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Regulation of short-distance transport of RNA and protein

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The intercellular trafficking of proteins and RNAs has emerged as a novel mechanism of cell–cell communication in plant development. Plasmodesmata (PD), intercellular cytoplasmic channels, have a central role in cell–cell trafficking of regulatory proteins and RNAs. Recent studies have demonstrated that plants use either a selective or a non-selective PD trafficking pathway for regulatory proteins. Moreover, plants have developed strategies to regulate both selective and non-selective movement. Recent work has focused especially on integrating the recent understanding of the function and mechanisms of intercellular macromolecule movement through PD.

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Abbreviations

CmPP	*Cucurbita maxima* phloem protein
CmPSRP1	*Cucurbita maxima* phloem SMALL RNA BINDING PROTEIN1
GFP	green fluorescent protein
ISE1	*INCREASED SIZE EXCLUSION LIMIT OF PLASMODESMATA 1*
KN1	KNOTTED1
LFY	LEAFY
Me	*Mouse ears*
MP	movement protein
NCAP	non-cell-autonomous protein
NCAPP1	NON-CELL AUTONOMOUS PATHWAY PROTEIN1
PD	plasmodesmata
PME	pectin methyl esterase
PTGS	post-transcriptional gene silencing
RNP	ribonucleoprotein complex
SAM	shoot apical meristem
SEL	size exclusion limit
SHR	SHORT ROOT
STM	SHOOT MERISTEMLESS
TMV	tobacco mosaic virus
TNT	tunneling nanotubules

Introduction

Intercellular communication that delivers crucial information for position-dependent specification of cell fate is an essential biological process in multicellular organisms. In animals, the understanding of ligand–receptor- and gap-junction-mediated communication pathways is well established. Moreover, recent observations of intercellular nanotubular structures suggested that animal cells have the potential to exchange macromolecules symplasmically [1••]. In plants, two pathways for intercellular communication are also evident: the ligand–receptor-mediated apoplastic pathway and the plasmodesmata (PD)-mediated symplasmic pathway. Evidence suggests that regulatory proteins and RNAs can traffic symplasmically through PD and play a crucial role in cell–cell communication. Excellent comprehensive reviews are available [2–5]. In this review, we focus on recent advances that have addressed the trafficking modes, regulatory mechanisms and biological significance of short-distance transport of proteins and RNAs.

Non-cell-autonomous proteins and RNA

Non-cell-autonomous proteins (NCAPs) represent all proteins that can traffic between plant cells [6••]. There are three classes of NCAPs: viral proteins, regulatory proteins and phloem proteins (Table 1). Viral movement proteins (MPs), one of the best-characterized protein families, are known to interact with PD to increase their size exclusion limit (SEL), to traffic themselves and to facilitate spread of the virus [7–9]. Regulatory NCAPs have an important role in the determination of cell fate. The first endogenous protein shown to traffic from cell to cell was the maize homeodomain protein KNOTTED1 (KN1), which is involved in the maintenance of the shoot apical meristem (SAM) [10]. Its *Arabidopsis* homologs, KNAT1/BREVIPEDICELLUS and SHOOT MERISTEMLESS (STM), were also shown to move in the SAM [11•]. Regulatory proteins that are involved in flowering or root cell differentiation, such as LEAFY (LFY), DEFICIENS, GLOBOSA, CAPRICE and SHORT ROOT (SHR), also act as NCAPs [12–16]. These NCAPs traffic over a range of between one and a few cell layers, and thus might function as short-distance signals. Phloem proteins are the third category of NCAPs. Sieve-tube exudates are known to include a surprisingly large number of proteins and RNAs [17–20]. As sieve elements lose their nuclei during development, all proteins or RNAs in them are thought to be imported from the neighboring companion cells. Of these phloem proteins, only three have been characterized for their PD-gating ability (Table 1). Although they have a potential role as long-distance signals as well as in sieve-tube maintenance, the function

Table 1

Non-cell-autonomous proteins.

Protein	Method	Plasmodesmal regulation/ RNA trafficking	References
Viral proteins			
MP/CP	M, B, T	Gating/RNA	[57,58]
Plant transcription factors			
LFY/FLO	M, T, C		[13,59]
GLO	M, C		[12,14]
DEF	M, C		[12,59]
KN1/STM/KNAT1,	M, B, T, C	Gating/RNA	[10,11•,60,61]
SHR	T		[16,56••]
CPC	T		[15]
Phloem proteins			
CmPP16	M	Gating/RNA	[62]
CmPP36	M		[35]
HSC70	M	Gating	[33]
PP1, PP2	M	RNA	[22]
SUT1	T	RNA	[63]
TRX	M	Gating	[39]

Fluorescently labeled probes were introduced into target cells by: microinjection (M), microprojectile bombardment (B), expression as a transgene (T) or chimera formation (C). CP, coat proteins; CPC, CAPRICE; DEF, DEFICIENS; FLO, FLORICAULA; GLO, GLOBOSA; KNAT1, KN1-related homeobox protein 1 in *Arabidopsis thaliana*; PP, phloem protein; SUT1, SUCROSE TRANSPORTER1; TRX, thioredoxin h.

of these phloem proteins and RNAs remains largely unknown.

