The plant vascular system plays a pivotal role in the delivery of nutrients to distantly located organs. Recent discoveries have provided new insight into a novel role for plasmodesmata and the phloem in terms of the transport and delivery of information macromolecules (i.e. proteins and ribonucleoprotein complexes). Non-cell/organ-autonomous control over gene expression may function both in defense signaling and developmental programming in plants.

Introduction

The plant vascular system, comprised of phloem and xylem conducting elements, has evolved to mediate the exchange of essential nutrients between distantly located organs. The xylem transpiration stream moves up the plant via files of dead cells (i.e. mature tracheary and vessel elements) and this flow is driven by a tensional gradient (in the water potential) along the plant axis. In contrast, the phloem conduit is comprised of files of living cells that transport sugars via plasmodesmata, or through the apoplasm, via sugar transport systems located on the plasma membranes of the companion-cell–sieve-element (CC–SE) complex and surrounding meristematic or receiver cells (Figure 2b).

These operational features of the phloem are now well established. However, there is a central unresolved issue relating to the mechanism(s) by which the plant controls the allocation of resources (i.e. fixed carbon and nitrogen) to its multiple sinks (e.g. shoot and root apical tissues, the vascular cambium, developing organs, root, storage organs and so on). At issue is whether ‘sink strength’ (i.e. the capacity of a tissue to metabolize fixed carbon) controls the distribution of photosynthate or if the plant has evolved a system that operates, at the whole-plant level, to orchestrate the allocation of its resources between distantly located tissues/organs. In the latter case, one might anticipate that the vascular system would play a pivotal role in the delivery of signaling molecules involved in this global allocation process. In this review, we focus on the recent discovery of proteins and RNA molecules that circulate within the phloem and explore their possible involvement as components of a global information-processing network.

Evidence for inter-organ communication via the vascular system

Plants respond and adapt to environmental stimuli and challenges — such as light, water and nutrient stress, and pathogen attack — at the cellular, tissue and organisinal levels. A role for an inter-organ communication system was identified by classical studies of floral induction and has long been recognized. In such a communication system, a combination of a genetic program operating within the shoot apical meristem and an environmentally activated signal generated by a photoperiodic response within source leaves directs the induction of genes responsible for the switch to the reproductive phase (i.e. the conversion from vegetative to floral meristems). Compelling evidence exists for the delivery of a floral signal, via the phloem, to the vegetative meristem; despite intense investigation, however, the nature of this long-distance signaling molecule(s) remains to be elucidated [2–4].

A glimpse into the florigenic signaling pathway was recently provided by the characterization of the maize indeterminate1 (id1) mutant, which remains vegetative [5]. Expression of ID1, a putative transcription factor, was found to be restricted to non-vascular tissues in developing and mature leaves. This result established that ID1 acts in a non-cell/organ-autonomous manner and early in the florigenic signal cascade. Presumably, synthesis of ID1 within bundle-sheath cells leads to the activation of a relay signal that propagates inward to the CC where it gives rise to either the production of a florigenic signal or the release of such a signal into the SE (Figure 1). Dissection of the downstream signaling components associated with ID1 control of floral initiation in maize could well lead to the identification of this elusive phloem-mobile signaling molecule.
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The allocation of resources to the root system to restore the supply of the limiting resource [6]. It has long been known that the perception of water stress by the root system results in the release of the phytohormone abscisic acid (ABA) into the xylem transpiration stream [7]. The delivery of ABA into source leaves leads to a reduction in stomatal aperture, and hence conservation of available water. Under such stress conditions, a reduction in growth occurs simultaneously in roots and shoots. The fundamental question arises as to how the plant exerts a differential response in these distant tissues to achieve an increase in the root-to-shoot ratio. Current research is dissecting the complex interplay of ABA-regulated gene expression [8•], and such studies will eventually reveal whether ABA entry into and delivery via the phloem integrates this shift in the root-to-shoot ratio of water-stressed plants. Insight into the mechanism by which ABA might mediate such a shift in resource allocation is provided by the recent characterization of ICK1, a cyclin-dependent protein kinase inhibitor whose expression is induced by ABA [9]. On a speculative note, ICK1 (or another member of this gene family) may exert, directly or indirectly, differential control over the cell cycle and, hence, over the growth rates of root and shoot tissues (Figure 1).

In response to pathogen attack, plants have been shown to mount a systemic defense response [10,11]. In this situation, phloem-mobile signals also appear to function in the activation of defense genes within non-infected tissues (Figure 1). For example, in solanaceous species, the damaging of tissues by chewing insects results in the systemic induction of proteinase inhibitors. In this case, the
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Phloem-mobile signal appears to be an 18-mer polypeptide, systemin, which is produced in the CCs [12] from pro-systemin by proteolytic cleavage [13]. A membrane-associated receptor for systemin was recently identified [14•], and its activation appears to cause the upregulation of the mitogen-activated protein (MAP) kinase and jasmonate signaling pathways [10]. Thus, upon its release from the phloem, systemin likely activates these signaling cascades within vascular cells. The signals are then relayed out into the surrounding tissues where defense genes are turned on.

