plant level, expression of TMV MP causes a dramatic shift in the root-to-shoot ratio, with an ~50% decrease in root biomass in the TMV MP transgenic lines (8). Grafting experiments demonstrated that transgenic scion tissues, expressing TMV MP, induced the same change in root-to-shoot ratio when grafted onto wild-type tobacco rootstocks (8).

Involvement of the SE-CC is indicated by tissue-specific expression of TMV MP in potato, using the phloem-specific *rolC* promoter. In these transgenic potato lines, source leaves displayed elevated sugar/biomass accumulation and a concomitant reduction in the root-to-shoot dry-weight ratio (2). Of particular note is the finding that when these TMV MP transgenic potato lines were grown under short-day (tuber-inducing) conditions, tubers displayed higher dry matter partitioning (increased harvest index) as compared with control lines. Interestingly, tuber initiation is correlated with a shift from an apoplasmic to a symplasmic pathway for phloem unloading (149). It would be intriguing to learn if the observed influence of TMV MP over carbon partitioning to the tuber were due to the promotion of an endogenous long-distance signal that regulates PD properties in the tuber tissues to increase the import of photoassimilates. In any event, these changes in photoassimilate partitioning further support the

Split-root studies: an experimental system in which the plant's root system is divided and placed into two different aqueous compartments; one contains the control, whereas the other has a test solution. This system is used to investigate signaling that occurs between roots and shoots

notion that NCAPs, like the TMV MP, can exert long-distance effects over carbon allocation.

Expression of a pathogenesis-related maize gene, *PRms*, results in profound physiological changes to the plant. Immunolocalization and in situ hybridization studies established that *PRms* is an NCAP, as it was detected within PD and traffics cell to cell through parenchyma cells of the vascular cylinder, a cell type not actively transcribing *PRms* (103). Ectopic expression of *PRms*, with the *CaMV35S* promoter, resulted in a 20-fold increase in sugar export from the petiole of transgenic tobacco source leaves and an accelerated growth rate (104). Support for the notion that the phloem plays a role in these *PRms*-induced changes in photoassimilate partitioning was provided by the fact that *PRms* was detected within the CC-SE PD, in the phloem translocation stream, and in wild-type scions grafted onto *PRms* transgenic tobacco stocks (16). Elucidation of the mechanism by which *PRms* causes these changes in the regulation of resource allocation/growth rate, at the whole-plant level, will likely yield further insights into the operation of long-distance signaling as well as provide a means to genetically engineer plant productivity.

Nutrient Signaling

Shoots exercise regulatory control over the nitrogen acquisition activity of the root system by a feedback mechanism involving root-shoot (xylem-phloem) communication. Plants respond locally to differences in nitrogen availability by increased levels of nitrate uptake and lateral root proliferation. Split-root experiments demonstrate that regulation of this process involves not only local but also long-distance signaling. In such studies, different parts of the root mass are exposed to either high or low nitrate levels. Consistent with a whole-plant response, nitrate starvation in one part of the root is compensated for

by increased uptake from another root zone (19, 41).

The rhizobia-legume symbiotic system provides further evidence for the operation of whole-plant signaling over nodule primordia proliferation. Split-root experiments, in which nitrogen was supplied to only one part of the root biomass, clearly demonstrated an inhibition to the proliferation of nodule primordia on the untreated roots (19). The molecular components regulating this phenomenon, autoregulation of nodulation (AON), are now under intensive investigation. Soybean mutants, defective in AON, supernodulated and have increased numbers of lateral root hairs. Reciprocal grafting experiments involving these mutants and wild-type genotypes revealed that it is the leaf genotype that exerts control over the proliferation of nodule primordia. Map-based cloning led to the identification of a *CLAVATA1*-like gene, called *Glycine max NODULE AUTOREGULATION RECEPTOR KINASE* (*GmNARK*). As *CLV1* functions as a receptor in a receptor-ligand (*CLV3*)-mediated signaling cascade in the *Arabidopsis* SAM (33), this homology suggests a regulatory system involving a small extracellular (xylem-mobile) ligand, sent by the root and recognized by *GmNARK* in the shoot.