Plasmodesmal components and regulators

PDs provide a unique symplasmic pathway for intercellular macromolecule trafficking. The identification of structural and functional components of PD remains a major challenge in plant biology. To date, about a dozen proteins have been found in and around PD, including pectin methyl esterase (PME), actin, myosin, centrin, calreticulin, calcium-dependent and -independent protein kinases and several proteins of unknown function. As calcium is known to modify the PD SEL, calcium-dependent phosphorylation of the actin–myosin machinery might be important for NCAP trafficking [2]. Recently, Escobar *et al.* [21•] developed a wonderful functional genomics approach to screen plasmodesmal-targeting proteins using the transient viral expression of a green fluorescent protein (GFP)-tagged cDNA library. Using this high-throughput assay, 12 fusion proteins from about 20 000 infection foci were localized to PD. Even though the functional significance of these proteins needs to be determined, further large-scale genomic approaches will shed light on these elusive tunnels that interconnect plant cells.

Genetic approaches also hold great promise for the discovery of PD structural and regulatory proteins. The *INCREASED SIZE EXCLUSION LIMIT OF PLASMODESMATA 1* (*ISE1*) gene is required for the regulation of PD SEL during embryogenesis [22]. *ISE1* encodes a DEAD-box RNA helicase localized to puncta in the plant cell wall (P Zambryski, I Kim, personal communication). As the helicase domain in several plant viruses is essential for cell–cell movement and there is no structural alteration in the PD of *ise1* mutants, it was suggested that the *ISE1* gene plays a regulatory role in PD function. Despite the increase in the number of known PD-localized proteins, much work remains to be done to identify the complete set of structural and regulatory PD elements, which are suggested to have similar mechanistic complexity to the 120-MDa nuclear pore complexes. Further genetic, proteomic and biochemical approaches will be necessary to describe the entire PD network. High-throughput proteomics approaches using PD enriched cell walls are expected to be especially valuable resources to screen PD components [23].

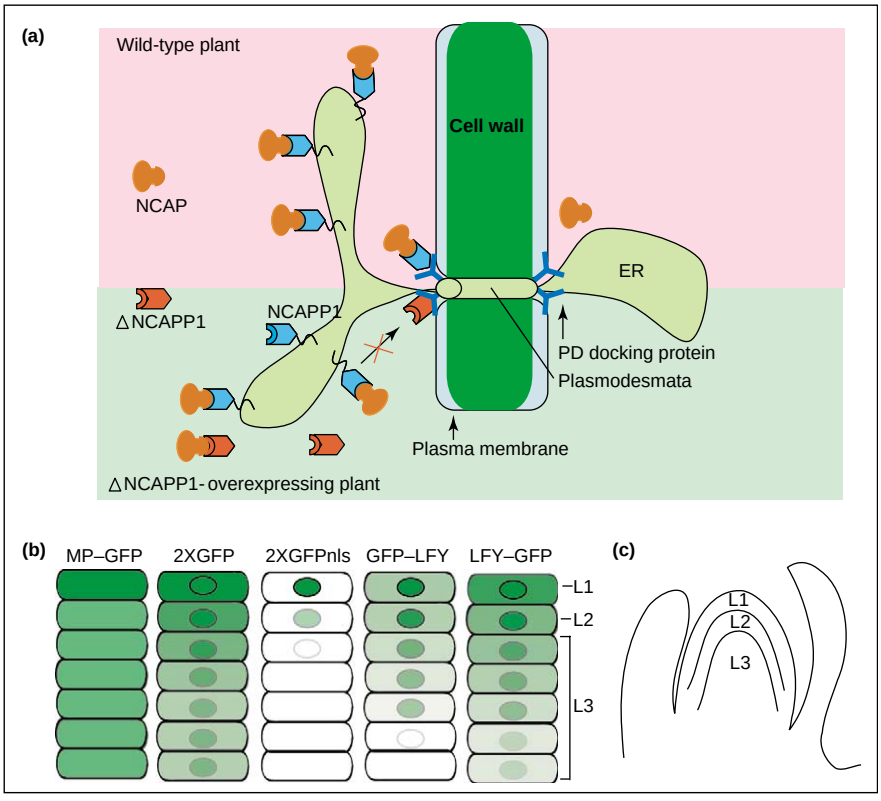
Mechanisms of plasmodesmal macromolecular trafficking

Two modes of movement through PD have been proposed. Selective trafficking involves specific interactions between a signal motif of NCAPs and endogenous factors, accompanied by PD SEL increase. By contrast, non-selective trafficking is diffusion-based and depends upon the endogenous PD SEL.

The best-understood case of selective trafficking is the trafficking of viral MPs that have plasmodesmal gating activity [7]. Viral MPs interact with chaperones, the cytoskeleton, PME and nuclear proteins [24–29]. Although the role of microtubules in intercellular MP trafficking remains controversial [29,30], the actin–myosin machinery is required for cell–cell movement of the viral replication complex [31]. Interaction of a DNAJ-like chaperone with MP suggested that chaperone activity is required for partial unfolding of NCAPs before their passage through PD [24]. This is consistent with findings that the beet yellow virus HSP70 homologue is essential for viral movement [32] and that plant HSP70s function as PD SEL modifiers [33]. PME was suggested to be an MP receptor [26,29,30]. MP movement can be affected by phosphorylation [34] or by interaction with either a microtubule-associated protein, MBP2C, or a leucine-zipper homeodomain protein, HFi22 [25,27].

