The Plant Cell, S303–S325, Supplement 2002, www.plantcell.org

Plasmodesmata: Pathways for Protein and Ribonucleoprotein Signaling

Valerie Haywood, Friedrich Kragler, and William J. Lucas[1]

Section of Plant Biology, Division of Biological Sciences, 1 Shields Avenue, University of California, Davis, California 95616

INTRODUCTION

A new paradigm is emerging in plant biology in which proteins and ribonucleoprotein (RNP) complexes play non-cell-autonomous roles in contributing to the control over developmental and physiological processes. Plasmodesmata (PD), the intercellular organelle(s) of the plant kingdom, create the pathway for the cell-to-cell trafficking of these information macromolecules. This functional property of PD also is thought to have potentiated the development of the angiosperm phloem sieve-tube system that serves as an effective conduit for the inter-organ delivery of proteins and RNP complexes (Lucas et al., 2001; Ruiz-Medrano et al., 2001). In this review, we examine the supporting evidence that underlies the existence and function of this supracellular informational signaling pathway.

BIOGENESIS AND DYNAMICS OF PD

To comprehend the role of non-cell-autonomous signaling molecules requires an understanding of both the pathway taken and the means by which such movement is regulated. The structure and development of PD has been the subject of numerous reviews (Lucas et al., 1993; Kragler et al., 1998a; Ding et al., 1999; Zambryski and Crawford, 2000; Blackman and Overall, 2001; Ehlers and Kollmann, 2001). The essential features are that PD can be (a) inserted into the cell wall either during cytokinesis (termed primary PD) or across an existing wall (termed secondary PD); (b) structurally modified and/or occluded during developmental programs; and (c) removed/replaced to adjust the extent to which a set of neighboring cells are interconnected (Figure 1). Mapping of tissue/organ-specific PD densities has implicated tight genetic control over the processes that effect dynamic changes in PD distribution, but these genetic elements remain uncharacterized (Robards and Lucas, 1990). In any event, such studies have established that a combination of primary and secondary PD establish pathways within meristematic and mature plant tissues whose main functions are the trafficking of nutrients and positional information.

[1] To whom correspondence should be addressed. E-mail wjlucas@ucdavis.edu; fax 1-530-752-5410.

Article, publication date, and citation information can be found at www.plantcell.org/cgi/doi/10.1105/tpc.000778.

POSITIONAL-DEPENDENT CONTROL IN PLANT DEVELOPMENT

Cell Fate in the Shoot Apical Meristem Involves Cell-to-Cell Signaling of Transcription Factors

Divisions of cell initials, or stem cells, located in the shoot and root apical meristems (SAM and RAM, respectively) give rise to multiple cell lineages that collectively differentiate to form new organs. The varying rates of division and differentiation account for the unique features of each organ formed and must be perfectly synchronized to perform normal developmental programs. Orchestration of these events requires the intercellular exchange of signaling molecules, including phytohormones (Golz and Hudson, 2002; Nakajima and Benfey, 2002) and macromolecules. The concept of positional-dependent control in plant development was founded on investigations of the SAM of flowering plants. The angiosperm SAM is generally comprised of three distinct layers (L1, L2, and L3; Figure 2A) in which the cells are interconnected by primary and secondary PD (Figure 2B). Studies using a combination of genetic mutants and grafting techniques revealed that although the contribution of cells from each layer varies considerably, with respect to tissue development, morphologically normal organs are consistently produced (Satina and Blakeslee, 1941; Stewart et al., 1974). Moreover, cells incorporated into another layer, by atypical divisions, acquire the characteristics associated with cells from that layer (Stewart and Dermen, 1975). Collectively, such findings indicate that cell fate can be controlled by the exchange of positional information between neighboring cells.

The intercellular exchange of developmental signals can occur by two different routes; cell–cell, via the apoplasm, and cell-to-cell, through PD (Figure 2C). Thus, positional information could be transmitted through a combination of receptor-ligand–mediated signaling cascades (Clark, 2001)

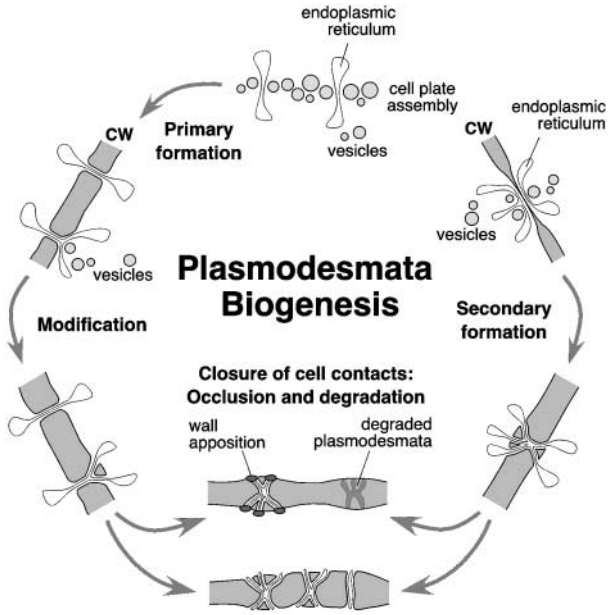

Figure 1. Formation of Primary and Secondary PD.

Formation of primary and secondary PD, in conjunction with PD occlusion and degradation, allows the plant to adjust the extent of the symplasmic/supracellular pathway interconnecting the cells of a tissue. CW, cell wall. (Adapted from Kragler et al., 1998a.)

and/or PD-based trafficking of proteins/RNP complexes. Phenotypic analyses of mosaic maize plants carrying the dominant *Knotted-1* (*Kn1*) mutation established that division patterns of wild-type epidermal cells can be altered when *Kn1* is ectopically expressed in the underlying cells (Hake and Freeling, 1986; Sinha and Hake, 1990). These studies demonstrated that a genetic element could exert control over developmental processes in a non-cell-autonomous manner. KN1 belongs to the homeobox gene family and has now been identified as a transcriptional regulator (Vollbrecht et al., 1991; Reiser et al., 2000). The non-cell-autonomous action of KN1, together with the fact that it could be detected within the nuclei of target cells (Jackson et al., 1994), implicated the involvement of the cell-to-cell signaling pathway in determining cell fate. Such signaling agents are hereafter be referred to as non-cell-autonomous proteins (NCAPs).

The delivery of information macromolecules, within a developing organ, is likely a highly regulated process in order for development to proceed along its normal path. An elegant series of experiments using Antirrhinum genetic chimeras, carrying transposon-induced mutations in the floral homoetic gene *FLORICAULA (FLO)*, demonstrated that control over both spatial and temporal aspects of signaling is critical for proper development to occur. FLO has been shown to regulate meristem identity, because loss of its activity results in the conversion of floral meristems into secondary inflorescence meristems (IM) (Figure 2D) (Carpenter and Coen, 1990). Floral development was almost fully rescued when *FLO* was expressed in the L1 layer, whereas restoration in the L2 or L3 layer gave rise to severely malformed flowers (Figure 2D) (Carpenter and Coen, 1995; Hantke et al., 1995). These results demonstrated that irrespective of the cell layer in which *FLO* is expressed, floral meristem identity was restored, consistent with the hypothesis that FLO acts as a NCAP (see also Table 1). The variation in floral organ development exhibited in relation to the layer in which *FLO* expression was restored suggests the operation of a cellular mechanism that can regulate the trafficking of information macromolecules during development. A further inference that can be drawn here is that either the secondary PD interconnecting the various cell layers of the meristem (Figure 2B) or factors in the cytoplasm of these cells (or in combination) can impose directional properties to NCAP trafficking.

Directional signaling was also observed in periclinal chimeras of the Antirrhinum floral organ identity genes *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*). In stark contrast to *FLO*, L1 expression of *DEF* or *GLO* failed to restore normal floral organ identity (Perbal et al., 1996; Efremova et al., 2001). Non-cell-autonomous action by DEF and GLO was seen only when expression took place in both L2 and L3 layers (Perbal et al., 1996). Interestingly, expression of *DEF* and *GLO* in the L1 of Arabidopsis plants carrying a mutation in *APETALA3* (the *DEF* ortholog) restored normal floral organ development (Efremova et al., 2001). Thus, in Arabidopsis, the putative regulatory elements involved in cell-to-cell trafficking of NCAPs may not exert effective control over DEF/GLO movement. In addition, *LEAFY* (*LFY*), the Arabidopsis ortholog to *FLO*, was shown to be fully effective irrespective of the layer in which it was expressed in *lfy* mutant plants (Sessions et al., 2000). These observed differences in the efficacy of rescuing a developmental program may reflect subtle requirements for successful interaction between the putative directional control system and the particular NCAP.

Microinjection experiments performed with these same non-cell-autonomous transcription factors demonstrated their ability to traffic cell to cell through PD (Table 1). However, within the heterologous tissues used for these experiments, these NCAPs were rarely observed to accumulate within the nuclei of the cells. This again suggests that the extent to which a particular NCAP will move through a tissue is controlled by a range of factors that can regulate entry into the nucleus, retention within the cytosol, or delivery to PD located within specific cellular boundaries. Clearly, there is a pressing need to develop a more complete understanding of the molecular events involved in controlling the movement of macromolecules engaged in the delivery of positional information.

Cellular Coordination Insurance

Given that most of the above-described genes appear to be expressed in all cell layers of the SAM, the question arises as to why there would be any need for such transcription factors to undergo cell-to-cell movement within the mer-

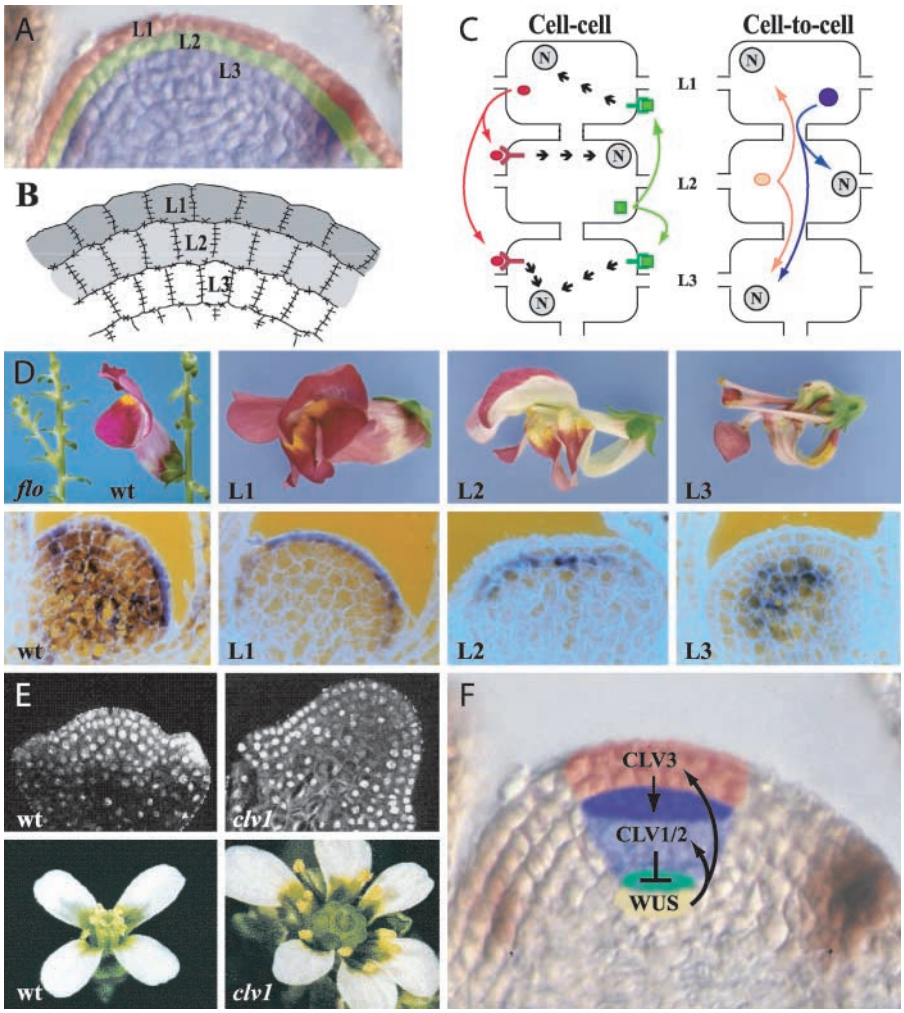

Figure 2. Non-Cell-Autonomous Signaling Molecules Mediate Control over Developmental Processes in the SAM.

(A) The dicot SAM is typically organized into three clonally distinct cell layers. Cell division in the L1 (pink) and L2 (green) occurs almost exclusively in the anticlinal plane, whereas cells of the L3 (purple) can divide in all planes. (Adapted from Bowman and Eshed, 2000.)
(B) Distribution of primary (−) and secondary (×) PD that interconnect the cells of the SAM. (Adapted from Lucas, 1995.)
(C) The possible intercellular pathways taken by non-cell-autonomous signaling molecules. Cell–cell signaling (left) takes place in the apoplasm and involves receptor–ligand-mediated interactions. Secreted ligands (red circle, green square) diffuse through the apoplasm and bind to plasma membrane–bound receptors, thereby activating downstream signaling cascades within the target cell. Cell-to-cell signaling (right) involves PD-mediated trafficking of information macromolecules. N, nucleus.
(D) Cell-to-cell signaling as exemplified by the effects on floral development by *FLO* expression in the SAM of Antirrhinum. Lack of *FLO* expression causes the production of secondary IM instead of flowers. The unstable *flo-613* mutation was caused by insertion of a transposable element, *Tam3*, into the second intron of *FLO*. Spontaneous *Tam3* excision can generate periclinal chimeras exhibiting revertant *FLO* sectors in the *flo-613* background (Carpenter and Coen, 1990). The degree to which normal floral development can be restored is here shown to depend on the layer of the SAM in which *FLO* is expressed. (Adapted from Carpenter and Coen, 1990, 1995; Hantke et al., 1995.)
(E) Phenotypes of wild-type (wt) and *clv1* mutant Arabidopsis plants. Loss of *CLV1* expression results in an enlarged SAM (at top) as well as increased production of floral organs (at bottom). (Adapted from Clark et al., 1993.)
(F) Cell–cell signaling in the SAM by the CLV regulatory pathway. (Adapted from Bowman and Eshed, 2000.)

istem. An answer to this question can be provided by analyzing the likely consequences of the activation of an inappropriate developmental program within a single cell, located, for example, in the L2 layer of an IM. Here, through an alteration in the rate of cell division, the progenitors of this aberrant cell might well displace the wild-type cells that would otherwise have been programmed to produce the reproductive structures. Thus, the absence of coordinated division within the meristem could lead to malformed flowers (see Figure 2D), with the most extreme case being infertility.