This xylem long-distance signaling system (root-to-shoot) would function to promote phloem-mobile signals that exercise homeostatic control over nodule meristem proliferation (130). Thus, in the presence of adequate inorganic nitrogen, the xylem-borne signal would be perceived by *GmNARK*, which would then activate a phloem-mobile signal(s) to downregulate nodule primordia proliferation in the roots (**Figure 4**). A central role for the SE-CC in this regulatory circuit awaits the demonstration that CC-specific expression of *GmNARK* restores AON in a soybean null mutant background. The possibility that the SE-CC plays a more significant role in both development of the nodule primordia and integration of nodule function on whole-plant

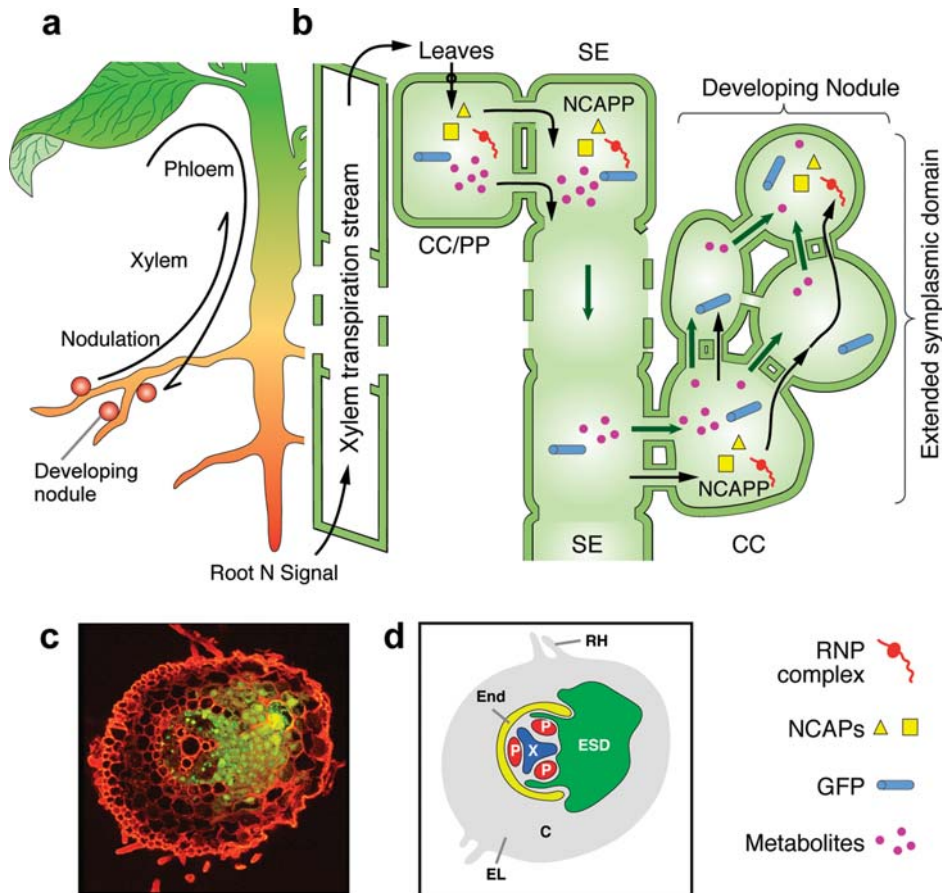


Figure 4

Xylem-phloem communication network: control over nitrogen fixation in the root system of the plant. (a) Plants use their vascular system to exchange information on the status of mineral nutrient acquisition by the root system and the growth requirements of the vegetative organs. (b) A symbiotic relationship with bacteria fixes N_2 into organic nitrogen; this process occurs in the roots in special structures called nodules (129). Coordination between nodulation and nitrogen demand for growth involves signal perception by a receptor kinase (GmNARK), located in the leaves (130), and phloem delivery of a feedback signal that exerts control over nodule development. One facet of this signaling system controls the formation of special symplasmic fields of cells that contain the nodule initials (28). (c) Transverse section of a nodule primordium from a *Medicago truncatula* root system inoculated with *Rhizobium*. Green fluorescence represents the cell-to-cell movement of green fluorescent protein (GFP) from the sieve tube system into all cells involved in the formation of the nodule primordium. GFP was produced in source tissues using a companion cell (CC)-specific promoter. Image reproduced from Reference 28, with permission. (d) Schematic illustration of the symplasmic field identified by the entry of GFP from the phloem translocation stream. Abbreviations are as follows: C, cortex; EL, epidermal layer; End, endodermis; ESD, extended symplasmic domain; P, phloem; RH, root hair; X, xylem.

physiology is indicated by the establishment of a new symplasmic domain in cells destined to become the nodule meristem (28).

What is the identity of the phloem-mobile regulators that control nutrient sig-

naling? The identification of small interfering (si)RNA and micro (mi)RNA in the phloem (167) is consistent with their involvement in the integration of whole-plant growth and development. In this regard, the phloem-mobile

Me: *Mouse ears* mutant in tomato

GAI/gai: wild-type and mutant form of the GIBBERELLIC ACID INSENSITIVE gene