Discovery of systemic gene silencing in plants

The involvement of RNA trafficking in phloem-mediated signaling has been implicated by the recent discovery that posttranscriptional gene silencing (PTGS) can operate on a whole-plant level. PTGS was first described in transgenic plants engineered to overexpress either endogenous or viral genes and was termed co-suppression [15,16]. This regulatory mechanism is now known, however, to be a general phenomenon in eukaryotes [17]. On the basis of extensive studies, it appears that this form of gene silencing involves the sequence-specific targeting and degradation of RNA...
molecules. Although the underlying mechanism(s) are still being resolved [18•], the basic processes have now been identified and include: transcription of the target RNA; detection and copying to complementary RNA (cRNA) via an RNA-dependent RNA polymerase [19••,20]; formation of an mRNA–cRNA duplex or double-stranded RNA (dsRNA) [21]; and duplex degradation, via a dsRNAse, to short 21- to 25-mer dsRNA fragments [22•]. These fragments may subsequently function in the degradation of the target mRNA [23•].

Evidence that PTGS can be relayed throughout the plant, via the phloem, was obtained by elegant experiments performed by Vaucheret and colleagues [24]. Transgenic tobacco lines overexpressing either nitrate reductase or nitrite reductase exhibited symptoms consistent with the co-suppression of both the trans and the endogenous copies of these genes [24]. Careful analysis of this phenomenon demonstrated that it was initiated within small groups of cells in source leaves but then transmitted to younger tissues, eventually causing gene silencing throughout all of the developing leaves. Grafting studies provided unequivocal proof that the propagation of PTGS within the plant represents a non-cell/organ-autonomous event. A sequence-specific signal for nitrate/nitrite reductase was shown to eventually gain access to the phloem translocation stream. Candidates for this long-distance signaling molecule(s) are the small dsRNA fragments [22•], their precursor dsRNA [29••], or the cRNA [30]. Confirmation of the nature of the signaling agent, along with the characterization of the protein machinery required for its cell-to-cell and long-distance transport, will provide an important foundation for the further manipulation of this novel whole-plant signaling pathway. Such knowledge will improve our understanding of pathogen defense as well as the epigenetic regulation of endogenous RNA.

**CC–SE complex: a site for protein/RNA exchange**

In addition to providing a symplasmic pathway for the diffusion of sugars, amino acids and ions, plasmodesmata are now known to engage in the selective trafficking of proteins and RNA–protein complexes [31]. This capacity was first established through detailed studies of plant viruses [32] and, subsequently, by studies of endogenous transcription factors, such as KNOTTED1 [33,34••]. The evolution of the ability of plant cells to exchange information macromolecules via plasmodesmata appears to have provided a framework for the development of the highly specialized CC–SE complex of the angiosperms. Here, when functional as a component of the sieve-tube system, each enucleate SE is supported by its ontogenetically related CC(s) through the interconnecting plasmodesmata (Figure 1).

Experimental confirmation that proteins present within the phloem translocation stream have the capacity to traffic through plasmodesmata was provided by microinjection studies [35]. Viral movement proteins and KNOTTED1 are known to interact with the plasmodesmal machinery to mediate their own cell-to-cell transport [32]. When introduced into a target cell phloem, proteins interacted with the plasmodesmal machinery in a similar way to cause an increase in the molecular size exclusion limit (SEL) of the plasmodesmata from 800 Daltons to approximately 25 kiloDaltons (kDa). Interestingly, although many phloem proteins appear to be restricted to the CC–SE complex, they displayed a clear capacity to move from cell to cell when microinjected into non-phloem tissues, such as mesophyll cells. This finding provided the first insight that the plasmodesmal machinery required for protein trafficking may well be common to all cellular boundaries, whereas movement between specific cell types (e.g. from CC to SE) might be regulated by control over protein access to plasmodesmal chaperones and docking proteins. In any event, phloem exudates contain a complex array of many hundreds of proteins that are constantly being exchanged between SE and CC along the translocation pathway [36,37]. This high level of protein trafficking between the CC–SE plasmodesmata should result in an almost ‘permanent’ increase in the SEL across this special boundary. Although fraught with technical problems, microinjection experiments performed on a functional SE–CC system have indeed confirmed that the interconnecting plasmodesmata have SEL values in the order of 25 kDa [38].