Table 1. Cell-to-Cell Movement Capacity of Viral and Endogenous Proteins

Probe	Method[a]	Movement (%)	Extent[b]	References
Viral movement proteins				
RCNMV MP	MI	70–80	E	Fujiwara et al. (1993)
BDMV MP	MI	80–90	E	Noueiry et al. (1994)
TMV MP	MI	70–90	E	Waigmann et al. (1994); Kragler et al. (1998b); Kragler et al. (2000)
CMV MP	MI	70–80	E	Ding et al. (1995), Kragler et al. (1998b)
GUS-TMV MP	MI	80	1 cell	Waigmann and Zambryski (1995)
35S::TMV MP:GFP	BB	62	1–3 cells	Crawford and Zambryski (2001)
35S::CMV MP:GFP	BB	56	1–3 cells	Itaya et al. (1998)
Endogenous transcription factors				
KN1	MI	70–88	E	Lucas et al. (1995); Kragler et al. (1998b)
KN1 mutant M6	MI	10	1 cell	Lucas et al. (1995)
GST-KN1	MI	70	E	Kragler et al. (1998b)
35S::KN1:GFP	BB	14	1–2 cells	Kim et al. (2002)
FLO	MI	70–80	E	Mezitt and Lucas (1996)
LFY	MI	70–80	E	L.A. Mezitt and W.J. Lucas, unpublished data
ML1::LFY	In vivo	100	E	Sessions et al. (2000)
GLO	MI	75	E	Kragler et al. (1998b)
DEF	MI	70–80	E	Mezitt and Lucas (1996)
Phloem proteins				
PP2	MI	80–85	E	Balachandran et al. (1997)
RPP13-1	MI	65	E	Ishiwatari et al. (1998)
CmPP16	MI	90	E	Xoconostle-Cázares et al. (1999)
CmPP16 + RNA	MI	70–80	E	Xoconostle-Cázares et al. (1999)
CmPP36	MI	0	NM	Xoconostle-Cázares et al. (2000)
ΔNCmPP36	MI	90	E	Xoconostle-Cázares et al. (2000)
Heterologous proteins and fluorescent probes				
FITC-dextran (10 kD)	MI	10	1–2 cells	Wolf et al. (1989); Noueiry et al. (1994)
GUS	MI	0	NM	Waigmann and Zambryski (1995)
GST	MI	10	1–2 cells	Kragler et al. (1998b)
GFP	MI (2 min)	0	NM	Oparka et al. (1999)
GFP	MI (24 hr)	66	E	Oparka et al. (1999)
35S::GFP	BB (>24 hr)	0	NM	Itaya et al. (1998)
35S::GFP [c]	BB (24 hr)	34/21	1–2 cells	Crawford and Zambryski (2001)
35S::GFP [c]	BB (24 hr)	100/88	E/1–2 cells	Oparka et al. (1999)
35S::NLS:GFP	BB (24 hr)	17	1–2 cells	Crawford and Zambryski (2000)
35S::ER:GFP	BB (24 hr)	0	NM	Oparka et al. (1999)
35S::GFP:GFP [c]	BB (24 hr)	30/2	1–2 cells	Crawford and Zambryski (2000, 2001)
SUC2::GFP	In vivo	100	E	Imlau et al. (1999); Oparka et al. (1999)

[a] Fluorescently labeled probes introduced into target cell by microinjection (MI), biolistic bombardment (BB) or expression as a transgene (in vivo).
[b] Extent of radial movement by the probe from the target cell: E, extensive movement through five to 10 cells; NM, no movement.
[c] Immature and mature tobacco leaves, respectively, were used in these experiments.

Perhaps the intercellular movement of signals, involved in the establishment of organ identity, provides insurance that all cells within this developmental field are indeed synchronized with respect to a given program (Wu et al., 2002).

The premise on which the above question was founded is that in situ hydridization methods, used to detect the distribution of transcripts within the SAM/IM, provide valid information as to the specific cells in which transcription of a particular gene is occurring. However, cell-to-cell and long-distance transport of endogenous RNA has now been demonstrated to occur in plants (Lucas et al., 1995; Ruiz-Medrano et al., 1999; Kim et al., 2001). Thus, the observed cellular patterns of RNA accumulation in the SAM/IM will not always reflect the actual site(s) of transcription. In some cases, the presence of a specific transcript may reflect a localized region of promoter activity together with the subsequent trafficking of the transcript, as an RNP, through PD. Such a scenario would be consistent with the concept that plants use NCAPs/RNPs as signaling molecules to ensure synchronization of fields of cells involved in developmental events.

Cell–Cell Signaling in the SAM

The occurrence of cell–cell signaling within the meristem has recently been demonstrated by studies on the Arabi-

dopsis *CLAVATA* (*CLV*) genes that appear to be involved in maintaining the stem cell population within the SAM (Clark, 2001). A loss of function in *CLV1*, *CLV2*, or *CLV3* results in an enlarged meristem, with an increased number of floral organs relative to wild-type flowers (Figure 2E) (Clark et al., 1993, 1995; Kayes and Clark, 1998). The *CLV* genes are expressed in small, overlapping domains corresponding to the location of the nondifferentiated stem cells (Figure 2F); they have been proposed to negatively regulate cell division and identity through antagonistic interactions with positive regulators, such as *WUSCHEL* (Brand et al., 2000). CLV1 encodes a leucine-rich-repeat receptor kinase and, together with CLV2 and other cellular components, is thought to form a plasma membrane–associated protein complex (Clark et al., 1997). Because CLV3 encodes a putative extracellular protein, acts non-cell-autonomously, and associates with the active CLV1 complex, it has been suggested that it functions as an extracellular ligand (see Figure 2C) (Fletcher et al., 1999; Jeong, et al., 1999; Trotochaud et al., 2000).

Confirmation of this proposed cell–cell signaling pathway requires the subcellular localization of the CLV gene products. In addition, it will be interesting to determine the distances over which diffusion of CLV3 can serve as an effective delivery mechanism for the activation of the CLV1 signal cascade. It seems likely that such cell–cell signaling would be highly limited in range, hence the small domain of cells in the SAM controlled in this manner. The nature of the feedback signal and the path taken (Figure 2F) remain to be elucidated. On a speculative note, this signaling agent might well be a NCAP/RNP complex, produced in the L3, that traffics through PD to regulate *CLV3* expression.

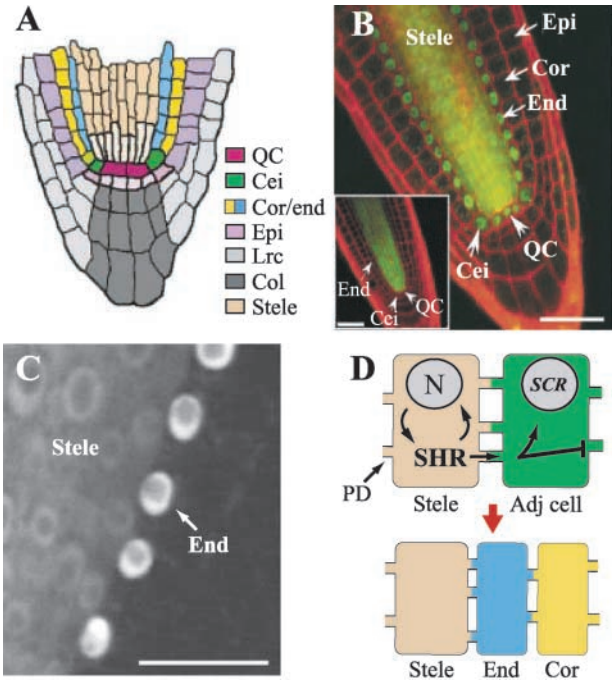

Figure 3. Cell Fate Determination in the RAM Involves Cell-to-Cell Trafficking of Information Macromolecules.

(A) Diagram illustrating, in longitudinal section, the cell types and their arrangement in the root tip of Arabidopsis.

(B) Longitudinal confocal images of transgenic Arabidopsis roots illustrating the transcriptional (inset) and translational patterns of *SHR*:*GFP* expression. Red indicates propidium iodide–stained cell walls. Bars = 50 μm.

(C) Longitudinal confocal (multiphoton) image of the Arabidopsis *SHR*:*GFP* line shown in **(B)** revealing strong accumulation of SHR-GFP in the endodermal nuclei. (**[A]** to **[C]** Adapted from Nakajima et al., 2001.) Bar = 25 μm.

(D) Model illustrating the non-cell-autonomous role played by SHR in endodermal development. SHR, produced in the stele, can either enter the nucleus of these cells or traffic to the adjacent cell layer via PD, where cell-specific factors then block further PD-mediated trafficking of SHR and direct its entry into the nucleus to activate *SCR* transcription. Cei, cortex/endodermis initial; Col, columella; Cor, cortex; End, endodermis; Epi, epidermis; Lrc, lateral root cap; N, nucleus.

Intercellular Signaling Orchestrates Development in the RAM

The RAM is comprised of a small group of slowly dividing cells, termed the quiescent center (QC), surrounded on all sides by cell initials (Benfey and Scheres, 2000). Divisions within these initials produce the highly organized files of different cell types that comprise the root (Figure 3A). Similar to the SAM, cell identity in the root appears to be determined by information provided from neighboring cells (van den Berg et al., 1995, 1997; Tsugeki and Fedoroff, 1999; Kidner et al., 2000). Evidence in support of this concept was provided by laser ablation experiments performed on the root tip of Arabidopsis. Laser ablation of QC cells resulted in differentiation of the adjacent cell initials that, under normal circumstances, would have remained undifferentiated (van den Berg et al., 1997). Furthermore, variations in cell division patterns, within the RAM, do not disrupt the highly organized cellular pattern of the root, revealing a plasticity of cellular differentiation comparable to that observed in the shoot (Kidner et al., 2000).

Given the similarities in cellular differentiation between the SAM and the RAM, it is reasonable to predict that the transmission of positional information in the root will also involve both cell–cell and cell-to-cell pathways. Studies on the genetic regulation of root hair development provided a further example for the existence of non-cell-autonomous control over patterns of cell differentiation. Root epidermal cells can develop into either hair or nonhair cells. In Arabidopsis, hair cells are positioned exclusively over anticlinal cell walls that are formed by pairs of adjoining cortical cells. The relative ratio of the transcription factors *WEREWOLF* and *CAPRICE* is thought to determine hair cell fate (Schiefelbein, 2000; Dolan and Costa, 2001). However, both factors are transcribed predominantly in nonhair cells, suggesting that at least one must act non-cell-autonomously in terms of controlling hair cell fate (Benfey and Scheres,

2000). A detailed analysis of PD distribution within this region of the Arabidopsis root (Zhu et al., 1998) has confirmed the symplasmic continuity within and between epidermal and cortical tissues. It now remains to be determined whether either transcription factor has the capacity to traffic through these PD.

An insightful series of experiments performed on the *short-root* (*shr*) mutant of Arabidopsis provided compelling evidence that SHR acts as a NCAP to convey positional information necessary for determining cell fate (Nakajima et al., 2001). In the *shr* mutant, the cortex/endodermal initial (Figure 3A) fails to undergo longitudinal divisions, resulting in the development of a root lacking the endodermis (Helariutta et al., 2000). Transformation of *shr* mutant lines with a *SHR:green fluorescent protein* (*GFP*) construct restored the wild-type developmental pattern and permitted identification of the cells in which the SHR-GFP accumulated (Figure 3B). Intercellular movement of SHR was inferred based on the finding that the SHR promoter was active only in the stele, whereas SHR-GFP was detected in cells of both the stele and the adjacent layer (Figure 3B).

Inspection of SHR-GFP accumulation within cells of the stele and the adjacent layer indicated a fundamental difference in the subcellular localization pattern of this putative transcriptional regulator (Figures 3B and 3C). Within the cells of the stele, SHR-GFP appeared to be distributed evenly between the cytoplasm and the nucleoplasm, whereas in cells of the QC, cortex/endodermal initial, and endodermis, the fluorescent signal was located almost exclusively over the nuclei. Of equal importance, the intercellular movement of SHR-GFP was confined to this neighboring layer of cells. As illustrated in Figure 3D, these results are consistent with the trafficking of SHR-GFP through the PD that interconnect the cells of the stele and the adjacent cell layer. The accumulation of SHR-GFP within the nuclei of the cortex/endodermal initial has been shown to activate the expression of *SCARECROW* (*SCR*), which then promotes cell division and differentiation (Di Laurenzio et al., 1996). Confinement of SHR-GFP to these cells likely reflects the involvement of at least two regulatory factors, one that directs the SHR-GFP into the nucleus and a second that restricts further outward intercellular trafficking by blocking SHR-GFP access to the PD. The identification of these putative regulatory elements would offer considerable insight into how plants evolved the capacity to use the cell-to-cell trafficking of NCAPs to orchestrate developmental processes.

PD-MEDIATED TRANSPORT OF MACROMOLECULES

Direct Evidence Provided by Viral Movement Proteins

A considerable body of genetic evidence has now accumulated to support the concept that plants use a combination of NCAPs and PD to communicate between cells. Experimental support for the concept that PD have the capacity to mediate the cell-to-cell trafficking of macromolecules was provided by studies into the mechanisms by which plant viruses move within host tissues (Deom et al., 1992; Lucas and Gilbertson, 1994; Carrington et al., 1996). Genetic studies identified viral-encoded proteins, termed movement proteins (MPs), which were shown to be essential for the cell-to-cell spread of infection. The link between these viral MPs and PD was established when it was discovered that expression of the *Tobacco mosaic virus* (TMV)–MP, within transgenic tobacco plants, resulted in an alteration in the functional properties of mesophyll PD. Under normal conditions, such PD restrict the size of molecules that can diffuse cell to cell to ~800 D (Robards and Lucas, 1990). However, in the presence of the TMV-MP, this size exclusion limit (SEL) was increased to a value in the range of 15 kD (Wolf et al., 1989).

Experiments using recombinant MPs provided direct evidence that these proteins have the capacity to interact with cellular components to mediate their transport through PD into neighboring cells (Figures 4A and 4B, Table 1). The inability of mutant forms of MP to move through PD demonstrated that a specific interaction is required for trafficking of these microinjected probes (Figure 4C; Fujiwara et al., 1993; Noueiry et al., 1994; Waigmann et al., 1994; Ding et al., 1995; Rojas et al., 1997; Lough et al., 1998). Irrefutable evidence that PD have the capacity to facilitate the transport of macromolecules was provided by studies involving MP–nucleic acid complexes. Introduction of differentially labeled MP and nucleic acid fluorescent probes resulted in the simultaneous transfer of both macromolecules into the surrounding cells (Lough et al., 1998). These results, in combination with the proven capacity of the viral MP to form stable MP–nucleic acid complexes, in vitro (Citovsky et al., 1992; Fujiwara et al., 1993; Kiselyova et al., 2001), established that PD serve as the conduit for cell-to-cell transport of MPs and MP–nucleic acid complexes (Table 1).

Two additional lines of evidence confirmed this conclusion. First, a number of mutant viruses lacking functional coat protein have been shown to retain the capacity to establish a local infection (Lucas and Gilbertson, 1994; Carrington et al., 1996; Gilbertson and Lucas, 1996). In such situations, because viral particles cannot be formed within the cell, the cell-to-cell spread of infection must be based on the transport of a MP–nucleic acid complex. Second, biolistic experiments confirmed that when produced in vivo, a GFP-tagged MP could move into the surrounding cells via PD (Table 1, Figure 4D). In contrast, bombardment of *GFP*::*YellowFP*, which results in the synthesis of an equivalently sized protein to the MP-GFP, led to the confinement of the fluorescent signal to the targeted epidermal cell (Kim et al., 2002). Collectively, these studies have established that viral MPs have the capacity to interact with PD to (a) induce an increase in SEL; (b) mediate their own transport into the neighboring cell; and (c) potentiate the cell-to-cell movement of the viral infectious agent, in the form of a MP–nucleic acid complex (Figure 4F).