miR395, predicted to target ATP sulphurylases (APS1, APS3 and APS4) (72), is potentially one such phloem-borne molecular regulator that acts within the whole-plant nutritional control system. APS activity is the first step leading to sulfate assimilation in the root. Accumulation of miR395 is influenced by SO_4^{2-} availability in the media to which the roots are exposed. In addition, miR395 levels are inversely proportional to the level of the target APS transcripts (72). Thus, the implication is that a shoot-derived and phloem-borne signal, miR395, regulates SO_4^{2-} uptake by modifying the level of APS expression. Experimental proof of this hypothesis would be the first demonstration of systemic control of a biological process by an miRNA mobile in the phloem translocation stream.

Leaf Architecture

Recent studies implicate a phloem long-distance RNA-based information network in the control over leaf shape. Such a system may explain, at least in part, the plasticity in leaf shape exhibited by plants. Graft transmissibility of tomato *Mouse ears* (*Me*) transcripts [*PFP-LeT6* fusion transcripts (22)] into wild-type scions was highly correlated with the appearance of the *Me* leaf developmental phenotype (77). Both the localization of *Me* transcripts, within the wild-type scion SAM, and the phenotype of the developing leaves reflect a property of the transcript to be translocated from stock to scion, through the phloem and post-phloem transport to the SAM.

A detailed analysis of *GIBBERELLIC ACID INSENSITIVE* (*GAI*) in pumpkin and tomato revealed similar functional properties of these phloem-mobile transcripts on leaf shape (60). *GAI* is an important negative regulator of GA signaling (116) and, thus, because GA influences a range of plant processes (122), long-distance translocation of *GAI* mRNA may fine-tune processes taking place in mature and developing organs.

Recessive loss-of-function *gai* alleles exhibit dark-green dwarfed phenotypes (83). Gain-of-function alleles, carrying mutations within the *DELLA* domain that is required for GA perception, result in a semidominant dwarf phenotype (116). Both *gai*-associated developmental phenotypes and transcript localization data, revealing a capacity of *Cm-GAIP* transcripts to traffic from the SE-CC into the meristem (125), implicated *GAI* as a regulator of developmental processes operating through the long-distance communication pathway. Heterologous expression of an engineered gain-of-function form of *Cm-GAIP*, carrying a mutated *DELLA* domain (Δ *DELLA-gai*), induced leaf-shape phenotypes in tomato and *Arabidopsis*. Parallel grafting experiments in both plant systems revealed that the Δ *DELLA-gai* transcripts had the capacity to traffic across the graft union and induce pronounced changes in leaf development. Interestingly, these phenotypes were restricted to sink regions of newly formed leaves on the scion; continued scion growth led to the restoration of wild-type leaves. This phenomenon likely reflects the influence of endogenous tomato *GAI* transcripts whose entry into the scion phloem translocation stream would dilute the influence of the stock-derived Δ *DELLA-gai* transcripts, thereby restoring normal patterns of leaf development.

