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Plasmodesmata form and function

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Intercellular transport via plasmodesmata controls cell fate decisions in plants, and is of fundamental importance in viral movement, disease resistance, and the spread of RNAi signals. Although plasmodesmata appear to be unique to plant cells, they may have structural and functional similarities to the newly discovered tunneling nanotubes that connect animal cells. Recently, proteins that localize to plasmodesmata have been identified, and a microtubule-associated protein was found to negatively regulate the trafficking of viral movement proteins. Other advances have delivered new insights into the function and molecular nature of plasmodesmata and have shown that protein trafficking through plasmodesmata is developmentally regulated, opening up the possibility that the genetic control of plasmodesmal function will soon be understood.

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Abbreviations

CPC	CAPRICE
ER	endoplasmic reticulum
GFP	green fluorescent protein
HSP70	heat-shock protein 70
ise1	*increased size exclusion limit 1*
KN1	KNOTTED1
LFY	LEAFY
MP	movement protein
NCAPP1	NON-CELL-AUTONOMOUS PATHWAY PROTEIN 1
PD	plasmodesmata
SEL	size exclusion limit
TEM	transmission electron micrograph
TGB	triple-gene block
TMV	tobacco mosaic virus
TNT	tunneling nanotube

Introduction

Plant cells are connected by cytoplasmic channels called plasmodesmata (PDs) that allow the transfer of nutrients and signals necessary for growth and development. PDs transverse the cell walls of neighboring cells, and inside the plasma membrane sleeve a proteinaceous rod called the desmotubule [1,2] (Figure 1) connects the endoplasmic reticulum (ER) of adjacent cells. The desmotubule may provide the surrounding plasma membrane with stability [1] and may also be important in regulating permeability.

Molecules are thought to traffic through the cytoplasmic channels between the desmotubule and the plasma membrane, either by a non-targeted or passive mechanism, if they are under the size exclusion limit (SEL) of the channel [3], or by a selective and regulated mechanism, if they possess an intrinsic trafficking signal(s) [4]. Other possible mechanisms are also discussed below. The SEL is developmentally regulated and decreases during development. This change is correlated with a change in PD morphology from simple channels to branched PD structures [5].

Plant development is reliant on intercellular communication through PDs. The first endogenous protein found to traffic cell-to-cell through PDs was the maize developmental homeodomain protein KNOTTED1 (KN1) [6,7]. Soon after, phloem proteins [8] were found to increase the PD SEL and to traffic cell-to-cell. The functional significance of the numerous phloem mobile proteins is still being elucidated. More recently, other developmental transcription factors such as DEFICIENS [9], SHORTROOT (SHR) [10], LEAFY (LFY) [11] and CAPRICE (CPC) [12] were also found to traffic and in some cases to mediate cell-fate decisions in destination cells.

Plant viruses also take advantage of PDs to spread their genomes from cell to cell. Movement proteins (MPs) are specialized viral proteins that increase the SEL [13,14] and permit viral genome transport [15,16,17]. MPs associate with both the cytoskeleton [18,19] and the ER [20,21,22]. The discovery of endogenous plant factors involved in MP function promises to greatly enhance our understanding of these elusive molecules [23••].

In this review, we will discuss recent advances in our understanding of the mechanisms of transport through PDs, how viruses use the PD machinery to traffic cell-to-cell, and the discovery of intriguing molecular players in PD function. We will also discuss recent choice experiments that demonstrate the considerable role of PDs in plant development.

Mechanisms of transport through plasmodesmata

Passive transport

PDs permit the passive transport of macromolecules if they are freely available in the cytoplasm and if their size

is below the SEL. Experiments involving either loading or microinjection of fluorescent probes indicated that these small dyes (~1 kDa) could move by diffusion (reviewed in [24]). In some cases, the SEL can be much larger than 1 kDa; for example the green fluorescent protein (GFP) can traffic freely in tissues such as petals, root tips and young rosette leaves [25]. Although passive transport through PDs is likely to be a result of diffusion, it may be selective, as is the case with animal gap junctions [26].

Active transport

The movement of viral MPs and endogenous proteins such as KN1 probably occurs via targeted mechanisms (reviewed in [27]). The hypothetical mechanisms of active, targeted movement through PDs are still under investigation. They include a PD-receptor-mediated mechanism where the non-cell-autonomous protein (any protein that traffics cell-to-cell through PDs) itself has a PD targeting signal, and a classical exo- and endocytosis mechanism [27]. We propose a novel hypothesis for transport through PDs by analogy to a novel mode of cell-to-cell transport that has recently been discovered in animals (to be discussed later in this section). These hypothetical mechanisms are distinct, but probably not mutually exclusive.