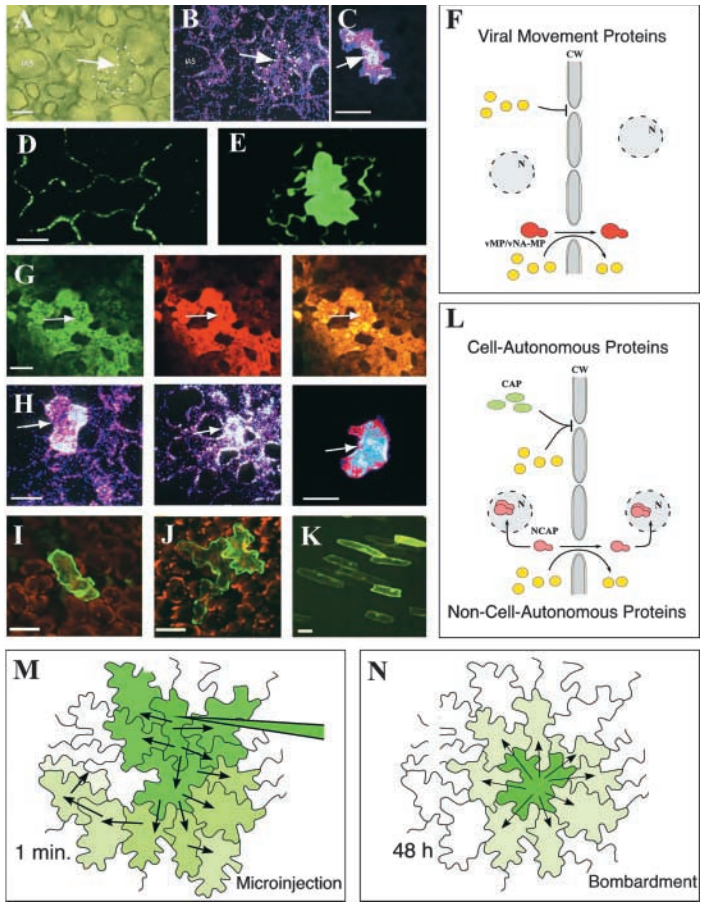

Figure 4. PD Potentiate Selective Cell-to-Cell Transport of Viral MPs/MP–Nucleic Acid Complexes and Endogenous Transcription Factors.

(A) and **(B)** Bright-field and fluorescent images, respectively, illustrating extensive cell-to-cell movement of a FITC-labeled viral MP after its injection into a *Phaeolus vulgaris* (bean) mesophyll cell. Arrows indicate injected cells. IAS, intercellular air space. (Adapted from Noueiry et al., 1994.)

(C) A mutation in this MP blocked its ability to move out of the injected cell. Arrows indicate injected cells. (Adapted from Noueiry et al., 1994.)

(D) Expression of TMV-MP-GFP in a tobacco epidermal cell, after biolistic bombardment, leads to cell-to-cell movement of this fluorescent probe. (Adapted from Crawford and Zambryski, 2001.)

(E) Control GFP bombardment experiment in which free GFP (27 kD) was produced in a tobacco epidermal cell (source leaf). Limited GFP diffusion into adjacent cells likely reflects low-frequency trafficking of endogenous NCAPs. (Adapted from Kotlizky et al., 2001.)

(F) Presence of viral MP (vMP) or MP–nucleic acid complexes (vNA-MP) (microinjected or produced in the infected cell) causes the dilation of PD microchannels, thereby permitting cell-to-cell movement of MP, MP–nucleic acid, and F-dextran/GFP probes (yellow circles). CW, cell wall; N, nucleus.

(G) Cell-to-cell trafficking of a tetramethylrhodamine isothiocyanate (TRITC)–labeled NCAP (left) permitted the simultaneous spread of an 11-kD FITC-labeled dextran (center); the yellow signal resulting from merged images (right) highlights the coupled nature of the TRITC-NCAP and FITC-dextran movement. Arrows indicate injected cell. (Adapted from Kragler et al., 1998b.)

(H) KN1 displays NCAP properties; microinjection of KN1-FITC (left) or KN1 plus 20-kD FITC-labeled dextran (center) resulted in the spread of fluorescence signal into the surrounding mesophyll cells, but movement was blocked in the case of the M6 KN1 mutant (right). Arrows indicate injected cell. (Adapted from Lucas et al., 1995.)

(I) to **(K)** Biolistic bombardment experiments confirm the capacity of NCAPs to traffic through PD. **(**Adapted from Kim et al., 2002.)

(I) Confinement to the target cell of the fluorescent signal associated with expression of GFP-YFP (52 kD) in epidermal cells of Arabidopsis.

(J) Parallel experiment to **(I)** demonstrating limited cell-to-cell movement of GFP-KN1 (~69 kD).

(K) Parallel experiment to **(J)** illustrating cell-to-cell movement of GFP-KN1 in onion root epidermal cells.

(L) Endogenously expressed or microinjected NCAPs interact with PD to induce microchannel dilation, thereby permitting their entry into the next cell as well as the co-diffusion of F-dextran/GFP probes (yellow circles). Cell-autonomous proteins (CAPs) lack this capacity to interact with PD. CW, cell wall; N, nucleus.

(M) and **(N)** Schematic illustrations of the patterns of NCAP cell-to-cell movement after delivery by microinjection or plasmid bombardment, respectively. In microinjection experiments, an NCAP generally spreads through some five cells within 1 min; by 10 min it will have moved out in a radial direction through some 10 cells. In bombardment experiments, NCAP-GFP expression takes 24 to 48 hr before a fluorescent signal can be detected, and then radial movement is often restricted to one or two cells.

Bars = 50 μm.

Endogenous Proteins on the Move

Studies performed on a number of plant transcription factors, such as KN1, FLO, LFY, GLO, and DEF, provided strong evidence that these endogenous proteins similarly have the capacity to interact with and move through PD (Table 1). As observed for viral MPs, introduction of such proteins resulted in (a) an increase in PD SEL; (b) the cell-to-cell transport of the probe; and (c) simultaneous spread of protein and SEL probes (Figures 4G and 4H, Table 1). Specificity of the interaction between the protein and the PD transport pathway was again confirmed using engineered KN1 mutant proteins (Figure 4H, Table 1). In a series of experiments using GFP-tagged KN1 expressed after biolistic delivery or tissue-specific expression within transgenic Arabidopis lines also provided independent confirmation that KN1 has the capacity to move cell to cell (Kim et al., 2002) (Figures 4I to 4K).

Control experiments performed with a range of fluorescent probes, including fluorescein isothiocyanate (FITC)–labeled dextrans (10 to 40 kD) and heterologous proteins derived from a variety of organisms, confirmed the requirement for specificity in terms of macromolecular trafficking through PD. A representative sampling of these controls is provided in Table 1. An interesting facet of these results was the observation that, whether the control probe is introduced by microinjection or produced within a bombarded cell, there was often a very low but detectable level of restricted movement into cells that adjoin the target cell. This likely represents the presence of cell-to-cell trafficking of endogenous NCAPs that induce an increase in PD SEL, thereby potentiating the diffusion of the control probe. This interpretation gains support from experiments performed with various forms of GFP. Here, it is interesting that both the frequency and extent to which free GFP is found to move appear to depend on the nature of the tissue used in the study. Generally, GFP is confined to single cells when introduced into mature epidermal cells (Itaya et al., 1998; Canto and Palukaitis, 1999; Lough et al., 2000; Satoh et al., 2000; Rojas et al., 2001; Tamai and Meshi, 2001a, 2001b). However, on occasion, free GFP appears to be able to move into the adjacent cell layer (see Figure 4E). Quite variable results have been obtained with developing leaf tissue (Table 1). At times, GFP has been reported to undergo very extensive cell-to-cell movement within such tissues (Oparka et al., 1999). Irrespective of this variation, expression and accumulation of GFP can serve as an effective reporter for the trafficking of NCAPs (Figure 4L) either within a tissue (Figure 4E) or at the whole-plant level (Imlau et al., 1999; Oparka et al., 1999).

A significant difference observed between microinjection and biolistic experiments relates to the extent to which the viral MPs and endogenous NCAPs move. When such proteins are introduced into a target epidermal/mesophyll cell within a source leaf, by microinjection, they readily move out into a number of neighboring cells (Table 1). In such cases, within a minute the protein can delineate a pathway of cell-to-cell movement involving trafficking through approximately five cells (Figure 4M). With longer times (5 to 10 min), these injected probes continue to move though additional cells, resulting in trafficking into and through ~10 cells. In contrast, GFP-tagged protein synthesized, in vivo, after plasmid bombardment into epidermal cells (source leaves), generally exhibits limited movement. Here, the fluorescent signal is typically detected in only one or two cells beyond the target cell, resulting in clusters of approximately eight fluorescently labeled epidermal cells (Figure 4N). These differences in the degree of movement may reflect (a) the nature of the probe (i.e., GFP-tag may impair the function of the protein); (b) the amount of protein present in the cytoplasm (i.e., rapid delivery versus in vivo protein synthesis); (c) specific activity of the fluorescent tag (i.e., multiple fluorochemical tags per protein versus a single chromophore in a GFP-tag); and (d) the cell types involved in assessing protein movement (i.e., nature and density of PD). Finally, the possibility should not be overlooked that environmental conditions may well influence the capacity and/or extent to which the PD, within a specific tissue, can mediate the trafficking of macromolecules.

Analysis of the spatial patterns of *KN1* RNA and protein distribution within the maize meristem also implicated KN1 as a NCAP (Jackson et al., 1994; Lucas et al., 1995) in that transcripts were not detected in the L1, whereas nuclear accumulation of KN1 was observed in all cell layers (Figure 5A). It is unfortunate that the physical dimensions of cells in such meristematic tissues precluded the direct delivery of fluorescently tagged KN1 into the tissue where it would normally exert its action. Thus, although NCAPs such as KN1 could be shown to move cell to cell when introduced into heterologous cell types (e.g., mesophyll and epidermal cells of leaves and roots) (Figures 4H to 4K, Table 1), it was critical that transport through PD be tested in the context of the normal site of signaling. Expression of *LFY* within the L1 of a *lfy* Arabidopsis mutant provided proof that this transcription factor (Lohmann et al., 2001) can undergo cell-to-cell transport into the underlying L2 and L3 layers (Sessions et al., 2000) (Figures 5B and 5C). Of equal importance, LFY retained its biological activity after transport, as downstream genes were activated, resulting in the restoration of normal floral development. It is also important to note that the extent to which LFY could traffic within these meristems was equivalent to that observed in microinjection experiments (cf. Figure 5C with Figures 4G, 4H, and 4M). Thus, studies performed with LFY and other NCAPs provided strong support for the hypothesis that PD can establish an effective pathway for non-cell-autonomous signaling in meristematic tissues.

MECHANISMS FOR MACROMOLECULAR TRANSPORT

Cell-to-Cell Transport: A Two-Step Process

In general, protein import into organelles is a sequential process involving exposure of a targeting motif, binding to a

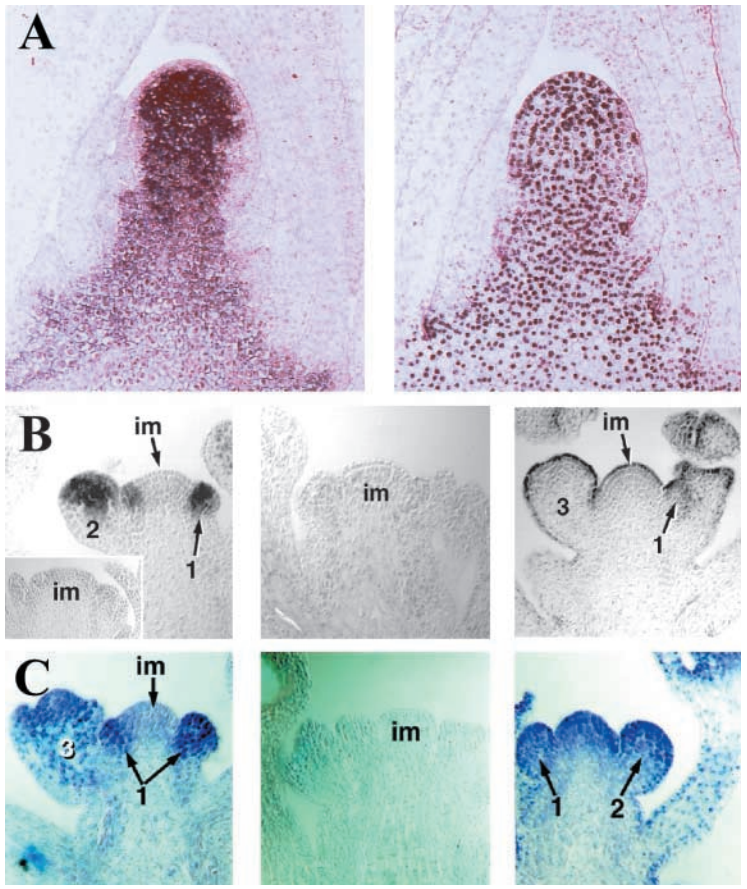

Figure 5. *KN1* and *LFY* Act as Non-Cell-Autonomous Transcription Factors.

(A) In the Zea mays, SAM *KN1* RNA can be detected only in the L2 layer (at left) whereas, by immunolocalization (at right), KN1 could be observed within the nuclei of cells located in the L1 layer. (Adapted from Lucas et al., 1995.)

(B) In wild-type Arabidopsis plants, *LFY* transcripts are detected in young floral buds of the inflorescence meristem (im) (at left) but are absent in plants carrying mutant *lfy* alleles (inset); at left, *lfy-30*; middle, *lfy-12*; at right, expression of *LFY* in a *ML1*::*LFY* transgenic *lfy-30* line resulted in confinement of transcripts to the L1 layer. Numbers indicate stages of flower development (Smyth et al., 1990). (Adapted from Sessions et al., 2000.)

(C) Immunodetection of LFY in the plant lines described in **(B)**. In wild-type plants, LFY was present in nuclei of all cells of young floral buds (at left), and as expected, LFY was absent in the *lfy-12* mutant (middle), but in the transgenic *ML1*::*LFY* line, LFY was detected in all cell layers of the IM and floral buds (at right). Numbers indicate stages of flower development. (Adapted from Sessions et al., 2000.)

translocation receptor complex, protein unfolding, and/or structural modifications to the translocation complex. Cell-to-cell transport of proteins, through the PD microchannel, appears to follow a similar process. All NCAPs and viral MPs examined thus far have been found to expose a motif(s) that can induce dilation of the PD microchannels. The simplest scenario is that this dilation is necessary and sufficient to allow protein movement (diffusion) into neighboring cells (Figure 4L). If this were the case, NCAP movement could be controlled by protein mobility within the cytoplasm (i.e., bound versus free) and the physical dimensions of the individual NCAP. Microinjection experiments proved that such cell-to-cell movement could indeed occur when a small protein (e.g., FITC-labeled soybean trypsin inhibitor [20 kD]) was introduced along with a NCAP; note that no movement occurred when this small protein was injected on its own (Lucas et al., 1995). However, although this model can account for some of the available data, there are a number of lines of evidence implicating a more complex process.