A developmental influence over the phloem-mediated delivery of Δ *DELLA-gai* transcripts was observed in studies performed with tomato. Careful analysis of wild-type scions, grafted onto Δ *DELLA-gai* stocks, failed to detect Δ *DELLA-gai* transcripts in developing fruit, whereas transcripts were readily detectable within young leaves located closer to the scion apex. The basis for selectivity is likely associated with an as yet unidentified motif associated with the Δ *DELLA-gai* transcript. Consistent with this notion, control grafting experiments, incorporating either *CaMV-35S*- or *Suc2*-mediated *GFP* expression, revealed *GFP* protein but not *GFP*

transcripts in scion phloem/tissue. Collectively, these features indicate that, as with CmPP16-1 and PSTVd RNA, Δ DELLA-*gai* mRNA translocation is a property of the transcript itself and that mass flow consideration alone cannot account for the observed selective trafficking within the plant (60).

Pathogen Defense

Systemic response to pathogen attack includes an epigenetic process called post-transcriptional gene silencing (PTGS) that results in sequence-specific degradation of target RNA. Once again, evidence supporting a whole-plant response involves use of grafting techniques. In the seminal works of Palauqui et al. (115), spontaneously silencing plants, activated to target either nitrate reductase or nitrite reductase, when used as a rootstock, efficiently induced PTGS in non-silenced scion tissues. Transmission of the silencing state to the scion was proven by both a marked reduction in transcript accumulation and by the development of chlorosis that is associated with a loss of nitrate or nitrite reductase activity.

In another study, *Agrobacterium*-mediated transient expression was used to activate PTGS in GFP-expressing plants (150). Again, the silencing signal was graft transmissible and initiated PTGS in scion tissues. The persistence of fluorescence signal in syplasmically isolated guard cells confirmed a role for PD in translocation of the silencing signal (150). Establishment of PTGS was correlated with the accumulation of 19–25 nucleotide (nt) siRNA (55). The observed sequence specificity of the silencing signal implied the involvement and phloem mobility of an RNA species. Evidence is now accumulating that siRNA comprises a component of this systemic silencing signal.

Direct analysis of phloem sap has been used to test the hypothesis that the systemic silencing signal is carried by small RNA species. As virus infection induces PTGS (156), phloem sap from virus-infected plants

was first examined and found to contain homologous ~21 nt siRNA (167). Cloning and sequencing of these siRNA species revealed approximately equal proportions of sense and antisense RNA, consistent with the involvement of double-stranded (ds) siRNA. Transgenic cucurbit lines expressing a viral CP provided further confirmation that siRNA moves in the phloem translocation stream. Here, phloem sap derived from spontaneously silencing CP lines revealed siRNA homologous to this transgene. Again, both sense and antisense siRNA species were identifiable by Northern blot analysis. However, RNase protection assays failed to identify a double-stranded (ds) siRNA duplex. In any event, these siRNA molecules were mobile across a graft union and, furthermore, their presence was highly correlated with induction of the silenced state within CP transgenic scion tissues.