MPs seem to piggyback on the endogenous trafficking machinery. This idea is supported by the fact that an MP-related *Cucurbita maxima* phloem protein, CmPP16, can traffic itself and RNA through PDs [35]. Recently, Lee *et al.* [6••] isolated a CmPP16-interacting protein, NON-CELL AUTONOMOUS PATHWAY PROTEIN1 (NCAPP1), and identified it as a candidate component of the plasmodesmal trafficking network. NCAPP1 probably functions as a mobile receptor that moves along the endoplasmic recticulum membrane to deliver its cargo to the PD (Figure 1a; [6••]). Interestingly, NCAPP1 is

Figure 1



Diffusion-based non-selective and receptor-mediated selective protein trafficking. **(a)** Model for NCAPP1 function in the selective trafficking pathway. NCAPP1 acts as a receptor carrier of NCAP that passes its cargo (NCAP) to plasmodesmal receptors. In transgenic plants overexpressing a dominant negative NCAPP1 mutant (ΔNCAPP1), this pathway is blocked by the formation of dysfunctional complexes between ΔNCAPP1 and the PD docking protein. Modified with permission from [6••]. **(b)** Correlation between cytoplasmic localization and trafficking capacity of 2xGFP, 2xGFPnls (nuclear localization signal) and LFY–GFP fusions (N-terminal and C-terminal GFP fusions) are schematized. In GFP–LFY, GFP is fused to the N-terminus of LFY, in contrast, LFY–GFP is a C-terminal GFP fusion to LFY. Green circles represent nuclear GFP fluorescence. The intensity of green color outside of the nuclei represents the level of cytoplasmic GFP. **(c)** SAM structure in *Arabidopsis thaliana*. L1 and L2 are single cell layers and L3 consists of several cell layers.

related to GP40, which is localized to the nuclear periphery and might play a role in nuclear protein import [36]. This observation supports the idea that plasmodesmal trafficking might be mechanistically related to nucleo-cytoplasmic trafficking through nuclear pores [37]. Plants overexpressing a dominant negative mutantion of NCAPP1 showed a loss of organ symmetry, fusion of organs and altered cell-fates in the leaf epidermis, highlighting the developmental significance of the NCAPP1-associated trafficking pathway. The mutant version of NCAPP1 also inhibited the cell–cell trafficking of CmPP16 and tobacco mosaic virus (TMV) MP as well as their PD gating ability. This effect was specific as the PD-gating activity of KN1 was not blocked. These results support the presence of selective trafficking pathways, and suggest that multiple networks mediate movement through PD.

Since the selective trafficking of NCAPs was postulated, efforts have been made to identify selective-trafficking signal motifs. To date, trafficking-signal peptides that are necessary and sufficient for intercellular trafficking have not been isolated. Studies using thioredoxin h or MPs suggested that the signal peptide might have either a three-dimensional structure or a combination of a primary sequence and a three-dimensional structure [7,38,39]. A carboxy-terminal 99-amino-acid domain of TMV MP is required to increase the PD SEL [7]. A potential peptide signal (the SVR motif) was identified from a CmHSP70 using deletion analysis. However, this 25-amino-acid motif operates only within the context of the HSP70 chaperone machinery, as it can induce the trafficking of human HSP70 but not of heterologous proteins [40].

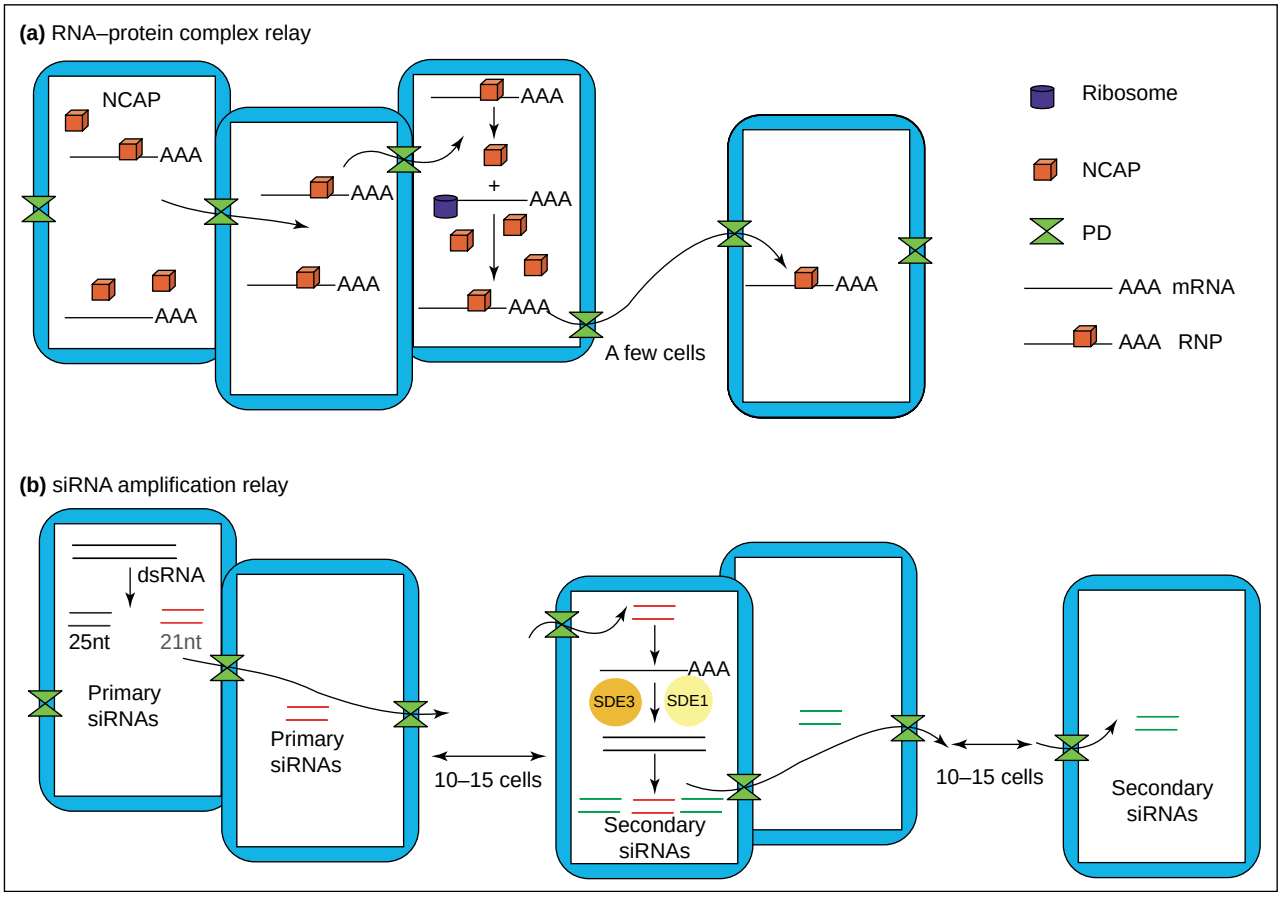
Besides selective protein trafficking, plant cells can also exchange proteins by diffusion. The use of GFP as a reporter greatly intensified our understanding of this mode of non-selective intercellular protein trafficking. GFP movement between plant cells suggested a dynamic regulation of PD SEL [41,42,43•]. As GFP is not an