A direct interaction between an exposed motif on the NCAP/MP and a PD constituent (e.g., putative receptor protein) that functions in the control of microchannel dilation is implicated by studies conducted with cross-linked and gold-bound probes (Figure 6A; Kragler et al., 1998b). These experiments revealed that the NCAP-induced increase in PD SEL could be uncoupled from the transport process per

se because the introduction of cross-linked KN1 resulted in the dilation of the microchannel (detected by movement of 10-kD F-dextran) but retention of KN1 within the injected cell. A similar result was observed when gold particles coated with KN1 or MPs were injected into cells. Here, gold-KN1/MP probes too large to enter the microchannel acted to block the trafficking of free KN1/MP but were still competent to induce an increase in the SEL (Kragler et al., 1998b). On the basis of these findings, it would appear that the PD protein(s) involved in mediating the SEL increase most likely resides in the proximity of the PD orifice (Figure 6A).

A second step in the process of NCAP transport through the PD microchannel involves a degree of protein unfolding. Insight into this requirement was gained by examining the competence of structurally modified proteins to move cell to cell (Kragler et al., 1998b). For example, cross-linked KN1 (incapable of unfolding) was no longer able to move through PD (Figure 6A). In addition, when small gold particles (1.4

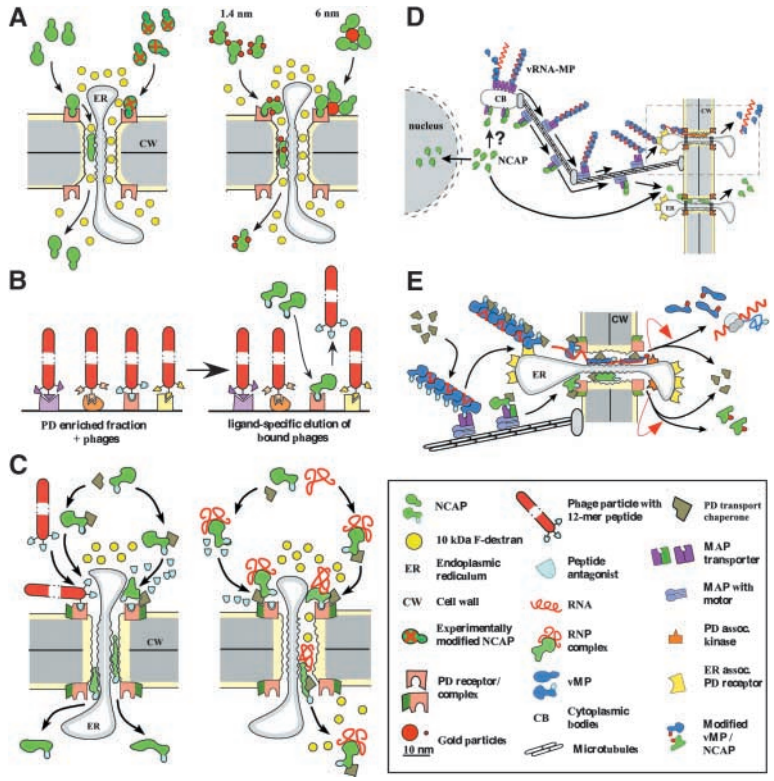

Figure 6. Dissection of Steps Required for MP/NCAP/RNP Complex Trafficking through PD.

(A) A two-step process of protein unfolding and binding to PD SEL motif established by studies performed with structurally modified (cross-linked) NCAP (at left) and gold-conjugated/bound NCAP/MP (at right). The SEL motif was placed at the PD orifice; activation by MP/NCAP permits diffusion of the F-dextran probe. (Adapted from Kragler et al., 1998b.)

(B) Schematic illustration of a phage display assay used to isolate peptide antagonists that could interfere with MP/NCAP transport through PD. (Adapted from Kragler et al., 2000.)

(C) Requirement for a molecular chaperone and a PD receptor complex founded on competitive interactions between MP/NCAP/RNP probes and specific peptide antagonists. (Adapted from Kragler et al., 2000.)

(D) Intracellular steps likely involved in MP/NCAP/RNP complex delivery to the vicinity of the PD orifice. During viral infection, MP–nucleic acid complexes appear to assemble at ER-derived cytoplasmic bodies (CB) before interacting with the microtubule-associated proteins (MAP)/microtubule-based cytoskeleton. This motor system is then thought to deliver the MP–nucleic acid complex to the cell periphery. A similar situation may well be used to control the delivery of NCAPs/RNP complexes to the nucleus or specific cellular interfaces.

(E) Regulation of RNP complex delivery to and translocation through the PD trafficking pathway. Receptor(s) located on the ER, in the immediate proximity to PD, may mediate the docking/delivery of an RNP complex to the PD orifice, where it then engages the SEL and translocation machinery. Structural modifications to the MP/NCAP, by a PD kinase, may be an essential step in the dissociation of the MP–nucleic acid/RNP complex so that the non-cell-autonomous RNA can bind to the translational machinery. Phosphorylation may also block further cell-to-cell transport of a NCAP (Lee and Lucas, 2001).

nm) were covalently linked to KN1, this modification greatly slowed down but did not block KN1 transport through the microchannel. Therefore, the dimensions of this KN1-gold complex appear to be close to the physical limits of the dilated PD microchannels that allow transport of macromolecules. Thus, NCAP transport appears to involve physical changes in both the protein and the PD microchannel.

Peptide Antagonists Block SEL Increase

Confirmation that protein transport can be separated from microchannel dilation was provided by experiments aimed at identifying the putative PD receptors involved in mediating NCAP transport. As illustrated in Figure 6B, a modified phage-display assay was used to identify small peptides having the capacity to bind proteins contained within a cell wall fraction enriched for components of the PD transport machinery (Kragler et al., 1998b, 2000). Microinjection of either phages, carrying a specific 12-mer peptide homologous to a short N-terminal KN1 sequence motif, or synthesized KN1 peptides, blocked the SEL increase mediated by KN1 (Figure 6C). In these peptide antagonist experiments, the inhibition of microchannel dilation was detected by the inability of 10-kD F-dextran to move through the PD. Here, it is of interest that the presence of these peptide antagonists did not prevent the cell-to-cell transport of KN1. Such studies provided a clear demonstration that an increase in PD SEL is not essential for KN1 transport through PD. However, it should be stressed that in the presence of the peptide antagonist, KN1 movement was confined to cells immediately adjacent to the target cell, most likely reflecting some form of structural damage to the protein during translocation through the constricted microchannels. Finally, experiments performed with these peptide antagonists revealed that the cell-to-cell transport of a KN1-RNA complex only occurs when the PD microchannel can be dilated (Figure 6C). This suggests that an increase in SEL may well be a prerequisite for the transport of RNP complexes.

Potential PD Targeting Motifs

The concept that PD can establish pathways for the delivery of NCAPs/MPs and RNP complexes is now well established. However, in contrast to the situation for intracellular transport of macromolecules, where targeting motifs (signal peptides) have been well documented (Keegstra and Cline, 1999; Jans et al., 2000; Holroyd and Erdmann, 2001), to date, equivalent simple PD targeting motifs have not been identified. Mutational analyses performed on viral MPs indicated that complex structural motifs, rather than simple, short, signal sequences, may well be required to transport these proteins into neighboring cells. For example, microinjection experiments revealed that the region of the TMV-MP that is necessary for transport through PD overlaps with the viral RNA binding domain and constitutes approximately one-third of the total protein (residues 110 to 226; Waigmann et al., 1994). Interestingly, small deletions within this region, as well as at the N terminus outside of this domain, have been shown to inhibit TMV-MP transport and viral infection.

An N-terminal TMV-MP^{NT-1} deletion mutant (delta amino acids 3 to 5) is of particular interest in regard to cell-to-cell trafficking. This mutant form of the TMV-MP was retained within the cytoplasm in association with the cytoskeleton, and specifically, the microtubules, and thus did not gain access to the PD when ectopically expressed after biolistic-mediated delivery of a plasmid carrying this gene into epidermal cells (Kotlizky et al., 2001). However, a TMV-MP N-terminal deletion mutant (delta amino acids 1 to 110), lacking the same residues, retained the capacity both to induce an increase in PD SEL and to mediate its movement though PD when microinjected into mesophyll cells (Waigmann et al., 1994). This discrepancy can be easily explained, and highlights the advantages and disadvantages of the current methods available for studying cell-to-cell transport of NCAPs and MPs. In contrast to studies on protein trafficking to organelles such as the nucleus, mitochondrion, or chloroplast, where in vitro experiments can be conducted in the absence of the complexity of the cytoplasm, the transport events involving PD can only be executed in vivo. Thus, when the TMV-MP^{NT-1} is expressed within a cell, after biolistic-mediated transfection, it becomes retained at the level of the cytoskeleton and therefore never gains access to the PD; hence its potential to interact with PD cannot be tested. However, a protein that is microinjected into a cell is able to interact simultaneously at all potential cytoplasmic sites involved in protein delivery to the PD orifice. These studies highlight the need to use all available tools to dissect the functional domains within any NCAP/MP.

The potential complexity associated with PD targeting motifs has also been demonstrated by studies conducted on the CMV-MP (Li et al., 2001). A small deletion within the N terminus of this MP (the M8 mutant) imparted a directionality to viral spread. Trafficking of this mutant MP into surrounding epidermal cells was greatly impaired, whereas movement into and through the underlying mesophyll cells was unaffected. Experiments performed with the MP of *Red clover necrotic mosaic virus* (RCNMV) have similarly underscored the complexity of tissue-specific movement function (Wang et al., 1998). Here, mutant viruses carrying single-point mutations in the RCNMV-MP demonstrated that PD–MP interactions are both tissue- and species-specific in nature. Certain classes of RCNMV-MP mutants were able to move through mesophyll PD but were unable to pass through those connecting the companion cell–sieve element (CC-SE) complex. These results suggest that distinct PD targeting motifs are used by MPs to mediate cell-to-cell transport in a tissue-specific manner.

Control of Transport Capacity through NCAP/MP Modification

The intracellular targeting of specific proteins can be regulated through various forms of structural modification, including proteolytic processing. A similar role for proteolytic processing, in potentiating protein transport across specific cellular boundaries, was demonstrated by the characterization of *CmPP36*, a 36-kD cytochrome b_5 reductase whose expression is confined to the CC in the phloem of pumpkin (*Cucurbita maxima*). Analysis of the pumpkin phloem sap revealed the presence of only an N-terminally truncated (31 kD) form of this CmPP36. Microinjection experiments confirmed that this N-terminally processed protein had the capacity to induce an increase in PD SEL and move cell to cell, whereas the full-length form displayed neither activity (Xoconostle-Cázares et al., 2000). Thus, CmPP36 appears to be an example of an NCAP whose capacity for targeting to and/or transport through PD microchannels is controlled by proteolytic processing. Future studies will establish the extent to which this form of NCAP modification is employed in controlling cell-to-cell transport of proteins and RNP complexes.

Protein phosphorylation has also been implicated in the regulation of the intracellular targeting of specific proteins. The potential for regulating MP/NCAP function, through phosphorylation, was realized when the TMV-MP was observed to carry potential phosphorylation domains that were recognized by cell wall–associated protein kinases (Citovsky et al., 1993). The concept that MP–protein kinase recognition serves to regulate MP cell-to-cell transport gained support from experiments in which amino acid substitutions were engineered within the C-terminal phosphorylation domain of the TMV-MP (Waigmann et al., 2000). These mutant forms of the TMV-MP reflected, to varying degrees, amino acid substitutions that mimicked phosphorylated residues. Microinjection and infection studies performed with these modified TMV-MPs supported the conclusion that MP phosphorylation can inhibit transport through PD in a species-dependent manner. Although cell wall extracts prepared from two plant species, *Nicotiana tabacum* and *N. benthamiana*, could phosphorylate the TMV-MP, the differential movement of the phosphorylation-mimicking mutants could be attributed to species-specific effectors involved in the regulation of MP transport through PD (Waigmann et al., 2000).

How Do Macromolecules Enter the PD Translocation Pathway?

Intracellular protein and RNA distribution is a highly regulated process and involves numerous components including chaperones and the cytoskeletal network. In animal cells, a number of transcription factors have been shown to be located to specific sites within the cell through the formation of mRNA-protein complexes that are recognized by a cytoskeletal-based delivery system (Bassell et al., 1999; Jansen, 2001). A similar mechanism may apply in terms of the delivery of NCAPs/RNP complexes and MPs/MP–nucleic acid complexes to specific regions within the cytoplasm located adjacent to the PD orifice (Figure 6D). Localization studies performed on TMV-infected protoplasts and tissues indicated that the TMV RNA and its MP were co-localized to both ER-derived vesicles and microtubules (Heinlein et al., 1995, 1998; McLean et al., 1995). The microtubule network has been suggested to function in the delivery of TMV MP–nucleic acid complexes from these ER-derived vesicles to the cell periphery (Heinlein et al., 1998; Más and Beachy, 1999; Boyko et al., 2000). Once in close proximity to the PD, this complex may then be recognized by a series of putative PD-associated receptors involved in mediating the subsequent transport of the complex into the adjacent cell (Figure 6E).

A question that clearly needs to be addressed is whether all MPs/NCAPs use this entry into the PD translocation pathway. A number of viral MPs have now been demonstrated to associate with the microtubule-based cytoskeletal delivery system (Reichel et al., 1999). To date, neither the CMV-MP nor KN1, when tagged with GFP, have been found to co-localize with microtubules (Canto et al., 1997; Kim et al., 2002). However, a novel cytoskeletal-associated protein has been found to interact with KN1 (F. Kragler and W.J. Lucas, unpublished data). Little information is also available concerning the role of microfilaments in MP/NCAP delivery. Thus, it is too early to classify the general components required for delivery to the cell periphery/PD orifice. Future studies founded on experimental systems developed to study equivalent processes in animal cells are likely to prove very fruitful.

VASCULAR-MEDIATED INTER-ORGAN COMMUNICATION

Vascular Architecture

Intercellular communication, via macromolecules, can play a pivotal role in regulating developmental events at the tissue and organ levels. But to what extent do plants use such molecules to coordinate events occurring within distantly located organs? It is axiomatic that the evolution of long-distance communication networks was essential for the successful colonization of the land by higher plants, allowing for the efficient exchange of nutrients and signaling molecules between distantly located plant organs. The xylem functions to transport water and minerals absorbed by the roots to aerial portions of the plant, whereas the phloem carries photoassimilates from their site of production in source leaves to actively growing and storage tissues (Figure 7A). Collectively referred to as the vascular system, this noncirculatory conducting network functions at the whole plant level to coordinate developmental and physiological processes through substrate delivery.