Consistent with the concept of selective si/miRNA trafficking within an RNA-based information network, microinjection experiments demonstrated that 25 nt siRNA molecules do not move through PD by simple diffusion. Rather, the capacity of siRNA to traffic between cells appears to be mediated by a small RNA-binding protein, CmPSRP1, identified from pumpkin phloem sap. Proteins having similar properties were detected within the phloem sap of cucumber and lupin, consistent with the notion that a similar mechanism operates in all plants. Binding studies revealed that CmPSRP1 has a 1000-fold higher affinity for single-stranded (ss) siRNA over ds siRNA. Furthermore, CmPSRP1 exhibited substrate specificity in that it bound, preferentially, to small RNA molecules in the size range of 20–40 nt. In addition, microinjection experiments indicated that CmPSRP1 only mediates the trafficking of ss siRNA. Collectively, these findings are consistent with ss siRNA acting as the phloem-mobile silencing signal; however, a role for ds siRNA species should not be excluded from further consideration.

Indirect evidence in support of siRNA as the systemic silencing molecule is provided by a number of studies employing different experimental systems. For example, a positive reporter system was developed to reveal tissues in which PTGS had been activated (73, 80). Microprojectile bombardment was used to introduce specific forms of RNA and DNA homologous to the target gene. Double-stranded siRNA, but not sense or antisense ss siRNA, induced effective systemic gene silencing. In another experimental system, utilizing GUS transgenic sunflower, infiltration into the apoplasm of 21–23 nt RNA, isolated from GUS-silenced tissues, efficiently induced systemic silencing of GUS in newly developing leaves located above the point of infiltration (62). Although the mechanism by which these small RNA species may enter the symplasm remains to be elucidated, if applicable to other plants this infiltration method could provide an effective means with which to explore the events underlying systemic silencing.

Virus-encoded counter measures act directly on the capacity of the plant to utilize the systemic silencing signal (37, 119, 123, 151, 152). Systemic transmission of silencing signals is blocked by expression of the *Cucumber mosaic virus* (CMV) 2b gene, a PTGS suppressor, in vascular tissues through which the signal would otherwise be translocated (53). The inhibitory effect of the CMV 2b suggests a need for some form of relay/amplification along the phloem pathway. Use of the ds siRNA-sequestering tombusvirus-derived p19 suppressor of PTGS abolishes both local (63) and systemic PTGS (86). Collectively, these observations confirm the importance of siRNA in PTGS and are consistent with its role as the long-distance signaling agent. Clearly, these viral suppressors of PTGS have proven extremely useful in dissecting the different stages of the amplification and transmission of the PTGS signal.

Exclusion from the meristem of the systemic silencing signal (150), and most viruses

(99), is consistent with the operation of a regulatory component in the RNA-based information superhighway (74). However, such a surveillance system would have to allow for the observed selective translocation of endogenous mRNA into the meristem (60, 77, 125). Support for this hypothesis was provided through ectopic expression of a potyvirus *TGB1* gene that induced a marked change in the establishment of organ polarity; this viral gene gave rise to radially symmetric leaves (42). Expression of the *TGB1* in the apex appeared to disarm the plant's surveillance system as a range of viruses were able to penetrate all the way into the SAM. Interestingly, entry of either the potyvirus or a *TGB1*-derived silencing signal into the meristem induced transgene silencing that then restored normal patterns of lateral organ development. Here, as virus entry into the plant meristem was dependent on expression of the *TGB1*, induction of PTGS removed both the transgene and the viral-derived *TGB1*, resulting in oscillating patterns of radially symmetric and normal leaves. These results provide support for the existence of a surveillance field regulating the entry of silencing signals and viral RNA into the meristem (42, 95).

“PHLOEMICS”: FUNCTIONAL GENOMICS OF THE PHLOEM

Vascular Genomics

Understanding the molecular basis for the integration of long-distance communication networks will require knowledge of the cognate component parts: Identification of the appropriate genes should assist in the assignment of function. To this end, specialized expressed sequence tag (EST) databases will need to be developed to better define: (a) genes specifically expressed within the plant vasculature system, (b) mRNA and si/miRNA molecules that are mobile in the phloem, and (c) the full complement of proteins that operate in the long-distance signaling pathway.