Many of the proteins present within the phloem sap are likely to play a role in the maintenance of the enucleate sieve-tube system and, thus, are not signaling molecules. The systemic delivery of a putative RNA signaling molecule via the phloem would, however, likely involve a protein(s) that could traffic through the CC–SE plasmodesmata as an RNA–protein complex. One candidate protein, the 16 kDa phloem protein from Cucurbita maxima (CmPP16), was recently identified [39••]. Interestingly, CmPP16 appears to be distantly related to the viral movement protein encoded by the red clover necrotic mosaic virus. The two proteins have similar properties in that they both bind RNA in a non-sequence-specific manner, mediate their own cell-to-cell movement, and potentiate the...
trafficking of a ribonucleoprotein complex through plasmodesmata. Homologues of the CmPP16 appear to be present in a wide range of plant species and so analysis of its function in long-distance transport should be possible through both mutant and PTGS analyses.

**Sub-set of RNA unique to the phloem translocation stream**

Heterografting experiments performed using cucumber (as scion) and pumpkin (as stock) established that both the CmPP16 and its mRNA move within the long-distance translocation stream (Figure 3a••,d [39••]). As phloem exudates collected from the heterografted cucumber scion were devoid of cucumber homologues of the CmPP16 proteins (Figure 3c), it would appear that proteins and/or RNA derived from the pumpkin stock have the capacity to influence transcription/translation within the scion CC system [39••]. The discovery of a unique sub-set of mRNA transcripts within phloem sap collected from pumpkin plants [40••] added support to the concept that the phloem serves as a conduit for inter-organ delivery of RNA. Indeed, a detailed analysis of one such transcript, the *CmNACP* encoding a putative transcription factor, demonstrated that it could be both delivered via the phloem into cucumber scion tissues and trafficked beyond into tissues such as the cucumber axillary meristem (Figure 3b). Future experiments will need to focus on providing clear evidence that these phloem-borne RNA molecules actually function within the recipient tissues to orchestrate physiological and/or developmental events.

**Selective versus indiscriminate exchange of macromolecules between CC and SE**

A number of studies have reported the presence of a specific protein/RNA within the SE although its transcript/protein was confined to the CC (Figure 2c). These observations suggest that the plasmodesmal-mediated exchange of macromolecules (i.e. proteins and RNA) between the CC and the SE is likely to be highly regulated. Experiments conducted on transgenic plants expressing GFP under a CC-specific promoter have, however, raised doubts concerning the selectivity of this plasmodesmal boundary [41••,42]. The basis for this concern relates to the fact that GFP enters and moves within the phloem translocation stream, is delivered into various sink tissues and then moves out into developing organs (i.e. sink leaves, flowers and so on) [41••,43]. It is important to note, however, that the GFP in these plant lines is being driven by a strong promoter; that the resultant protein is cylindrical in nature, with dimensions (i.e. diameter 2 nm, length 4 nm) close to those presumed to exist in undilated plasmodesmal microchannels; and that the SEL of the CC–SE plasmodesmata is almost constantly increased to approximately 25 kDa. Hence, this foreign protein likely builds up in the CC until there is sufficient free GFP within the cytoplasm to diffuse across the dilated plasmodesmal microchannels into the SE. In this case, the GFP would function as a valuable reporter for changes in plasmodesmal dilation (analogous to the use of fluorescently labeled dextrans; see [44,45]), rather than as a *bona fide* example of indiscriminate exchange of proteins across the CC–SE boundary.

**Post-phloem movement of macromolecules**

The ability of GFP to move beyond the phloem into sink tissues provided an important insight into the likely dynamic role of plasmodesmata in orchestrating post-phloem delivery of photosynthate and/or macromolecules [41••,43]. Caution must be exercised, however, when

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**Figure 3**

Heterografting experiments demonstrate the long-distance translocation of proteins and RNA molecules via the phloem. (a) Schematic illustration of a cucumber scion grafted onto a pumpkin stock. Phloem sap generated in the pumpkin source leaves passes across the graft union (gu) into the scion from which it can be collected for analysis by excising specific tissues (broken arrow). (b) Detection of *CmNACP1* transcripts, by in situ RT-PCR, within the phloem and post-phloem cucumber axillary meristem (CAM) of a heterografted scion. (Reproduced from [40••], with permission.) (c) Western and (d) RT-PCR analyses demonstrate the presence of CmPP16 proteins and CmPP16 transcript within the phloem sap of a cucumber scion (lane 3). Control experiments illustrated in lanes 1 and 2 represent phloem sap collected from pumpkin (scion and stock) and cucumber (scion and stock) tissues. (Reproduced from [39••], with permission.) AM, apical meristem; LM, lateral meristem; LP, leaf primordium; P, phloem; Pe, petiole; RM, root meristem.
extrapolating the spatial distribution of GFP within such sink tissues to predict the non-selective movement of endogenous (macro)molecules. This problem was addressed by Fisher and Cash-Clark [46••] in an elegant study in which they used severed aphid styles as a means with which to pressure-inject a range of fluorescent tracers and proteins directly into functional sieve tubes of the wheat grain. Post-phloem movement of small F-dextrans (of 3 kDa, with Stokes’ radius of approximately 1.2 nm) could be routinely detected, whereas the diffusion pattern of larger molecules was limited, irregular and highly dependent on the duration over which the experiment was conducted. Perhaps most importantly, these experiments underscored the importance of considering the molecular dimensions of the specific reporter probes used in studies relating to plasmodesmal properties.