In angiosperms, the phloem is made up of two main cell types called sieve elements (SEs) and companion cells (CCs). These cells are intimately connected to one another at maturity through specialized, branched PD across their adjoining cell walls, creating the CC-SE complex (Figures 7B and 7C). The conduit for the phloem is comprised of individual SEs, interconnected, through sieve plate pores (Figure 7D), to form the sieve-tube system. During differentiation, the SE undergoes a partial apoptotic program in which the cellular contents become highly simplified, including removal of the vacuole, plastid reduction and simplification, ribosomal degradation (or severe reduction), and nuclear degeneration. Conversely, the CC is densely cytoplasmic and exhibits a high rate of cellular activity. The CCs appear to function as the control center for the phloem, synthesizing proteins and RNP complexes involved in both the physiological maintenance of the enucleate SE (Figure 7E) and long-distance communication (Figure 7F).

Trafficking of Macromolecules between the CC-SE Complex

The integrity of the SE plasma membrane is crucial for generating and sustaining the osmotic gradients within the phloem. Because the enucleate SE is presumably incapable of protein synthesis and yet remains viable for extended periods, its functionality is thought to depend on continual support being provided by the CC (Oparka and Turgeon, 1999; van Bel and Knoblauch, 2000). Analyses of phloem exudates, collected from several plant species, have revealed the presence of a large number of soluble proteins present within the phloem translocation stream (Fisher et al., 1992; Golecki et al., 1998, 1999; Schobert et al., 1998). Labeling studies demonstrated that these phloem proteins are continually being turned over as they move along the translocation pathway (Fisher et al., 1992). This result most likely reflects the process of protein exchange between the CC-SE complex. Detailed analyses of several phloem proteins revealed that their expression is confined to the CC, indicating that protein synthesis occurs here before entry into the SE (Bostwick et al., 1992; Ishiwatari et al., 1998; Golecki et al., 1999; Xoconostle-Cázares et al., 2000).

Direct evidence in support of the hypothesis that macromolecules can traffic between the CC-SE complex was provided by microinjection experiments involving phloem proteins (Table 1). As with experiments aimed at characterizing the movement capacity of NCAPs that function within the SAM, difficulties were also encountered in accessing the CC-SE complex, and thus, microinjections were performed on heterologous cell types (e.g., into mesophyll cells). Such experiments demonstrated that phloem proteins have the capacity to increase PD SEL and potentiate their own cell-to-cell transport. Finally, the concentration at which these phloem proteins displayed these properties was estimated to be in the range of 10 to 200 nM (Balachandran et al., 1997). Thus, phloem proteins appear to exhibit a high affinity for the mesophyll PD trafficking machinery, a property that likely can also be extrapolated to the CC-SE PD.

Selectivity of trafficking was demonstrated by experiments conducted with a 13-kD rice phloem protein, RPP13-1, belonging to the *thioredoxin h* gene family (Ishiwatari et al., 1995). *RPP13-1* is expressed exclusively in the CC (Figure 8A), and RPP13-1 can mediate its own cell-to-cell transport when microinjected into tobacco mesophyll cells (Figure 8B) (Ishiwatari et al., 1998). However, both the bacterial homolog and a mutant form of RPP13-1 (which retained biological activity) failed to move from the injected cell, again establishing that cell-to-cell transport is a highly selective/regulated process (Figures 8C and 8D). Detailed functional and structural analyses performed on this RPP13-1 revealed that recognition by the PD trafficking machinery must involve structural, rather than simple targeting, motifs. The absence, or alteration, of such motifs in the highly homologous bacterial thioredoxin protein would account for its inability to move cell to cell (Ishiwatari et al., 1998).

Is Protein Exchange through CC-SE PD Regulated?

Extrapolation of information gained from studies on PD located within other plant tissues, such as the mesophyll, the SAM, and the RAM, would indicate that the CC-SE PD similarly engage in the selective trafficking of proteins. Given the high number of proteins that appear to cross this boundary (several hundred) and the capacity of many to induce a significant increase in the SEL of mesophyll PD (20 to 40 kD), it would seem imperative for the plant to have evolved a mechanism to regulate trafficking through these PD. Interestingly, an entirely opposite view has been proposed in which it has been suggested that the contents of the phloem sap "reflects the flotsam produced by CCs along the phloem transport pathway" and thus serves as a "sewage system" (Oparka and Santa Cruz, 2000). This rather interesting view is based on the premise that unless a protein in the CC is anchored, it will in all probability enter the SE by default through the dilated PD. The experimental basis for this notion was the observation that, when highly expressed in the CC, GFP can enter the SE and then be translocated to sink tissues (Imlau et al., 1999; Oparka et al., 1999). Here, it is important to note that as the result of the physical dimensions of GFP (cylindrical molecule; diameter, 3 nm; length, 4 nm [Phillips, 1997]), it, like the 10-kD F-dextran, can diffuse through the PD microchannels that are being dilated during the trafficking of macromolecules (see Figures 4E and 4L).

An additional concern regarding the selectivity of protein entry into the SE relates to the methods used to sample the phloem translocation stream. Excision of plant organs to collect phloem exudate undoubtedly causes damage to the severed tissues and results in contamination of the collected sap. The extent to which this wounding process alters the protein composition of the phloem translocation

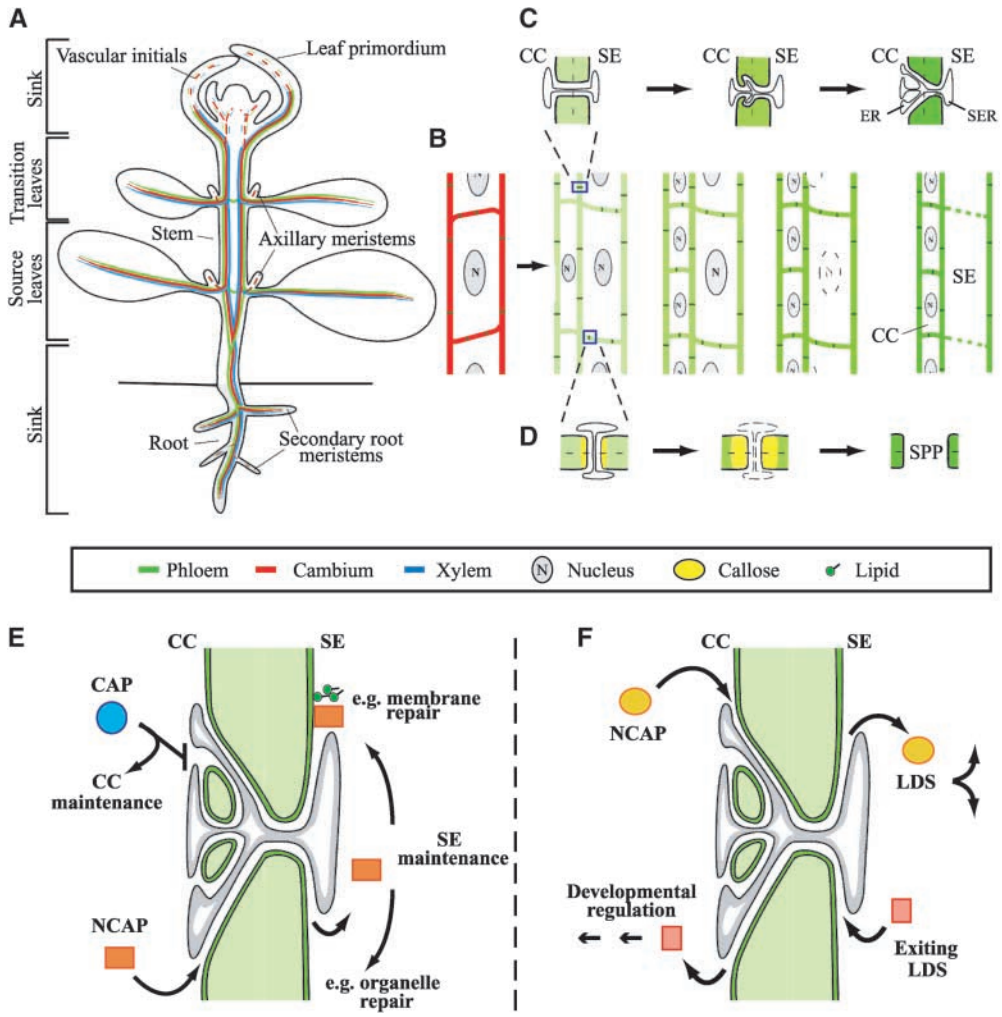

Figure 7. Plant Vascular Network and the Development and Function of the Sieve-Tube System.

(A) The noncirculatory vascular network interconnects distantly located plant organs, providing pathways for the exchange of nutrients and information molecules. The phloem carries photosynthate produced in source leaves to various sinks, such as young, developing tissues (e.g., SAMs and RAMs), and nonphotosynthetic tissues (e.g., roots and floral organs). In developing organs, vascular initials (dashed lines) derived from cellular differentiation establish the vascular routes needed to support these young tissues.

(B) Schematic of the developmental steps involved in the formation of functional enucleate sieve tubes.

(C) During formation of the mature CC-SE complex, specialized, branched PD develop between these two cell types, presumably allowing for the highly controlled exchange of macromolecules into and out of the phloem.

(D) The end walls of the individual SEs form the sieve plates. During SE differentiation, PD located in the transverse walls are structurally modified to produce enlarged pores, called sieve plate pores (SPPs). As the SEs expand, callose is deposited around these PD, and its subsequent removal results in the formation of plasma membrane–lined sieve plate pores that create an open pathway for the pressure-driven flow of assimilates. (**[A]** to **[D]** Adapted from Lucas et al., 2001.)

(E) and **(F)** Models depicting the dual roles played by the CC-SE PD.

(E) Cellular maintenance within the enucleate sieve-tube system is achieved by the production of NCAPs, within the CCs, followed by delivery to and transport through the CC-SE PD. CAPs needed for cellular functions within the CCs are either incapable of cell-to-cell transport or their cellular distribution precludes such transport through PD.

(F) Selective exchange of long-distance signals (LDS), in the form of NCAPs and/or RNP complexes, mediated by the CC-SE PD. Upon arrival at the appropriate target tissue(s), these information macromolecules exit the sieve-tube system to participate in regulation of physiological/developmental events. ER, endoplasmic reticulum; SER, sieve element reticulum; SPP, sieve plate pore.

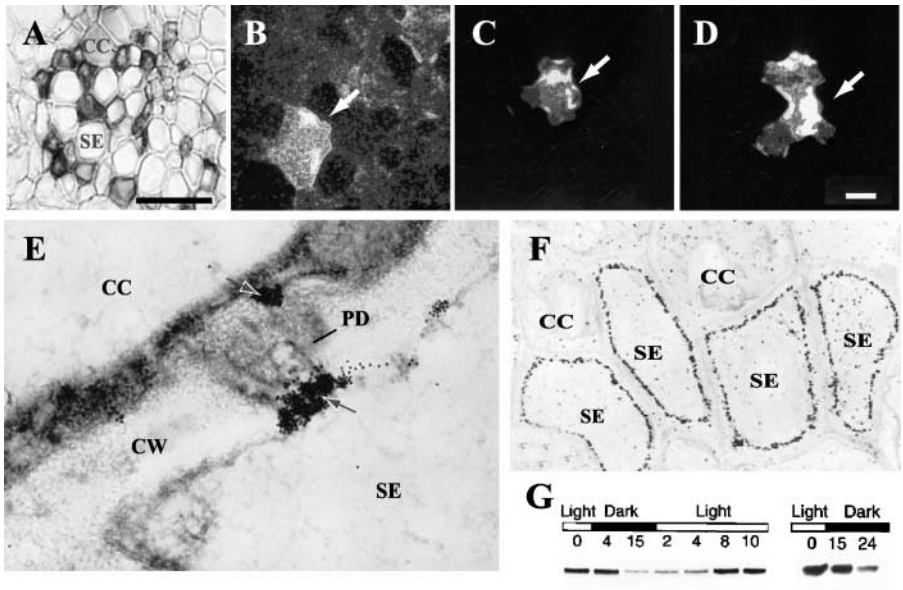

Figure 8. Function of CC-SE PD in the Operation of the CC-SE Complex.

(A) Confinement of *RPP13-1* mRNA to CCs in rice stems, as demonstrated by in situ hybridization. Bar = 20 μm.
(B) FITC-labeled RPP13-1 microinjected into a tobacco mesophyll cell (arrow).
(C) Mutant form of RPP13-1 incapable of cell-to-cell movement.
(D) *Escherichia coli* homolog of RPP13-1 lacks capacity to move through mesophyll PD. (**[A]** to **[D]** Adapted from Ishiwatari et al., 1998.)
(E) *SUT1* mRNA detected by in situ hybridization in CCs and SEs of potato leaf tissue. Note the strong labeling observed at the PD orifices (arrows).
(F) Immunogold labeling of SUT1 in potato petiole phloem observed almost exclusively at the SE plasma membrane.
(G) RNA gel blot hybridization of *SUT1* mRNA (at left) and Western analysis of SUT1 (at right) demonstrate light-dependent turnover of transcript and protein. (**[E]** to **[G]** Adapted from Kühn et al., 1997.)
Bar in **(D)** = 50 μm for **(B)** to **(D)**.

stream has been studied by using heterografting techniques. The protein profiles of phloem sap collected from the stock and the scion generally exhibit a very high degree of similarity (Tiedemann and Carstens-Behrens, 1994; Golecki et al., 1998). Therefore, because the sap within the scion phloem is derived predominantly from the stock (when sap is drawn from close to the graft union), the presence of stock proteins within this scion exudate (Golecki et al., 1999) indicates that wounding per se cannot be the sole basis for protein entry into the phloem sap. Collectively, these studies support the hypothesis that the proteins within the phloem sap are bone fide constituents of the long-distance translocation stream. These findings are also consistent with the evolution of a mechanism that likely arose to limit the perturbation within the angiosperm phloem caused during herbivory/mechanical damage. Indeed, intuitively, the physical features of the CC-SE PD are commensurate with a pressure-driven sealing mechanism (Figure 7E).

The above studies revealed the capacity of phloem proteins to traffic cell to cell and further support the occurrence of PD-mediated macromolecular exchange between the CC-SE complex. The mechanisms regulating protein entry into the SE remain to be elucidated. Because these proteins are often confined to the CC-SE complex, a mechanism must exist to control their delivery to the appropriate cell boundary. Cell-specific chaperones, in combination with the cytoskeleton, may well mediate this delivery to the PD at the CC-SE boundary (Figures 6D and 6E).