A full understanding of the plant vasculature must include analysis of xylem function and the role it plays in coordinating whole-plant processes, including growth and development. Xylogenesis (61) and root-shoot feedback systems, controlling branching, nutrient sensing, and resource allocation attest to the importance of a fully integrated approach (15, 18). Recent studies to define the profile of genes active in the vasculature have been based on laser-capture microdissection of phloem-enriched cells from rice and maize (6, 108) and sequence analysis of a cDNA library derived from the “stripped” petiole vascular tissues of celery (148). Macro/microarrays have been employed to better define the slate of vasculature-associated genes. As the number of sequenced plant genomes steadily increases, eventually we will have a solid foundation for a more comprehensive analysis of a wider range of plant vascular systems. An integration of bioinformatics and vascular studies will most surely accelerate progress in this important area of plant biology.

Phloem Transcriptome Contains Both mRNA and Small RNA

Current approaches to defining phloem-mobile transcripts include profiling the mRNA population within the SE-CC complex (6, 17, 67, 148) and direct analysis of transcripts present in the phloem translocation stream (60, 125, 163). The concept of mRNA mobility and an initial characterization of the transcript profile were provided by direct analysis of phloem exudates collected from cucurbits. Based on these studies, the phloem sieve tube transcriptome appears to be comprised of >1500 different mRNA molecules (W.J. Lucas & T.J. Lough, unpublished data), plus many thousands of small RNA species (167).

Insect laser-based stylectomy-assisted extraction of rice phloem sap has been used to obtain limited amounts of mRNA (127).

Other workers have taken a glass microcapillary approach to collect the contents of CCs that were identified by GFP expression driven by a CC-specific promoter. In this case, cellular extracts were not further purified prior to RT-PCR amplification using a range of different primer sets (17). A somewhat similar approach was used to isolate CC protoplasts that were expressing GFP; in this study, RNA was extracted and a cDNA library was constructed to allow the cloning of K⁺ transporters (67). Interestingly, stylectomy-based analysis of barley phloem sap revealed the presence of mRNA encoding a number of integral membrane proteins (36); the K⁺ transporter, HAKT1, could not be detected, consistent with the hypothesis that the PD interconnecting the CC-SE complex mediate selective trafficking of mRNA.

Given the importance of the CC-SE complex, in both phloem transport of photoassimilates and delivery of macromolecules involved in long-distance signaling, it would seem timely to launch efforts aimed at developing complete transcriptomes for a range of plant species. Laser microdissection techniques performed on plants for which genomes have already been sequenced would probably be the most expedient strategy to adopt. Parallel analyses performed on phloem sap derived using insect stylectomy as well as from natural phloem bleeders, such as lupin, castor bean, and the cucurbits, would also serve as an invaluable resource for the development of the phloem database. In view of the positive attributes of the cucurbits, and the fact that their genome size is similar to that of *Arabidopsis*, a cucurbit-based genome sequencing project would accelerate progress in this emerging frontier.

Phloem Proteome

Direct analysis of phloem sap indicates that the phloem proteome is comprised of more than 1500 species present within the translocation stream of the angiosperms (50, 51, 89,

Phloem transcriptome: a database containing sequence information for all RNA species, including mRNA, small interfering (si)RNA, and micro(mi)RNA, present within the sieve tube system

167). Development of the above-mentioned vascular and phloem transcriptomes would underpin identification of these proteins. Here, mass spectrometry approaches would allow for the efficient identification of individual proteins comprising the phloem proteome (10).

Proof of the benefits of this approach is derived from direct analysis of cucumber and pumpkin phloem sap identifying proteins contributing to stress and defense (154) and an antioxidant defense system (50, 51, 155). Comparative proteomics would allow the further identification and characterization of proteins whose entry into the phloem sieve tube system is induced by a range of inputs, including pathogen challenge, nutrient stress, etc. Lastly, this database would greatly facilitate the identification of peptide signaling components that may participate in signaling cascades associated with such processes as flowering control (64) or systemic acquired resistance (147).