endogenous plant protein, it must move by a non-selective mechanism. Recently, Wu *et al.* [44••] proposed that LFY, a transcription factor that activates floral homeotic genes, also moves in a non-selective manner, mediated by simple diffusion. A strong correlation between the cytoplasmic localization and trafficking ability of GFP–LFY fusions was observed, as reported for other diffusion-based movement (Figure 1b; [45,46]). Moreover, LFY does not appear to have a specific trafficking signal motif because all GFP fusions with LFY fragments were able to move. This suggests that diffusion is the default state for some proteins, unless they have a retention mechanism that targets them to a specific compartment or complex. However, diffusion-based LFY movement seems to be regulated in a tissue- or PD-dependent manner. For example, GFP–LFY fusions move more easily between apical–basal layers than in a lateral direction, and LFY cannot move into the inflorescence meristem from the floral meristem. This and other studies indicate that some PD do not permit the free movement of LFY, suggesting that LFY trafficking occurs in selective symplasmic borders consisting of a symplasmic domain in the floral meristem [13].

RNA/DNA trafficking and its regulation

Viral RNAs and DNAs, viroids and some selected endogenous RNAs traffic between cells by way of plasmodesmal or phloem symplasmic networks [31,47•,48•]. RNA trafficking was first found in the study of viral infection, during which viral RNA genomes move as ribonucleoprotein complexes (RNPs) with MP or the virus replication complex [31,49,50]. Recent characterization of a bipartite RNA sequence motif that is required for the directional trafficking of the potato spindle tuber viroid from bundle sheath cells to mesophyll cells suggested that this motif is involved in specific interactions with endogenous factors [47•]. The unidirectional-trafficking motif suggests either that the plasmodesmal orifice in a

Figure 2



RNP relay and siRNA amplification relay. **(a)** RNP relay model. NCAPs such as KN1 can bind to its RNA and facilitate RNA trafficking. In source cells expressing the NCAP, it binds to its RNA and moves to neighboring cells as RNPs. In target cells with low levels of RNP, the RNP dissociates and initiates translation. Increased NCAP protein concentration stimulates reassociation with its RNA, which then moves to neighboring cells. **(b)** siRNA amplification relay model. Silencing can spread to 10–15 cells in the absence of amplification, through the trafficking of primary 21 nt siRNAs. Extensive cell–cell movement is mediated by further spread of secondary 21 nt siRNA synthesized *de novo* by the action of SDE1 and SDE3. Modified with permission from [54••].

cell boundary has a molecular composition that varies among different cell types or that each cell type has unique factors that interact with specific RNA motifs to potentiate trafficking.

The mRNA of the tomato KN1-related homolog gene, *LeT6*, can also traffic from cell to cell. Kim *et al.* [51] observed that the movement of a PYROPHOSPHATE-DEPENDENT PHOSPHOFRUCTOKINASE–LeT6 fusion RNA from a *Mouse ears* (*Me*) stock into a wild-type graft scion induced *Me* morphology in the scion shoot, suggesting that *Me* RNA functions as a long-distance developmental signal. However, it is unclear how *Me* transcripts traffic between cells. Some plant NCAPs, including KN1, can specifically bind to RNA and facilitate its trafficking [10]. Ribonucleoprotein trafficking is proposed to be mediated by an NCAP-dependent relay mechanism as shown in Figure 2a.

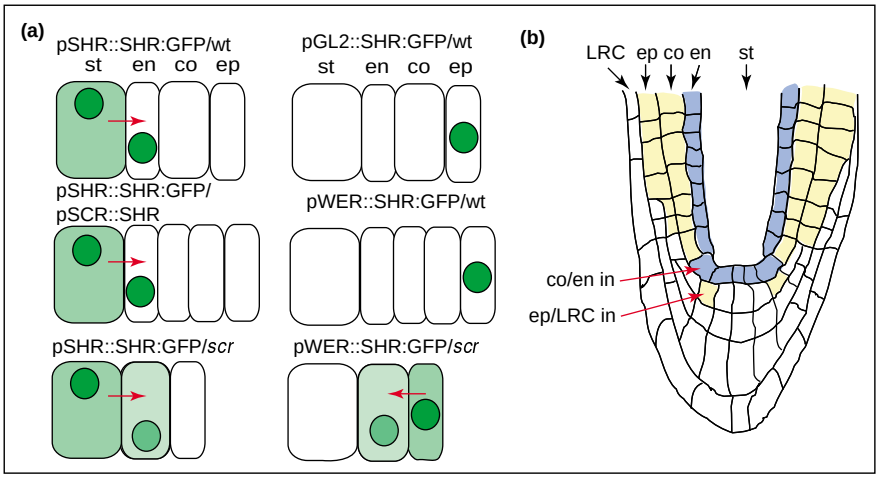
In addition, trafficking of mRNA fragments might have an important role in the regulation of gene expression by means of post-transcriptional gene silencing (PTGS) pathways. PTGS or RNA interference (RNAi) is a sequence-specific RNA degradation pathway that is propagated systemically in animals and plants. Recently, it has been reported that the cells of animals such as *Caenorhabditis elegans* express a receptor-like dsRNA transporter that is essential for systemic RNAi responses [52,53••]. To date, this kind of protein has not been reported in the plant kingdom. Himber *et al.* [54••] showed that the extensive cell–cell movement of RNA silencing is the result of relay amplification of the 21 nt siRNA and of reiterated short-distance signaling events (Figure 2b). This group has obtained enhanced and defective systemic silencing ethyl methanesulfonate (EMS) mutants (O Voinnet, personal communication); the molecular characterization of these mutants will disclose the trafficking machinery of siRNAs.