Suc Transporter-1 Raises the Specter of Ribosomes in the Phloem

Perhaps the most perplexing evidence involving macromolecular exchange between the CC-SE complex comes from studies on the Suc transporter-1 (SUT1), an integral membrane protein involved in phloem loading and export of Suc from source leaves (Ward et al., 1998). Although transcription occurs in the CCs, surprisingly, in situ hybridization experiments detected *SUT1* mRNA in both CCs and SEs (Kühn et al., 1997). Furthermore, intense signal was detected within both orifices of the CC-SE PD, and a clear signal was also detected in the neighboring region of the CC cytoplasm as well as along the SE plasma membrane

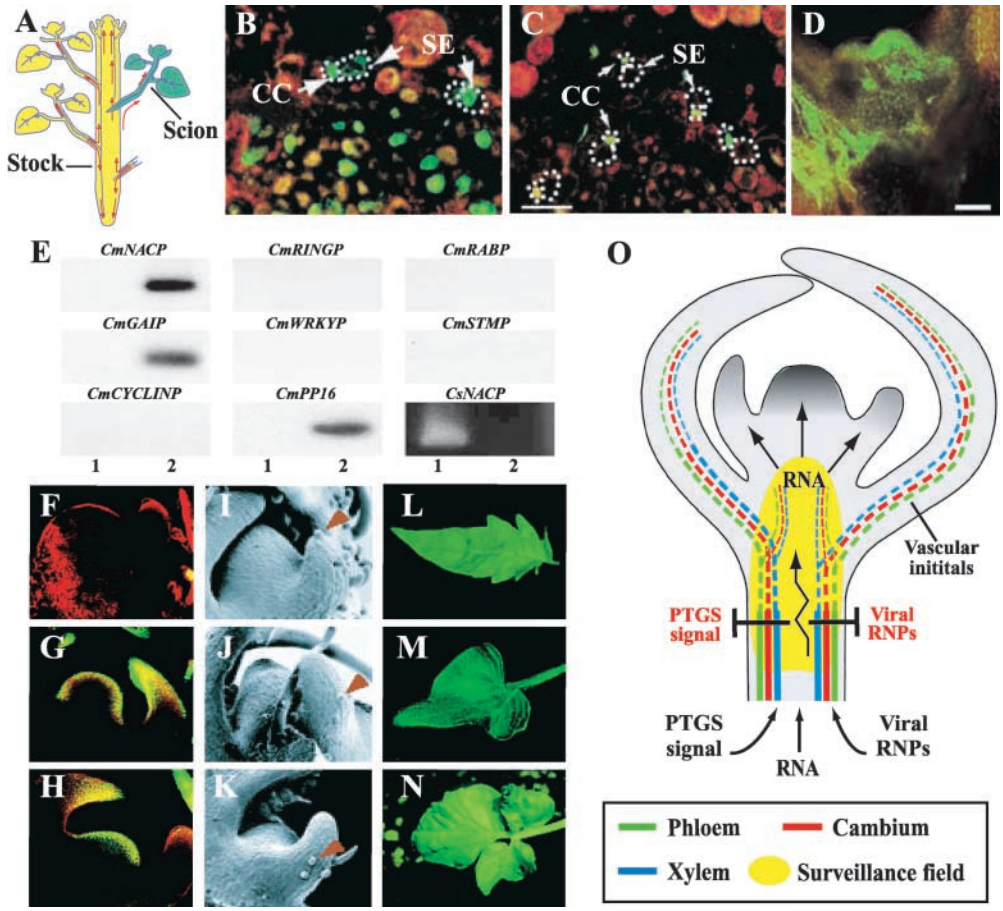

Figure 9. Developmental Regulation through Phloem-Mediated Translocation of mRNA Signals in Plants, as Demonstrated by Grafting Experiments.

(A) Diagramatic representation of heterografting system used to detect delivery of macromolecules in the phloem; arrows indicate the direction of translocation.

(B) In situ RT-PCR detection of *CmNACP* mRNA in CCs and enucleate SEs of pumpkin stem tissue. *CmNACP* gene-specific primers produced a green fluorescent signal when amplification of the transcript occurred. Red signal represents autofluorescence.

(C) Control experiment demonstrating detection of *C. maxima importin-*α transcripts over CCs of pumpkin stem phloem. Bar = 50 μm for **(B)** and **(C)**.

(D) Long-distance translocation of *CmNACP* transcripts, as demonstrated by the accumulation of *CmNACP* mRNA within the axillary meristem of a *Cucumis sativus* (cucumber) scion grafted onto a pumpkin stock. Bar = 100 μm.

(E) Selective trafficking/entry of phloem transcripts from the pumpkin stock into the shoot apex of the cucumber scion. Transcripts for *CmNACP*, *CmGAIP*, and *CmPP16* could be amplified by RT-PCR performed on apical tissue from cucumber scions (lane 2), whereas five other transcripts, present in the phloem sap of pumpkin, could not be detected in these same scion apical tissues. Products were not amplified using the same primers in control experiments performed on apical tissues from nongrafted cucumber plants (lane 1). *C. sativa NACP* (*CsNACP*) was amplified from cucumber (lane 1) but not from pumpkin (lane 2) apical tissue. (**[A]** to **[E]** Adapted from Ruiz-Medrano et al., 1999.)

(F) to **(N)** Developmental changes in control tomato scion tissue correlated with translocation of *PFP-LeT6* fusion RNA from the stock of mutant *Me* plants. (**[F]** to **[N]** adapted from Kim et al., 2001.)

(F) *PFP-LeT6* fusion transcripts were not detected by in situ RT-PCR performed on the shoot apex of wild-type tomato plants. Red signal is produced by tissue autofluorescence.

(G) Detection of *PFP-LeT6* RNA in the shoot apex and leaf primordia of *Me* plants using gene-specific primers and in situ RT-PCR to produce a green fluorescent signal. Overlap of green and red signals produces a yellow color.

(H) *PFP-LeT6* transcripts detected in the apical tissues of wild-type scions grafted onto *Me* stocks.

(I) Scanning electron micrograph showing trichome initiation at the tip of a wild-type leaf primordium.

(J) Development of trichomes is delayed on *Me* mutant plants, because trichomes are initiated in the middle region, rather than the tip, of developing leaf primordia.

(K) Trichome development is similarly delayed on wild-type scions grafted onto *Me* stocks.

(L) Leaflet of wild-type tomato with a pinnate veination pattern and acute lobes.

(M) Phenotype of a leaflet from a *Me* mutant plant with cordate, unlobed morphology and a palmate veination pattern.

(N) Leaflet from a wild-type scion grafted onto a *Me* mutant stock exhibiting a *Me*-like phenotype.

(Figure 8E). Immunolocalization studies suggested that SUT1 accumulates exclusively in the SE (Figure 8F), and further, both protein and mRNA were shown to be under diurnal regulation (Figure 8G). Thus, it would seem that the CC-SE complex has the capacity to turn over both *SUT1* mRNA and SUT1 located within the SE plasma membrane. Equally important, these results provided an experimental foundation for the hypothesis that the CC-SE PD can mediate the transport of endogenous RNP complexes.

These results need to be considered in the framework of the generally accepted notion that the enucleate SE does not engage in protein synthesis. From this perspective, why would the plant traffic *SUT1* mRNA into the SE? One possibility is that the continual PD-mediated trafficking of proteins and RNP complexes, between the CC-SE complex, may allow for nonspecific movement of cell-autonomous mRNA. Such an explanation could account for a low level of contamination, but the high levels of *SUT1* transcript detected in the SE clearly contradict such an explanation. An alternate hypothesis can be offered based on recent heterografting experiments (R. Ruiz-Medrano, B. Xoconostle-Cázares, and W.J. Lucas, unpublished data). Here, it was shown that *SUT1* mRNA actually moves within the phloem translocation stream, and thus, these transcripts could serve as long-distance signaling molecules. In this case, *SUT1* mRNA would not be translated in the SE; rather, SUT1 would be produced within the CC followed by its translocation into the SE using the continuity established by the ER/plasma membrane of the CC-SE PD. Finally, the possibility that mature SEs contain the machinery capable of translating *SUT1* mRNA should not be discounted at this time.

Phloem-Mobile RNA Mediates Systemic Acquired Gene Silencing

It has long been known that viruses use the phloem to establish a systemic infection and, furthermore, that the systemic movement of some coat protein deletion mutant strains implicated the sieve-tube system in the long-distance delivery of MP-viral nucleic acid complexes (Gilbertson and Lucas, 1996). Additional experimental support for the hypothesis that plants use the phloem pathway for the delivery of RNA-based signals is founded on the discovery that plants use an epigenetic process, termed post-transcriptional gene silencing (PTGS), that results in the sequence-specific degradation of targeted mRNA (see Mlotshwa et al., 2002, in this issue). Several experimental approaches have provided clear evidence that the phloem functions in the systemic transmission of epigenetic phenotypes attributable to PTGS. In the case of transgenic plants overexpressing nitrate or nitrite reductase, signs of spontaneous gene silencing (chlorosis resulting from a perturbation in nitrogen availability) were detected within an expanding cluster of cells present within a source leaf (Palauqui et al., 1996, 1997). This phenotype was then observed to propagate up the plant axis in a pattern reflecting the pathway of phloem translocation. Heterografting experiments confirmed a role for the phloem in the systemic delivery of a sequence-specific PTGS signal (Palauqui et al., 1997).

Transgenic plants expressing *GFP* also provided a powerful experimental system to test the general applicability of the concept that the phloem functions as the conduit for systemic transmission of RNA-based signaling molecules. Here, *Agrobacterium* infiltration was used to allow a local transient production of *GFP* RNA within a cluster of mesophyll cells (Voinnet et al., 1998). Local PTGS of the *GFP* transgene was detected first by the loss of GFP fluorescence within this infiltrated region of the leaf; this silenced state then spread through cells connected by PD. Next, a sequence-specific PTGS signal entered the phloem of the source leaf, and its delivery to the upper developing leaves resulted in the establishment of systemic silencing of the heterologous *GFP* transgene. Collectively, such studies establish that the sieve-tube system, and in particular the properties of the CC-SE PD, creates a transport system that can mediate the delivery of RNA signaling molecules, although the exact nature of the systemic PTGS signal still remains to be identified (Lucas et al., 2001).

Endogenous MPs of the Phloem

The entry of RNA into the sieve-tube system would almost certainly require the involvement of a unique class of endogenous RNA binding proteins that can function as NCAPs. Recent evidence provided by the characterization of the 16-kD *C. maxima* phloem protein (*CmPP16*) demonstrated the presence of an RNA binding protein having properties consistent with an ability to mediate the long-distance transport of RNA

Figure 9. (continued).

(O) Model depicting the selective trafficking of macromolecules within the shoot apex. A surveillance field monitors the exit of macromolecules from the protophloem. Information macromolecules involved in developmental regulation, such as *CmNACP* and *PFP-LeT6* transcripts, are permitted to pass through the surveillance field and traffic through the cells of the apex, accumulating within the SAM proper and developing lateral organs. Aberrant or inappropriately delivered macromolecules (e.g., viral RNPs or systemic PTGS signals) detected by the surveillance system are degraded. (Adapted from Lucas et al., 2001.)

(Xoconostle-Cázares et al., 1999). CmPP16 was identified based on its structural (immunological) homology to the RC-NMV-MP, and was shown to be localized to the CC-SE complex and to share many properties in common with viral MPs. For example, CmPP16 has the capacity to (a) increase PD SEL; (b) mediate its own cell-to-cell transport; and (c) potentiate the intercellular trafficking of both sense and complementary RNA; this capacity of CmPP16 to traffic RNA was independent of the sequence reflected in the actual transcripts employed. Finally, heterografting experiments performed using pumpkin (stock) and cucumber (scion) demonstrated that both CmPP16 and its mRNA are translocated over long distances through the phloem.

Parallel studies performed using antibodies raised against other MPs suggest that the phloem sap likely contains additional RNA binding proteins. In vitro RNA binding studies performed with phloem sap–purified proteins have demonstrated that the pumpkin phloem translocation stream indeed contains additional RNA binding proteins that are currently being characterized (B.C. Yoo and W.J. Lucas, unpublished data). These findings provide insights into the mechanism(s) by which CmPP16 and other phloem RNA binding proteins may mediate the entry of transcripts, such as *SUT1* mRNA and the RNA species responsible for the delivery of the systemic gene-silencing signals, into the phloem translocation stream.

RNA as Long-Distance Information Macromolecules

In addition to delivering nutrients, the phloem is known to deliver to sink tissues a range of signaling molecules, including phytohormones (Jackson, 1997) and peptide hormones, like systemin (Ryan et al., 2002, in this issue). Sugars delivered by the phloem have also been implicated as developmental signals (Rolland et al., 2002, in this issue). A detailed analysis of phloem RNA, in which the question of wound-induced contamination was extensively examined, revealed that the pumpkin sap contains a unique population of transcripts, including >100 polyadenylated mRNA molecules (Ruiz-Medrano et al., 1999). In experiments parallel to those performed earlier on SUT1 (Kühn et al., 1997), Ruiz-Medrano et al. (1999) used in situ reverse transcriptase–mediated (RT)–PCR to confirm the location of these phloem-mobile transcripts within the functional sieve-tube system of control and heterografted cucurbits (Figures 9A to 9C). Heterografting experiments, using cucumber scions grafted onto pumpkin stocks, were employed to test whether representative samplings of these phloem transcripts were actually being translocated through the phloem. Founded on the ability of in situ RT-PCR to discriminate between phloem mobile transcripts that originate from the pumpkin stock, these studies clearly identified the presence of pumpkin mRNA within the CC-SE complexes of the cucumber scion. A pivotal finding from these grafting experiments was the discovery that *CmNACP* (for *C. maxima NAC DOMAIN PHLOEM*) mRNA could be traced along the translocation pathway of cucumber where it was found to exit the phloem and enter meristematic tissues (Ruiz-Medrano et al., 1999) (Figure 9D). Indeed, delivery and/or exit of these phloem-mobile transcripts into the shoot apex appeared to be regulated, because although such transcripts could be detected in the scion SEs, only a subset were detected in the scion apex (Figure 9E).

Additional evidence that phloem-mobile mRNA can play a role in developmental events was provided by grafting experiments conducted with tomato plants carrying the dominant gain-of-function leaf morphology mutation *Mouse ears* (*Me*) (Kim et al., 2001). This *Me* phenotype is caused by a gene fusion event between a gene encoding a glycolytic enzyme, *PHOSPHATE-DEPENDENT PHOSPHO-FRUCTOKINASE* (*PFP*), and a *KN1*-like homeobox gene, *LeT6* (Chen et al., 1997), resulting in the production of *PFP-LeT6* fusion transcripts. In situ RT-PCR analysis revealed the presence of this *PFP-LeT6* fusion RNA in the apex of *Me* plants (Figures 9F and 9G). *PFP-LeT6* RNA was demonstrated to be translocated into wild-type tomato scions grafted onto *Me* stocks. As in the *CmNACP* experiments, *PFP-LeT6* transcripts could be traced into the scion apex, where they accumulated in the SAM and developing leaf primordia (Figure 9H); note well the similarity between this pattern of *PFP-LeT6* RNA and that observed in the SAM of *Me* plants (Figure 9G). Phenotypic changes in the scion appeared to occur early in development, as evidenced by delayed trichome development on emerging leaf primordia (Figures 9I to 9K). In *Me* plants, leaves exhibited increased pinnation in addition to an alteration in leaflet shape (Figures 9L and 9M). Import and accumulation of the dominant gain-of-function *PFP-LeT6* transcripts was also shown to correlate with a similar alteration in scion leaf morphology (Figure 9N). Hence, the detection of *PFP-LeT6* transcripts in the scion apex, in conjunction with the observed morphological changes, provides strong support for the hypothesis that RNA can function as an information macromolecule to control developmental processes in distantly located tissues and organs.