On the basis of a number of studies using different techniques [40••,41••,46••,47–50,51•], the emerging paradigm is that plasmodesmata within sink tissues likely serve as dynamic pathways for the trafficking of proteins and protein–RNA complexes (Figure 2d). Clearly, these trafficking events will reflect the involvement of specific sets of molecules that are produced by the activation of discrete developmental programs. As a consequence, reporter probes such as GFP will need to be employed and the data interpreted in the light of the fact that various tissues and organs could easily display quite disparate characteristics in response to different environmental or developmental cues.

Conclusions and future directions

Evidence continues to accumulate in support of the hypothesis that the phloem plays an important role in the delivery of information molecules to distantly located plant organs. Of the many hundreds of proteins that have been detected in the phloem translocation stream, only a few have been characterized at the molecular level. Many of these proteins are involved in the maintenance of the enucleate SE-based sieve-tube system. Characterization of the genes encoding such proteins should provide important insights into the evolution of the processes that underlie CC–SE development. Knowledge from these studies will provide a foundation for the analysis of the mechanisms by which proteins and RNA are exchanged across the vital CC–SE boundary. Indeed, it will be of great interest to learn whether there are endogenous proteins/RNA (in addition to systemin-like peptide hormones [10]) that enter the phloem translocation stream purely by default, as recently proposed by Oparka and Santa Cruz [42].

Evidence that sequence-specific information macromolecules enter the phloem and circulate within the plant via the phloem has now accumulated to the point at which the paradigm can be considered beyond debate. One challenge for the future is the identification of the protein machinery that is responsible for the trafficking of RNA molecules into, along, and out of the phloem. The widespread presence of orthologues of the 16 kDa CmPP16 [39**] suggests that these RNA-trafficking proteins, as well as the underlying mechanisms for the long-distance transport of RNA–protein complexes, may well be conserved among the tracheophytes. The discovery that some plant viral-surveillance mechanisms are founded on PTGS, and that viruses can infect their host by blocking such mechanisms (i.e. by engaging in anti-surveillance), indicates the sophistication to which RNA can serve as a cell-to-cell/long-distance information macromolecule. Clearly, future experiments must now focus on identifying the manner in which the plant utilizes this potential.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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Voinnet O, Lederer C, Baulcombe DC: A viral movement protein prevents spread of the gene silencing signal in Nicotiana benthamiana. Cell 2000, 103:157-167. A specific form of PTGS, termed virus-induced gene silencing (VIGS), functions in plants as an anti-viral surveillance system. In this study, the authors demonstrate that a viral movement protein can counteract this surveillance system at the level of phloem and long-distance signaling to developing leaves.


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Hamilton AJ, Baulcombe DC: A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 1999, 286:950-952. The authors describe an important set of experiments that demonstrate the production during the propagation of PTGS of small (25 nucleotide) antisense fragments that may serve as a systemic signal.

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Voinnet O, Lederer C, Baulcombe DC: A viral movement protein prevents spread of the gene silencing signal in Nicotiana benthamiana. Cell 2000, 103:157-167. A specific form of PTGS, termed virus-induced gene silencing (VIGS), functions in plants as an anti-viral surveillance system. In this study, the authors demonstrate that a viral movement protein can counteract this surveillance system at the level of phloem and long-distance signaling to developing leaves.


This study reports the results of a meticulous analysis of plasmodesmal distribution within the plant meristem carried out as the phloem delivers the floral induction signal(s). A temporal rise and fall in plasmodesmal density was observed during the shift from vegetative to floral meristem, suggesting that this developmental transition requires new or supplementary machinery for the cell-to-cell trafficking of information molecules.


The authors demonstrate that SE entry of a phloem sap protein, the CmPP36 of pumpkin, requires the cleavage of a 5 kDa amino-terminal membrane-anchoring motif. Interestingly, experiments involving microinjection into mesophyll cells revealed that the proteolytically cleaved, but not the full-length, CmPP36 was able to traffic through plasmodesmata. In addition, an NADH-binding motif was shown to be related to motifs within both CmPP16 and a viral movement protein, thus strengthening the hypothesis that the virus obtained movement function by the acquisition of host genes.