Biological function of intercellular macromolecule trafficking

Despite the potential importance of the trafficking of endogenous transcription factors, the specific significance of intercellular trafficking is not always clear. The expression of different target genes, depending on trafficking-mediated gradients of NCAP concentration, might be important for cell differentiation. For example, a KN1 gradient either between the SAM and the P_0 domain or at the base of older leaf primordia is speculated to control boundary-specific target genes [55]. Another function of protein trafficking is in the specification of cell fate. Benfey and colleagues [16,56••] showed that movement of SHR protein from the stele is limited to the adjacent endodermal layer, whereas the competence to respond to SHR extends beyond the zone of movement. This emphasizes the functional role of this tightly regulated SHR trafficking in generating the correct root radial pattern (Figure 3).

Another possible role of movement could be the coordination of cell fate within the expression domain. For example, LFY RNA and protein are expressed in the same domain within young flowers, thus LFY trafficking might function as a redundant safety mechanism to

Figure 3



SHR trafficking pattern in root tissues. **(a)** Cytoplasmic/nuclear expression and trafficking of SHR. SHR–GFP fusion (SHR:GFP) was expressed under the control of stelar cell-specific promoter (pSHR) or epidermal specific promoters (pGL2, pWER) in wild-type (/wt), *scarecrow* mutant (/*scr*) or a transgenic *Arabidopsis* (/pSCR::SHR) expressing SHR under the control of *SCARECROW* promoter (pSCR). The darkness of the green circles represents the concentration of nuclear GFP. The *scr* mutation alters the subcellular localization of SHR and its trafficking ability. Green cells represent strong or weak cytoplasmic GFP. Arrows show protein movement. **(b)** SHR competence in regulating root radial patterning. SHR movement is restricted to adjacent cells (blue), but the competence to respond to SHR extends beyond the zone of movement (yellow). co, cortex; en, endodermis; ep, epidermis; in, initial; LRC, lateral root cap; st, stele. Modified from [56••].

ensure coordination of cell fate in the flower bud [13]. However, LFY movement is also required for normal flower development, suggesting the importance of protein trafficking *per se* [44••]. Similarly, the overexpression of a non-trafficking version of KN1 fusion did not cause the KNOX overexpression phenotype [11•]. These observations suggest that protein trafficking *per se*, possibly accompanied by post-translational protein modification during trafficking, might be a prerequisite for some functions of NCAPs. It is now thought that the cell–cell trafficking of plant proteins has multiple functions in specifying the correct developmental program.

Conclusions and future perspectives

Clearly cell–cell communication by way of plasmodesmal protein and RNA trafficking has a pivotal role in plant cell biology and development. However, its mechanism and regulation are still mostly a black box. Thus, a future challenge will be to understand how macromolecular trafficking occurs and how it is regulated. To identify NCAPs and trafficking RNAs as well as their modes of trafficking on a full-genome scale is an enormous task. It will be important to determine whether the diffusion-based movement of regulatory proteins through PD is restricted to a few unique proteins or if it is widespread. Identification of trafficking signal motifs or domains will be essential to elucidate selective trafficking mechanisms. Combined proteomic/genomic, molecular and biochemical approaches will be required to develop a complete picture of the PD structural components and functional regulators. To explore these once obscure channels is an exciting and essential endeavor that will continue to reveal the wonders of plant cell biology.

Update

Yoo *et al.* [64••] recently showed that pumpkin phloem sap contains an endogenous population of small RNA species, predominantly in the form of single-strand RNA (ssRNA) ranging from ~18 to 25 nucleotides, which might serve as the systemic signals. Microinjection studies demonstrated that single- or double-stranded RNA cannot simply diffuse between tobacco cells. They identified *Cucurbita maxima* phloem SMALL RNA BINDING PROTEIN1 (CmPSRP1), which binds selectively to 25-nucleotide ssRNAs and that can mediate the cell–cell trafficking of ssRNA, but not double-strand RNA or long mRNA. As CmPSRP1 probably acts during systemic RNAi by mediating cell–cell trafficking of ssRNA between companion cells and the sieve tube system, additional small RNA binding proteins for local transmission of RNAi must be required.

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References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. •• Rustom A, Saffrich R, Markovic I, Walther P, Gerdes H: **Nanotubular highways for intercellular organelle transport**. *Science* 2004, **303**:1007-1010.
This study reports a novel type of intercellular symplasmic communication channel, termed tunneling nanotubes (TNTs). These nanotubes can be formed *de novo* and are detected in various cultured animal cells including PC12, HEK and NRK, suggesting that TNTs exist in tissues *in vivo*. Uni-directional actin-based vesicle and organelle transport occurs by way of TNTs, but neither the small dye molecule calcein nor cytosolic GFP passes through these nanotubes. This surprising discovery provides a new paradigm for intercellular communication between animal cells.