A Priority Delivery System for Long-Distance Transcripts

The selective nature of macromolecular transport out of the phloem into the shoot apex has been previously observed for a number of processes. For example, within apical tissues, cell-to-cell movement of systemically infecting viruses is hindered upon exit from the phloem (Gilbertson and Lucas, 1996; Wang et al., 1996; Jones et al., 1998). In addition, the systemic signal propagating PTGS appears to be able to enter into developing leaves but is normally excluded from the vegetative meristem (Ruiz et al., 1998). In contrast, the observed accumulation patterns of phloem-translocated RNA, within the shoot apex (Figures 9D and 9H), indicate the ability of these transcripts to move through the post-phloem tissues, via PD, to gain access to the cells of the meristem. Such differences in macromolecular transport

suggest the presence of a surveillance field, within the shoot apex, which monitors the cell-to-cell movement of NCAPs and RNP complexes as they exit the phloem (Figure 9O). The basic elements of this surveillance system will likely incorporate the known properties associated with regulated trafficking of NCAPs/RNP complexes through PD and the initiation/propagation of PTGS (Lucas et al., 2001).

The observed patterns of *CmNACP* and *PFP-LeT6* RNA accumulation within the terminal tissues of the apex implicate the action of some form of specialized delivery system. Delivery from the terminal phloem through to the L1 layer of the SAM requires the passage of NCAPs/RNP complexes through some 30 to 50 cells, depending on the plant species. In moving along this route, transcripts destined for the SAM also encounter the "tide" of cell division that effectively inserts additional cell boundaries through which these signaling molecules must pass. It may be that macromolecules capable of accessing the meristem display a specific structural motif(s) that acts as a "zip code," allowing the recognition and vectorial transport on the PD translocation machinery present within these tissues. This SAM surveillance field and delivery system could function as an effective control system to protect the meristem from input signals that would otherwise perturb cell proliferation and cell fate determination.

Future Directions

An expanding body of evidence provides support for the hypothesis that plants use a combination of PD and the phloem sieve-tube system to effect non-cell-autonomous, or supracellular, control over developmental processes. This having been said, it is also fair to say that the discoveries that led to this new paradigm have raised far more questions than answers. Although extensive studies have been conducted on PD distribution within various plant organs, the genetic basis underlying control over PD density/turnover remains to be elucidated. Similarly, compelling evidence has been collected in support of the concept that PD mediate the trafficking of macromolecules, but currently almost no information is available at the molecular level concerning the nature of the putative chaperones and receptors involved in mediating these trafficking events. Even the most basic of questions need to be resolved concerning how NCAPs/RNP complexes are recognized by the PD translocation machinery. Clearly, it will be impossible to advance our understanding of the role played by this unique signaling pathway until the component parts have been isolated and characterized. An integrated approach, using in vitro and in vivo experimental systems, reverse genetics, and novel highly sensitive methods for detection of intracellular and cell-to-cell transport of NCAPs is required to dissect the molecular components of this trafficking pathway.

The function of RNA as a long-distance signaling molecule opens up an entirely new facet of plant biology. A complete characterization of the phloem proteins and RNP complexes that move within the phloem translocation stream should provide the foundation necessary for elucidating the mechanisms that underlie this novel communication pathway. Studies aimed at elucidating the molecular determinants that control the entry and exit of RNP complexes, across the CC-SE boundary, as well as those that act within the proposed surveillance field, should eventually provide the knowledge necessary to allow the manipulation of this inter-organ information signaling pathway. Ultimately, such studies should provide insight into how the plant has evolved to respond globally to localized conditions such as environmental inputs and pathogen attack.

ACKNOWLEDGMENTS

We offer our apologies to the colleagues whose work could not be properly discussed due to space limitations. We thank David Jackson for providing results prior to publication. Work in our laboratory on PD and the supracellular nature of plants was supported by grants from the National Science Foundation (Grant No. IBN-9900539) and the U.S. Department of Energy Office of Basic Energy Sciences (Grant No. DE-FG03-94ER20134).

Received November 29, 2001; accepted March 17, 2002.

REFERENCES

Balachandran, S., Xiang, Y., Schobert, C., Thompson, G.A., and Lucas, W.J. (1997). Phloem sap proteins from *Cucurbita maxima* and *Ricinus communis* have the capacity to traffic cell to cell through plasmodesmata. Proc. Natl. Acad. Sci. USA **94,** 14150–14155.

Bassell, G.J., Oleynikov, Y., and Singer, R.H. (1999). The travels of mRNA through all cells large and small. FASEB J. **13,** 447–454.

Benfey, P.N., and Scheres, B. (2000). Root development. Curr. Biol. **10,** R813–R815.

Blackman, L.M., and Overall, R.L. (2001). Structure and function of plasmodesmata. Austr. J. Plant Physiol. **28,** 709–727.

Bostwick, D.E., Dannenhoffer, J.M., Skaggs, M.I., Lister, R.M., Larkins, B.A., and Thompson, G.A. (1992). Pumpkin phloem lectin genes are specifically expressed in companion cells. Plant Cell **4,** 1539–1548.

Bowman, J.L., and Eshed, Y. (2000). Formation and maintenance of the shoot apical meristem. Trends Plant Sci. **5,** 110–115.

Boyko,V., Ferralli, J., Ashby, J., Schellenbaum, P., and Heinlein, M. (2000). Function of microtubules in intercellular transport of plant virus RNA. Nat. Cell Biol. **2,** 826–832.

Brand, U., Fletcher, J.C., Hobe, M., Meyerowitz, E.M., and Simon, R. (2000). Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity. Science **289,** 617–619.

Canto, T., and Palukaitis, P. (1999). Are tubules generated by the

3a protein necessary for cucumber mosaic virus movement? Mol. Plant-Microbe Interact. **12,** 985–993.

Canto, T., Prior, D.A., Hellwald, K.H., Oparka, K.J., and Palukaitis, P. (1997). Characterization of cucumber mosaic virus. IV. Movement protein and coat protein are both essential for cell-to-cell movement of cucumber mosaic virus. Virology **237,** 237–248.

Carpenter, R., and Coen, E.S. (1990). Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus.* Genes Dev. **4,** 1483–1493.

Carpenter, R., and Coen, E.S. (1995). Transposon induced chimeras show that *floricaula*, a meristem identity gene, acts non-autonomously between cell layers. Development **121,** 19–26.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K., and Schaad, M.C. (1996). Cell-to-cell and long-distance transport of viruses in plants. Plant Cell **8,** 1669–1681.

Chen, J.-J., Janssen, B.J., Williams, A., and Sinha, N.A. (1997). A gene fusion at a homeobox locus: Alterations in leaf shape and implications for morphological evolution. Plant Cell **9,** 1289–1304.

Citovsky, V., Wong, M.L., Shaw, A.L., Prasad, B.V., and Zambryski, P. (1992). Visualization and characterization of tobacco mosaic virus movement protein binding to single-stranded nucleic acids. Plant Cell **4,** 397–411.

Citovsky, V., McLean, B.G., Zupan, J.R., and Zambryski, P. (1993). Phosphorylation of tobacco mosaic virus cell-to-cell movement protein by a developmentally regulated plant cell wall–associated protein kinase. Genes Dev. **7,** 904–910.

Clark, S.E. (2001). Cell signalling at the shoot meristem. Natl. Rev. Mol. Cell Biol. **2,** 276–284.

Clark, S.E., Running, M.P., and Meyerowitz, E.M. (1993). CLAVATA1, a regulator of meristem and flower development in *Arabidopsis.* Development **119,** 397–418.

Clark, S.E., Running, M.P., and Meyerowitz, E.M. (1995). CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. Development **121,** 2057–2067.

Clark, S.E., Williams, R.W., and Meyerowitz, E.M. (1997). The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis.* Cell **89,** 575–585.

Crawford, K.M., and Zambryski, P.C. (2000). Subcellular localization determines the availability of non-targeted proteins to plasmodesmatal transport. Curr. Biol. **10,** 1032–1040.

Crawford, K.M., and Zambryski, P.C. (2001). Non-targeted and targeted protein movement through plasmodesmata in leaves in different developmental and physiological states. Plant Physiol. **125,** 1802–1812.

Deom, C.M., Lapidot, M., and Beachy, R.N. (1992). Plant virus movement proteins. Cell **69,** 221–224.

Di Laurenzio, L., Wysocka-Diller, J., Malamy, J.E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M.G., Feldmann, K.A., and Benfey, P.N. (1996). The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. Cell **86,** 423–433.

Ding, B., Li, Q., Nguyen, L., Palukaitis, P., and Lucas, W.J. (1995). Cucumber mosaic virus 3a protein potentiates cell-to-cell trafficking of CMV RNA in tobacco plants. Virology **207,** 345–353.

Ding, B., Itaya, A., and Woo, Y.M. (1999). Plasmodesmata and cell-to-cell communication in plants. Int. Rev. Cytol. **190,** 251–316.

Dolan, L., and Costa, S. (2001). Evolution and genetics of root hair stripes in the root epidermis. J. Exp. Bot. **52S,** 413–417.

Efremova, N., Perbal, M.-C., Yephremov, A., Hofmann, W.A., Saedler, H., and Schwarz-Sommer, Z. (2001). Epidermal control of floral organ identity by class B homeotic genes in *Antirrhinum* and *Arabidopsis.* Development **128,** 2661–2671.

Ehlers, K., and Kollmann, R. (2001). Primary and secondary plasmodesmata: Structure, origin, and functioning. Protoplasma **216,** 1–30.

Fisher, D.B., Wu, Y., and Ku, M.S.B. (1992). Turnover of soluble proteins in the wheat sieve tube. Plant Physiol. **100,** 1433–1441.

Fletcher, J.C., Brand, U., Running, M.P., Simon, R., and Meyerowitz, E.W. (1999). Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems. Science **283,** 1911–1914.

Fujiwara, T., Giesman-Cookmeyer, D., Ding, B., Lommel, S.A., and Lucas, W.J. (1993). Cell-to-cell trafficking of macromolecules through plasmodesmata potentiated by the red clover necrotic mosaic virus movement protein. Plant Cell **5,** 1783–1794.

Gilbertson, R.L., and Lucas, W.J. (1996). How do viruses traffic on the 'vascular highway'? Trends Plant Sci. **1,** 260–268.

Golecki, B., Schulz, A., Carstens-Behrens, U., and Kollmann, R. (1998). Evidence for graft transmission of structural phloem proteins or their precursors in heterografts of *Cucurbitaceae.* Planta **206,** 630–640.

Golecki, B., Schulz, A., and Thompson, G.A. (1999). Translocation of structural P proteins in the phloem. Plant Cell **11,** 127–140.

Golz, J.F., and Hudson, A. (2002). Signaling in plant lateral organ development. Plant Cell **14** (suppl.), S277–S288.

Hake, S., and Freeling, M. (1986). Analysis of genetic mosaics shows that the extra epidermal cell divisions in *KNOTTED* mutant maize plants are induced by adjacent mesophyll cells. Nature **320,** 621–623.

Hantke, S.S., Carpenter, R., and Coen, E.S. (1995). Expression of *floricaula* in single cell layers of periclinal chimeras activates downstream homeotic genes in all layers of floral meristems. Development **121,** 27–35.

Heinlein, M., Epel, B.L., Padgett, H.S., and Beachy, R.N. (1995). Interaction of tobamovirus movement proteins with the plant cytoskeleton. Science **270,** 1983–1985.

Heinlein, M., Padgett, H.S., Gens, J.S., Pickard, B.G., Casper, S.J., Epel, B.L., and Beachy, R.N. (1998). Changing patterns of localization of the tobacco mosaic virus movement protein and replicase to the endoplasmic reticulum and microtubules during infection. Plant Cell **10,** 1107–1120.

Helariutta, Y., Fukaki, H., Wysocka-Diller, J., Nakajima, K., Jung, J., Sena, G., Hauser, M.T., and Benfey, P.N. (2000). The *SHORT-ROOT* gene controls radial patterning of the *Arabidopsis* root through radial signaling. Cell **101,** 555–567.

Holroyd, C., and Erdmann, R. (2001). Protein translocation machineries of peroxisomes. FEBS Lett. **501,** 6–10.

Imlau, A., Truernit, E., and Sauer, N. (1999). Cell-to-cell and long-distance trafficking of the green fluorescent protein in the phloem and symplastic unloading of the protein into sink tissues. Plant Cell **11,** 309–322.

Ishiwatari, Y., Honda, C., Kawashima, I., Nakamura, S.-I., Hirano,

H., Mori, S., Fujiwara, T., Hayashi, H., and Chino, M. (1995). Thioredoxin h is one of the major proteins in rice phloem sap. Planta **195,** 456–463.

Ishiwatari, Y., Fujiwara, T., McFarland, K.C., Nemoto, K., Hayashi, H., Chino, M., and Lucas, W.J. (1998). Rice phloem thioredoxin h has the capacity to mediate its own cell-to-cell transport through plasmodesmata. Planta **205,** 12–22.

Itaya, A., Woo, Y.-M., Masuta, C., Bao, Y., Nelson, R.S., and Ding, B. (1998). Developmental regulation of intercellular protein trafficking through plasmodesmata in tobacco leaf epidermis. Plant Physiol. **118,** 373–385.

Jackson, D., Veit, B., and Hake, S. (1994). Expression of maize *Knotted1* related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. Development **120,** 405–413.

Jackson, M. (1997). Hormones from roots as signals for the shoots of stressed plants. Trends Plant Sci. **2,** 22–28.

Jans, D.A., Xiao, C.-Y., and Lam, M.H.C. (2000). Nuclear targeting signal recognition: A key control point in nuclear transport? Bioessays **22,** 532–544.

Jansen, R.-P. (2001). mRNA localization: Message on the move. Nat. Rev. Mol. Cell Biol. **2,** 247–256.

Jeong, S., Trotochaud, A.E., and Clark, S.E. (1999). The Arabidopsis *CLAVATA2* gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. Plant Cell **11,** 1925–1933.

Jones, A.L., Thomas, C.L., and Maule, A.J. (1998). *De novo* methylation and co-suppression induced by a cytoplasmically replicating plant RNA virus. EMBO J. **17,** 6385–6393.

Kayes, J.M., and Clark, S.E. (1998). CLAVATA2, a regulator of meristem and organ development in *Arabidopsis.* Development **125,** 3843–3851.

Keegstra, K., and Cline, K. (1999). Protein import and routing systems of chloroplasts. Plant Cell **11,** 557–570.

Kidner, C., Sundaresan, V., Roberts, K., and Dolan, L. (2000). Clonal analysis of the *Arabidopsis* root confirms that position, not lineage, determines cell fate. Planta **211,** 191–199.

Kim, M., Canio, W., Kessler, S., and Sinha, N. (2001). Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. Science **293,** 287–289.

Kim, Y.J., Yuan, Z., Cilia, M., Khalfan-Jagani, Z., and Jackson, D. (2002). Intercellular trafficking of a functional *KNOTTED1* green fluorescent protein fusion in *Arabidopsis.* Proc. Natl. Acad. Sci. USA, in press.