FUTURE PERSPECTIVES

Recent progress in our understanding of the processes that act to control macromolecular trafficking through PD have laid the foundation for the elucidation of the various mechanisms that operate to orchestrate NCAP exchange within the phloem long-distance signaling pathway. Assignment of function for specific phloem-mobile macromolecules

has now provided compelling proof of principle for the role played by the phloem in controlling physiological, developmental, and pathogenesis-related processes at the whole-plant level. These findings ensure a bright future for continued dissection of these long-distance communication networks.

The critical next steps include development of comprehensive databases of vascular genes and phloem-mobile transcripts, small RNA, and proteins. It is only in the context of this knowledge that we will be able to understand the various control points and regulatory pathways that utilize the phloem for signal transduction. Natural next steps then include developing protein interaction maps (11, 131) and unraveling the cascades of post-translational modifications involved in regulating signal propagation and/or transduction along each specific pathway. The establishment of such phloem proteomic and transcriptome databases will provide a critical resource for the development of a systems biology approach to the study of whole-plant biology. Studies of this nature will also yield important insights into the evolution of the phloem, both as a nutrient and information macromolecule delivery system. Finally, as plants allocate their resources on a whole-organismal basis, in response to multiple inputs, understanding the molecular basis for these decisions will ultimately enhance our capacity to bioengineer agriculturally important traits.

SUMMARY POINTS

1. Evolutionary development of the plant vascular system afforded a long-distance system for the delivery of both nutrients and information molecules.
2. Plasmodesmata (PD) mediate the local (cell-to-cell) exchange of non-cell-autonomous proteins (NCAPs) and ribonucleoprotein complexes, including transcription factors and RNA involved in developmental programs.
3. A combination of the enucleate sieve tube system of the phloem and the symplasmic domains established by PD allowed the angiosperms to develop an NCAP-based signaling network to integrate environmental cues at the whole-plant level.

4. Cell biologists have now identified and characterized a broad array of proteins and RNA species contained within the phloem translocation stream. The authenticity of these macromolecules as bona fide components of the phloem sap offers support for the hypothesis that they participate in an interorgan signaling network.
5. Plant viruses and viroids evolved the capacity to utilize the properties of PD and the phloem to establish systemic infections. As such, these viral systems provide an effective means with which to dissect the molecular determinants involved in the trafficking of macromolecules into specific tissues/cell types. Recent elegant work on viroid movement identified the first RNA motif involved in RNA trafficking across a specific cellular/PD boundary.
6. Florigen and tuberigen, long sought-after signals involved in floral and tuber induction, in response to day length, move through the phloem and may be transmitted as a long-distance protein and/or RNA signaling molecule(s).
7. Resource allocation and nutrient signaling are affected by specific NCAPs of endogenous and viral origins. One such NCAP, the maize pathogenesis-related protein, PRms, orchestrates profound changes in physiology and growth, apparently by manipulating control over resource allocation.
8. A proteomic approach to the study of phloem long-distance signaling is underway; however, to be most effective, this work will require database initiatives for a number of plant species. The same situation holds for efforts to develop phloem transcriptomes. Given the utility of these databases for a range of projects, including plant systems biology, an initiative is urgently needed at the international level to coordinate such programs.

FUTURE ISSUES TO BE RESOLVED

1. There is a need to develop a comprehensive database of phloem-mobile macromolecules, including mRNA, small RNA, and proteins; ideally, this would incorporate bioinformatic analysis of a number of different plant species. This resource will be essential for future systems biology applications.
2. We need to assign function for all phloem-mobile macromolecules; this information will provide a foundation for the dissection of the component parts involved in specific interorgan signaling pathways.
3. We need to identify the macromolecular determinants responsible for phloem-mediated selective delivery of information macromolecules to target tissues. Studies on the processes involved in maintaining the enucleate sieve tube system may provide important insights into the evolution of the angiosperm phloem.

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Provides evidence that FT forms a complex with FD, a bZIP transcription factor, to activate expression of *APETALA1* in cells that will form the floral meristem.

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