2. Oparka KJ: **Getting the message across: how do plant cells exchange macromolecular complexes?** *Trends Plant Sci* 2004, **9**:33-41.

3. Ding B, Itaya A, Qi Y: **Symplasmic protein and RNA traffic: regulatory points and regulatory factors**. *Curr Opin Plant Biol* 2003, **6**:596-602.

4. Haywood V, Kragler F, Lucas WJ: **Plasmodesmata: pathways for protein and ribonucleoprotein signaling**. *Plant Cell* 2002, **14**:S303-S325.

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

6. •• Lee J, Yoo B, Rojas M, Gomez-Ospina N, Staehelin L, Lucas W: **Selective trafficking of non-cell-autonomous proteins mediated by NtNCAPP1**. *Science* 2003, **299**:392-396.
This study first demonstrated that the selective intercellular trafficking of NCAPs is mediated by a receptor-like protein. NCAPP1 mutations selectively affected the gating ability of CmPP16 and TMV MP, but not KN1, suggesting the presence of multiple networks for plasmodesmal movement.

7. Waigmann E, Lucas WJ, Citovsky V, Zambryski P: **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* 1994, **91**:1433-1437.

8. Deom CM, Oliver MJ, Beachy RN: **The 30-kilodalton gene-product of tobacco mosaic-virus potentiates virus movement**. *Science* 1987, **237**:389-394.

9. Wolf S, Deom CM, Beachy RN, Lucas WJ: **Movement protein of tobacco mosaic-virus modifies plasmodesmatal size exclusion limit**. *Science* 1989, **246**:377-379.

10. Lucas WJ, Bouche-Pillon S, Jackson DP, Nguyen L, Baker L, Ding B, Hake S: **Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata**. *Science* 1995, **270**:1980-1983.

11. • Kim JY, Yuan Z, Jackson D: **Developmental regulation and significance of KNOX protein trafficking in *Arabidopsis***. *Development* 2003, **130**:4351-4362.
This paper demonstrates that the trafficking of KN1 and the related *Arabidopsis* proteins STM and KNAT1 is correlated with complementation of *stm-11* mutant phenotypes.

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

13. Sessions A, Yanofsky MF, Weigel D: **Cell–cell signaling and movement by the floral transcription factors LEAFY and APETALA1**. *Science* 2000, **289**:779-782.

14. Kragler F, Monzer J, Shash K, Xoconostle-Cazares B, Lucas WJ: **Cell-to-cell transport of proteins: requirement for unfolding**

and characterization of binding to a putative plasmodesmal receptor. *Plant J* 1998, **15**:367-381.

15. Wada T, Kurata T, Tominaga R, Koshino-Kimura Y, Tachibana T, Goto K, Marks MD, Shimura Y, Okada K: **Role of a positive regulator of root hair development, CAPRICE, in *Arabidopsis* root epidermal cell differentiation**. *Development* 2002, **129**:5409-5419.

16. Nakajima K, Sena G, Nawy T, Benfey P: **Intercellular movement of the putative transcription factor SHR in root patterning**. *Nature* 2001, **413**:307-311.

17. van Bel AJE, Ehlers K, Knoblauch M: **Sieve elements caught in the act**. *Trends Plant Sci* 2002, **7**:126-132.

18. Ruiz-Medrano R, Xoconostle-Cazares B, Lucas WJ: **Phloem long-distance transport of CmNACP mRNA: implications for supracellular regulation in plants**. *Development* 1999, **126**:4405-4419.

19. Walz C, Giavalisco P, Schad M, Juenger M, Klose J, Kehr J: **Proteomics of curcurbit phloem exudate reveals a network of defence proteins**. *Phytochemistry* 2004, **65**:1795-1804.

20. Balachandran S, Xiang Y, Schobert C, Thompson GA, Lucas WJ: **Phloem sap proteins from cucurbita maxima and ricinus communis have the capacity to traffic cell to cell through plasmodesmata**. *Proc Natl Acad Sci USA* 1997, **94**:14150-14155.

21. Escobar N, Haupt S, Thow G, Boevink P, Chapman S, Oparka K:
• **High-throughput viral expression of cDNA-green fluorescent protein fusions reveals novel subcellular addresses and identifies unique proteins that interact with plasmodesmata**. *Plant Cell* 2003, **15**:1507-1523.
This study presents a high-throughput screen for protein localization using a viral vector expressing a cDNA library as GFP fusions. A key element of this approach is to assay transiently expressed GFP–cDNAs from viral infection foci, overcoming the time-consuming conventional transgenic approach. This method has proved very useful for the discovery of novel proteins detected through their association with particular organelles.

22. Kim I, Hempel F, Sha K, Pfluger J, Zambryski P: **Identification of a developmental transition in plasmodesmatal function during embryogenesis in *Arabidopsis thaliana***. *Development* 2002, **129**:1261-1272.

23. Bayer E, Thomas CL, Maule AJ: **Plasmodesmata in *Arabidopsis thaliana* suspension cells**. *Protoplasma* 2004, **223**:93-102.

24. Soellick T, Uhrig JF, Bucher GL, Kellmann JW, Schreier PH: **The movement protein NSm of tomato spotted wilt tospovirus (TSWV): RNA binding, interaction with the TSWV N protein, and identification of interacting plant proteins**. *Proc Natl Acad Sci USA* 2000, **97**:2373-2378.