Kiselyova, O.I., Yaminsky, I.V., Karger, E.M., Frolova, O.Y., Dorokhov, Y.L., and Atabekov, J.G. (2001). Visualization by atomic force microscopy of tobacco mosaic virus movement protein-RNA complexes formed in vitro. J. Gen. Virol. **82,** 1503–1508.

Kotlizky, G., Katz, A., van der Laak, J., Boyko, V., Lapidot, M., Beachy, R.N., Heinlein, M., and Epel, B.L. (2001). A dysfunctional movement protein of tobacco mosaic virus interferes with targeting of wild-type movement protein to microtubules. Mol. Plant-Microbe Interact. **14,** 895–904.

Kragler, F., Lucas, W.J., and Monzer, J. (1998a). Plasmodesmata: Dynamics, domains and patterning. Ann. Bot. **81,** 1–10.

Kragler, F., Monzer, J., Shash, K., Xoconostle-Cázares, B., and Lucas, W.J. (1998b). Cell-to-cell transport of proteins: Requirement for unfolding and characterization of binding to a putative plasmodesmal receptor. Plant J. **15,** 367–381.

Kragler, F., Monzer, J., Xoconostle-Cázares, B., and Lucas, W.J. (2000). Peptide antagonists of the plasmodesmal macromolecular trafficking pathway. EMBO J. **19,** 2856–2868.

Kühn, C., Franceschi, V.R., Schulz, A., Lemoine, R., and Frommer, W.B. (1997). Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. Science **275,** 1298–1300.

Lee, J.-Y., and Lucas, W.J. (2001). Phosphorylation of viral movement proteins—Regulation of cell-to-cell trafficking. Trends Microbiol. **9,** 5–8.

Li, Q., Ryu, K.H., and Palukaitis, P. (2001). *Cucumber mosaic virus*–plant interactions: Identification of 3a protein sequences affecting infectivity, cell-to-cell movement, and long-distance movement. Mol. Plant-Microbe Interact. **14,** 378–385.

Lohmann, J.U., Hong, R.L., Hobe, M., Busch, M.A., Parcy, F., Simon, R., and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in *Arabidopsis.* Cell **105,** 793–803.

Lough, T.J., Shash, K., Xoconostle-Cázares, B., Hofstra, K.R., Beck, D.L., Balmori, E., Forster, R.L.S., and Lucas, W.J. (1998). Molecular dissection of the mechanism by which potexvirus triple gene block proteins mediate cell-to-cell transport of infectious RNA. Mol. Plant-Microbe Interact. **11,** 801–814.

Lough, T.J., Netzler, N.E., Emerson, S.J., Sutherland, P., Carr, F., Beck, D.L., Lucas, W.J., and Forster, R.L.S. (2000). Cell-to-cell movement of potexviruses: Evidence for a ribonucleoprotein complex involving the coat protein and the first triple gene block protein. Mol. Plant-Microbe Interact. **13,** 962–974.

Lucas, W.J. (1995). Plasmodesmata: Intercellular channels for macromolecular transport in plants. Curr. Opin. Cell Biol. **7,** 673–680.

Lucas, W.J., and Gilbertson, R.L. (1994). Plasmodesmata in relation to viral movement within leaf tissues. Annu. Rev. Phytopathol. **32,** 387–411.

Lucas, W.J., Ding, B., and van der Schoot, C. (1993). Tansley Review No. 58: Plasmodesmata and the supracellular nature of plants. New Phytol. **125,** 435–476.

Lucas, W.J., Bouché-Pillon, S., Jackson, D.P., Nguyen, L., Baker, L., Ding, B., and Hake, S. (1995). Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. Science **270,** 1980–1983.

Lucas, W.J., Yoo, B.-C., and Kragler, F. (2001). RNA as a long-distance information macromolecule in plants. Nat. Rev. Mol. Cell Biol. **2,** 849–857.

Más, P., and Beachy, R.N. (1999). Replication of tobacco mosaic virus on endoplasmic reticulum and role of the cytoskeleton and virus movement protein in intracellular distribution of viral RNA. J. Cell Biol. **147,** 945–958.

McLean, B.G., Zupan, J., and Zambryski, P.C. (1995). Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. Plant Cell **7,** 2101–2114.

Mezitt, L.A., and Lucas, W.J. (1996). Plasmodesmal cell-to-cell transport of proteins and nucleic acids. Plant Mol. Biol. **32,** 251–273.

Mlotshwa, S., Voinnet, O., Mette, M.F., Matzke, M., Vaucheret,

H., Ding, S.W., Pruss, G., and Vance, V.B. (2002). RNA silencing and the mobile silencing signal. Plant Cell **14** (suppl.), S289–S301.

Nakajima, K., and Benfey, P.N. (2002). Signaling in and out: Control of cell division and differtiation in the shoot and root. Plant Cell **14** (suppl.), S265–S276.

Nakajima, K., Sena, G., Nawy, T., and Benfey, P.N. (2001). Intercellular movement of the putative transcription factor SHR in root patterning. Nature **413,** 307–311.

Noueiry, A.O., Lucas, W.J., and Gilbertson, R.L. (1994). Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport. Cell **76,** 925–932.

Oparka, K.J., and Santa Cruz, S. (2000). The great escape: Phloem transport and unloading of macromolecules. Annu. Rev. Plant Physiol. Plant Mol. Biol. **51,** 323–347.

Oparka, K.J., and Turgeon, R. (1999). Sieve elements and companion cells—Traffic control centers of the phloem. Plant Cell **11,** 739–750.

Oparka, K.J., Roberts, A.G., Boevink, P., Santa Cruz, S., Roberts, I., Pradel, K.S., Imlau, A., Kotlizky, G., Sauer, N., and Epel, B. (1999). Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. Cell **97,** 743–754.

Palauqui, J.-C., Elmayan, T., Dorlhac de Borne, F., Crété, P., Charles, C., and Vaucheret, H. (1996). Frequencies, timing and spatial patterns of co-suppression of nitrate reductase and nitrite reductase in transgenic tobacco plants. Plant Physiol. **112,** 1447–1456.

Palauqui, J.-C., Elmayan, T., Pollien, J.-M., and Vaucheret, H. (1997). Systemic acquired silencing: Transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J. **16,** 4738–4745.

Perbal, M.-C., Haughn, G., Saedler, H., and Schwarz-Sommer, Z. (1996). Non-cell-autonomous function of the *Antirrhinum* floral homeotic proteins DEFICIENS and GLOBOSA is exerted by their polar cell-to-cell trafficking. Development **122,** 3433–3441.

Phillips, G.N., Jr. (1997). Structure and dynamics of green fluorescent protein. Curr. Opin. Struct. Biol. **7,** 821–827.

Reichel, C., Más, P., and Beachy, R.N. (1999). The role of the ER and cytoskeleton in plant viral trafficking. Trends Plant Sci. **4,** 458–462.

Reiser, L., Sanchez-Baracaldo, P., and Hake, S. (2000). Knots in the family tree: Evolutionary relationships and functions of knox homeobox genes. Plant Mol. Biol. **42,** 151–166.

Robards, A.W., and Lucas, W.J. (1990). Plasmodesmata. Annu. Rev. Plant Physiol. Plant Mol. Biol. **41,** 369–419.

Rojas, M.R., Zerbini, F.M., Allison, R.F., Gilbertson, R.L., and Lucas, W.J. (1997). Capsid protein and helper component–proteinase function as potyvirus cell-to-cell movement proteins. Virology **237,** 283–295.

Rojas, M.R., Jiang, H., Salati, R., Xoconostle-Cázares, B., Sudarshana, M.R., Lucas, W.J., and Gilbertson, R.L. (2001). Functional analysis of proteins involved in movement of the monopartite begomovirus, *Tomato Yellow Leaf Curl Virus.* Virology **291,** 110–125.

Rolland, F., Moore, B., and Sheen, J. (2002). Sugar sensing and signaling in plants. Plant Cell **14** (suppl.), S185–S205.

Ruiz, M.T., Voinnet, O., and Baulcombe, D.C. (1998). Initiation and maintenance of virus-induced gene silencing. Plant Cell **10,** 937–946.

Ruiz-Medrano, R., Xoconostle-Cázares, B., and Lucas, W.J. (1999). Phloem long-distance transport of *CmNACP* mRNA: Implications for supracellular regulation in plants. Development **126,** 4405–4419.

Ruiz-Medrano, R., Xoconostle-Cázares, B., and Lucas, W.J. (2001). The phloem as a conduit for inter-organ communication. Curr. Opin. Plant Biol. **4,** 202–209.

Ryan, C.A., Pearce, G., Scheer, J., and Moura, D.S. (2002). Peptide hormones. Plant Cell **14** (suppl.), S251–S264.

Satina, S., and Blakeslee, A.F. (1941). Periclinal chimeras in *Datura stramonium* in relation to development of leaf and flower. Am. J. Bot. **28,** 862–871.

Satoh, H., Matsuda, H., Kawamura, T., Isogai, M., Yoshikawa, N., and Takahashi, T. (2000). Intracellular distribution, cell-to-cell trafficking and tubule-inducing activity of the 50 kDa movement protein of *Apple chlorotic leaf spot virus* fused to green fluorescent protein. J. Gen. Virol. **81,** 2085–2093.

Schiefelbein, J.W. (2000). Constructing a plant cell. The genetic control of root hair development. Plant Physiol. **124,** 1525–1531.

Schobert, C., Baker, L., Szederkényi, J., Grossmann, P., Komor, E., Hayashi, H., Chino, M., and Lucas, W.J. (1998). Identification of immunologically related proteins in sieve-tube exudate collected from monocotyledonous and dicotyledonous plants. Planta **206,** 245–252.

Sessions, A., Yanofsky, M.F., and Weigel, D. (2000). Cell–cell signaling and movement by the floral transcription factors LEAFY and APETALA1. Science **289,** 779–781.

Sinha, N., and Hake, S. (1990). Mutant characters of *knotted* maize leaves are determined in the innermost tissue layers. Dev. Biol. **141,** 203–210.

Smyth, D.R., Bowman, J.L., and Meyerowitz, E.M. (1990). Early flower development in *Arabidopsis.* Plant Cell **2,** 755–767.

Stewart, R.N., and Dermen, H. (1975). Flexibility in ontogeny as shown by the contribution of the shoot apical layers to leaves of periclinal chimeras. Am. J. Bot. **62,** 935–947.

Stewart, R.N., Semeniuk, P., and Dermen, H. (1974). Competitions and accommodations between apical layers and their derivations in the ontogeny of chimeral shoot of *Pelargonium hortorum.* Am. J. Bot. **61,** 54–67.

Tamai, A., and Meshi, T. (2001a). Tobamoviral movement protein transiently expressed in a single epidermal cell functions beyond multiple plasmodesmata and spreads multicellularly in an infection-coupled manner. Mol. Plant-Microbe Interact. **14,** 126–134.

Tamai, A., and Meshi, T. (2001b). Cell-to-cell movement of *Potato virus X*: The role of p12 and p8 encoded by the second and third open reading frames of the triple gene block. Mol. Plant-Microbe Interact. **14,** 1158–1167.

Tiedemann, R., and Carstens-Behrens, U. (1994). Influence of grafting on the phloem protein patterns in Cucurbitaceae. I. Additional phloem exudates proteins in *Cucumis sativus* grafted on two cucurbita species. J. Plant Physiol. **143,** 189–194.

Trotochaud, A.E., Jeong, S., and Clark, S.E. (2000). CLAVATA3, a multimeric ligand for the CLAVATA1 receptor-kinase. Science **289,** 613–617.

Tsugeki, R., and Fedoroff, N.V. (1999). Genetic ablation of root cap cells in *Arabidopsis.* Proc. Natl. Acad. Sci. USA **96,** 12941–12946.

van Bel, A.J.E., and Knoblauch, M. (2000). Sieve element and companion cell: The story of the comatose patient and the hyperactive nurse. Austr. J. Plant Physiol. **27,** 477–487.

van den Berg, C., Willemsen, V., Hage, W., Weisbeek, P., and Scheres, B. (1995). Cell fate in the *Arabidopsis* root meristem determined by directional signalling. Nature **378,** 62–65.

van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P., and Scheres, B. (1997). Short-range control of cell differentiation in the *Arabidopsis* root meristem. Nature **390,** 287–289.

Voinnet, O., Vain, P., Angell, S., and Baulcombe, D.C. (1998). Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. Cell **95,** 177–187.

Vollbrecht, E., Veit, B., Sinha, N., and Hake, S. (1991). The developmental gene *Knotted-1* is a member of a maize homeobox gene family. Nature **350,** 241–243.

Waigmann, E., and Zambryski, P. (1995). Tobacco mosaic virus movement protein-mediated protein transport between trichome cells. Plant Cell **7,** 2069–2079.

Waigmann, E., Lucas, W.J., Citovsky, V., and Zambryski, P. (1994). Direct functional assay for tobacco mosaic virus cell-to-cell movement protein and identification of a domain involved in increasing plasmodesmal permeability. Proc. Natl. Acad. Sci. USA **91,** 1433–1437.

Waigmann, E., Chen, M.-H., Bachmaier, R., Ghoshroy, S., and Citovsky, V. (2000). Regulation of plasmodesmal transport by phosphorylation of tobacco mosaic virus cell-to-cell movement protein. EMBO J. **19,** 4875–4884.

Wang, H.-L., Gilbertson, R.L., and Lucas, W.J. (1996). Spatial and temporal distribution of bean dwarf mosaic geminivirus in *Phaseolus vulgaris* and *Nicotiana benthamiana.* Phytopathology **86,** 1204–1214.

Wang, H.-L., Wang, Y., Giesman-Cookmeyer, D., Lommel, S.A., and Lucas, W.J. (1998). Mutations in viral movement protein alter systemic infection and identify an intercellular barrier to entry into the phloem long-distance transport system. Virology **245,** 75–89.

Ward, J.M., Kühn, C., Tegeder, M., and Frommer, W.B. (1998). Sucrose transport in higher plants. Int. Rev. Cytol. **178,** 41–71.

Wolf, S., Deom, C.M., Beachy, R.N., and Lucas, W.J. (1989). Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. Science **246,** 377–379.

Wu, X., Weigel, D., and Wigge, P.A. (2002). Signaling in plants by intercellular RNA and protein movement. Genes Dev. **16,** 151–158.

Xoconostle-Cázares, B., Xiang, Y., Ruiz-Medrano, R., Wang, H.-L., Monzer, J., Yoo, B.C., McFarland, K.C., Franceschi, V.R., and Lucas, W.J. (1999). Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. Science **283,** 94–98.

Xoconostle-Cázares, B., Ruiz-Medrano, R., and Lucas, W.J. (2000). Proteolytic processing of CmPP36, a protein from the cytochrome b(5)-reductase family, is required for entry into the phloem translocation pathway. Plant J. **24,** 735–747.

Zambryski, P., and Crawford, K. (2000). Plasmodesmata: Gatekeepers for cell-to-cell transport of developmental signals in plants. Annu. Rev. Cell Dev. Biol. **16,** 393–421.

Zhu, T., O'Quinn, R.L., Lucas, W.J., and Rost, T.L. (1998). Directional cell-to-cell communication in the *Arabidopsis* root apical meristem. II. Dynamics of plasmodesmatal formation. Protoplasma **204,** 84–93.