25. Kragler F, Curin M, Trutnyeva K, Gansch A, Waigmann E: **MPB2C, a microtubule-associated plant protein binds to and interferes with cell-to-cell transport of tobacco mosaic virus movement protein**. *Plant Physiol* 2003, **132**:1870-1883.

26. Chen MH, Sheng J, Hind G, Handa AK, Citovsky V: **Interaction between the tobacco mosaic virus movement protein and host cell pectin methylesterases is required for viral cell-to-cell movement**. *EMBO J* 2000, **19**:913-920.

27. Desvoyes B, Faure-Rabasse S, Chen MH, Park JW, Scholthof HB: **A novel plant homeodomain protein interacts in a functionally relevant manner with a virus movement protein**. *Plant Physiol* 2002, **129**:1521-1532.

28. Matsushita Y, Deguchi M, Youda M, Nishiguchi M, Nyunoya H: **The tomato mosaic tobamovirus movement protein interacts with a putative transcriptional coactivator KELP**. *Mol Cells* 2001, **12**:57-66.

29. Mas P, Beachy RN: **Role of microtubules in the intracellular distribution of tobacco mosaic virus movement protein**. *Proc Natl Acad Sci USA* 2000, **97**:12345-12349.

30. Gillespie T, Boevink P, Haupt S, Roberts AG, Toth R, Valentine T, Chapman S, Oparka KJ: **Functional analysis of a DNA-shuffled movement protein reveals that microtubules are dispensable for the cell-to-cell movement of tobacco mosaic virus**. *Plant Cell* 2002, **14**:1207-1222.

31. Kawakami S, Watanabe Y, Beachy RN: **Tobacco mosaic virus infection spreads cell to cell as intact replication complexes**. *Proc Natl Acad Sci USA* 2004, **101**:6291-6296.

32. Peremyslov VV, Dolja VV: **Identification of the subgenomic mRNAs that encode 6-kDa movement protein and Hsp70 homolog of Beet yellows virus**. *Virology* 2002, **295**:299-306.

33. Aoki K, Kragler F, Xoconostle-Cazares B, Lucas W: **A subclass of plant heat shock cognate 70 chaperones carries a motif that facilitates trafficking through plasmodesmata**. *Proc Natl Acad Sci USA* 2002, **99**:16342-16347.

34. Waigmann E, Chen MH, Bachmaier R, Ghoshroy S, Citovsky V: **Regulation of plasmodesmal transport by phosphorylation of tobacco mosaic virus cell-to-cell movement protein**. *EMBO J* 2000, **19**:4875-4884.

35. Xoconostle-Cazares B, Ruiz-Medrano R, Lucas WJ: **Proteolytic processing of CmPP36, a protein from the cytochrome b_5 reductase family, is required for entry into the phloem translocation pathway**. *Plant J* 2000, **24**:735-747.

36. Heese-Peck A, Raikhel NV: **A glycoprotein modified with terminal N-acetylglucosamine and localized at the nuclear rim shows sequence similarity to aldose-1-epimerases**. *Plant Cell* 1998, **10**:599-612.

37. Lee JY, Yoo BC, Lucas WJ: **Parallels between nuclear-pore and plasmodesmal trafficking of information molecules**. *Planta* 2000, **210**:177-187.

38. Giesman-Cookmeyer D, Lommel SA: **Alanine scanning mutagenesis of a plant virus movement protein identifies three functional domains**. *Plant Cell* 1993, **5**:973-982.

39. Ishiwatari Y, Fujiwara T, McFarland KC, Nemoto K, Hayashi H, Chino M, Lucas WJ: **Rice phloem thioredoxin h has the capacity to mediate its own cell-to-cell transport through plasmodesmata**. *Planta* 1998, **205**:12-22.

40. Aoki K, Kragler F, Xoconostle-Cazares B, Lucas WJ: **A subclass of plant heat shock cognate 70 chaperones carries a motif that facilitates trafficking through plasmodesmata**. *Proc Natl Acad Sci USA* 2002, **99**:16342-16347.

41. Imlau A, Truernit E, Sauer N: **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* 1999, **11**:309-322.

42. Oparka KJ, Roberts AG, Boevink P, Santa Cruz S, Roberts I, Pradel KS, Imlau A, Kotlizky G, Sauer N, Epel B: **Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves**. *Cell* 1999, **97**:743-754.

43. Martens H, Hansen M, Schulz A: **Caged probes: a novel tool in**
• **studying symplasmic transport in plant tissues**. *Protoplasma* 2004, **223**:63-66.
This work introduced the first application of photolyzable caged probes to study plasmodesmal transport in plants. This novel technique provides a rapid method to measure the symplasmic network in plants without the disturbance of plasmodesmal permeability that occurs with microinjection.

44. Wu X, Dinneny J, Crawford K, Rhee Y, Citovsky V, Zambryski P,
•• Weigel D: **Modes of intercellular transcription factor movement in the *Arabidopsis* apex**. *Development* 2003, **130**:3735-3745.
The authors propose that some transcription factors move between cells by diffusion through plasmodesmata rather than selective trafficking. The authors were unable to find a selective trafficking signal on the LFY protein, but observed a strong correlation between movement and cytoplasmic localization of GFP fusions. This led them to propose that protein diffusion might be the default pathway.

45. Crawford KM, Zambryski PC: **Non-targeted and targeted protein movement through plasmodesmata in leaves in different developmental and physiological states**. *Plant Physiol* 2001, **125**:1802-1812.

46. Crawford KM, Zambryski PC: **Subcellular localization determines the availability of non-targeted proteins to plasmodesmatal transport**. *Curr Biol* 2000, **10**:1032-1040.

47. Qi Y, Pelissier T, Itaya A, Hunt E, Wassenegger M, Ding B: **Direct**
• **role of a viroid RNA motif in mediating directional RNA**

trafficking across a specific cellular boundary. *Plant Cell* 2004, **47**:1741-1752.
This study presents a viroid RNA motif that is required for directional RNA trafficking from bundle sheath cells to mesophyll cells, tissue specifically. The results demonstrate that RNA structural motifs can play a role in mediating trafficking of RNA in a tissue and/or development specific manner, and that the symplasmic network remodels its capacity to traffic RNAs during plant development.

48. Gilbertson R, Sudarshana M, Jiang H, Rojas M, Lucas W:
• **Limitations on geminivirus genome size imposed by plasmodesmata and virus-encoded movement protein: insights into DNA trafficking**. *Plant Cell* 2003, **15**:2578-2591.
DNA viruses can use RNA trafficking pathways to move cell–cell. Using recombinant geminiviruses, this work shows that DNA genome size is the crucial factor restricting entry into the PD.

49. Ding B, Li Q, Nguyen L, Palukaitis P, Lucas WJ: **Cucumber mosaic virus 3a protein potentiates cell-to-cell trafficking of CMV RNA in tobacco plants**. *Virology* 1995, **207**:345-353.

50. Fujiwara T, Giesman-Cookmeyer D, Ding B, Lommel SA, Lucas WJ: **Cell-to-cell trafficking of macromolecules through plasmodesmata potentiated by the red clover necrotic mosaic virus movement protein**. *Plant Cell* 1993, **5**:1783-1794.

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

52. Winston WM, Molodowitch C, Hunter CP: **Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1**. *Science* 2002, **295**:2456-2459.

53. Feinberg EH, Hunter CP: **Transport of dsRNA into cells by the**
•• **transmembrane protein SID-1**. *Science* 2003, **301**:1545-1547.
Previously, this research group cloned a gene, *SID-1*, encoding a putative transmembrane protein involved in systemic RNAi mechanisms in *C. elegans* [52]. The authors identify SID-1 as a protein that is not only required for systemic RNAi but is also sufficient to promote the uptake of dsRNA when ectopically expressed in cultured cells of *Drosophila* that lack systemic RNAi.

54. Himber C, Dunoyer P, Moissiard G, Ritzenthaler C, Voinnet O:
•• **Transitivity-dependent and independent cell-to-cell movement of RNA silencing**. *EMBO J* 2003, **22**:4523-4533.
This report demonstrates PTGS mechanisms and signals used for short- and long-distance RNAi. A primary 21 nt siRNA was shown to move 10–15 cells from its origin and act as a local RNAi signal. Secondary 21 nt siRNA, made by way of reamplification of signals, is required for more extensive movement. This short-range spread of silencing is distinct from long-distance systemic silencing, involving the production of the 25 nt siRNAs.

55. Jackson D: **Double labeling of KNOTTED1 mRNA and protein reveals multiple potential sites of protein trafficking in the shoot apex**. *Plant Physiol* 2002, **129**:1423-1429.

56. Sena G, Jung JW, Benfey PN: **A broad competence to respond**
•• **to SHORT ROOT revealed by tissue specific ectopic expression**. *Development* 2004, **131**:2817-2826.
This work reveals the importance of tightly regulated cell–cell trafficking of regulatory proteins using the GFP–SHR fusion expression under tissue specific promoters. The authors show that the competence to respond to SHR-mediated cell specification is widely distributed in the epidermis. Thus, SHR movement only one cell distant is essential for generating the correct root radial pattern.

57. Carrington JC, Kasschau KD, Mahajan SK, Schaad MC: **Cell-to-cell and long-distance transport of viruses in plants**. *Plant Cell* 1996, **8**:1669-1681.

58. Morozov SY, Solovyev AG: **Triple gene block: modular design of a multifunctional machine for plant virus movement**. *J Gen Virol* 2003, **84**:1351-1366.

59. Mezitt LA, Lucas WJ: **Plasmodesmal cell-to-cell transport of proteins and nucleic acids**. *Plant Mol Biol* 1996, **32**:251-273.

60. Hake S, Freeling M: **Analysis of genetic mosaics shows that the extraepidermal cell divisions in Knotted1 mutant maize plants are induced by adjacent mesophyll cells**. *Nature* 1986, **320**:621-623.

61. Kim JY, Yuan Z, Cilia M, Khalfan-Jagani Z, Jackson D: **Intercellular trafficking of a KNOTTED1 green fluorescent protein fusion in the leaf and shoot meristem of *Arabidopsis***. *Proc Natl Acad Sci USA* 2002, **99**:4103-4108.

62. Xoconostle-Cazares B, Xiang Y, Ruiz-Medrano R, Wang HL, Monzer J, Yoo BC, McFarland KC, Franceschi VR, Lucas WJ: **Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem**. *Science* 1999, **283**:94-98.

63. Kuhn C, Franceschi VR, Schulz A, Lemoine R, Frommer WB: **Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements**. *Science* 1997, **275**:1298-1300.

64. Yoo BC, Kragler F, Varkonyi-Gasic E, Haywood V, Archer-Evans S,
•• Lee YM, Lough TJ, Lucas WJ: **A systemic small RNA signaling system in plants**. *Plant Cell* 2004, **16**:1979-2000.
This study presents the identification of a population of small RNA species in plant phloem saps that might exert systemic control over plant development. Using a heterografting study, authors demonstrate that transgene-derived siRNA moves long-distance through the phloem. They isolated and characterized a *C. maxima* phloem protein, CmPSRP1, which might be a unique component of small RNA trafficking machinery. CmPSRP1 binds selectively to and could mediate the cell–cell trafficking of 25-nucleotide single strand RNA.