Exo-endocytosis

Baluska *et al.* [28] used fluorescent dye labeling to show that fluid phase endocytosis occurs in the PDs of maize root apices. Tubulo-vesicular compartments invaginated from the plasma membrane at acto-myosin-enriched pit-fields and at individual PDs. The formation of these compartments was blocked by latrunculin B, suggesting an actin-dependent mechanism, whereas microtubule disruption had no effect. The presence of endocytic vesicles at PDs suggests that PDs play a role in endomembrane trafficking or vesicle internalization, possibly at the site of vesicle docking, and in membrane fusion. The cell wall (ECM) is much thinner at sites where PDs are clustered, which may therefore coincidentally be good sites for exo- and endocytosis. If coupled with exocytosis, such a mechanism could result in protein translocation between plant cells, similar to a proposed mechanism for ENGRAILED homeodomain transport between animal cells [29].

Tunneling nanotubes

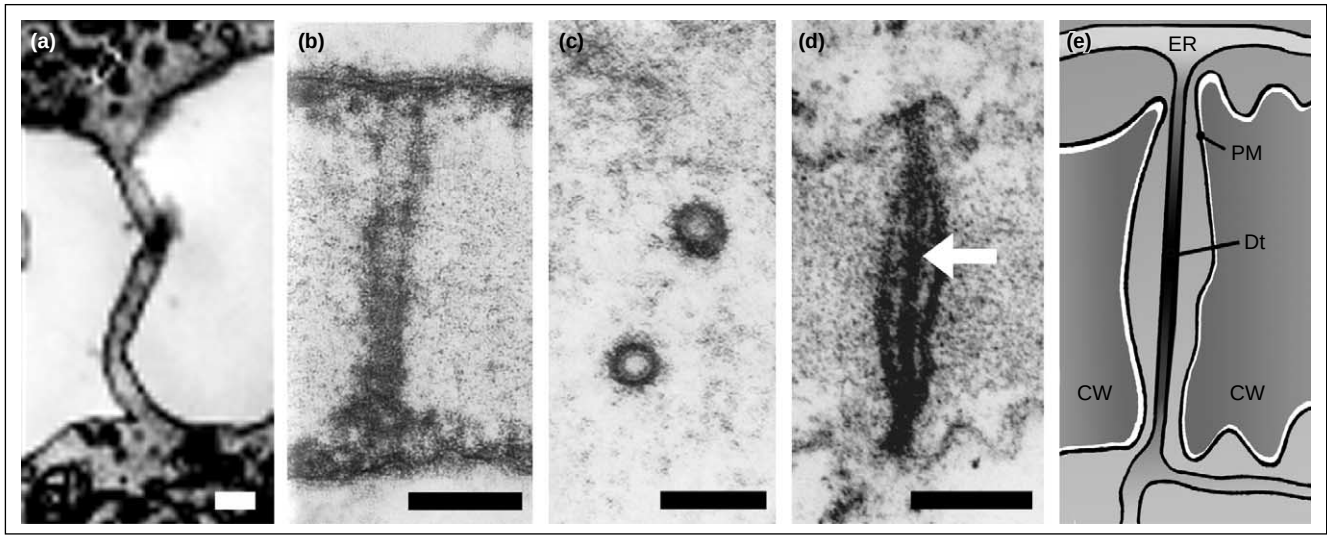
The discovery of a novel type of intercellular channel in animal cells [30••], called a 'tunneling nanotube' (TNT), may provide the first evidence that functionally similar modes of intercellular communication exist in plants and animals. TNTs were discovered during the imaging of fluorescently labeled lectin dyes in cultured rat cells [30••]. Independently of classical endo- and exocytosis, TNTs permit trafficking of endomembrane vesicles between cells, as shown by the presence of synaptophysin, a marker for early endosomes and endosome-derived synaptic-like microvesicles in TNTs [31]. However, neither GFP nor the small cytoplasmic dye calcein could move along the nanotubes, and the authors suggest that the dense packing of F-actin inside the nanotube may impose functional constraints on free diffusion. TNTs resemble PDs in some mechanistic aspects. For example, TNTs are sensitive to latrunculin B, and therefore probably use an F-actin-dependent transport mechanism, and viral transport through PDs is also sensitive to this inhibitor [32•]. Another parallel is that the microtubule cytoskeleton does not appear to be important for TNT or for PD transport [22,32•]. However, a major difference is that TNTs are transient and variable in location, whereas in plant cells PDs are thought to be stable. One possibility is that PDs could provide sites for nanotube formation between plant cells. The discovery of TNTs is exciting because it suggests a novel and testable hypothesis of macromolecular trafficking involving vesicular transport through PDs.

Although TNTs are structurally distinct from the PDs found in higher plants, they are more similar in structure to the PDs of *Chara corallina*, a characean algae thought to be a transition species between algae and higher plants [33] (Figure 1). PDs in *Chara* form after cytokinesis and, like TNTs, lack an ER desmotubule. An intriguing idea is that the primitive PDs in *Chara* are the precursors of higher plant PDs and also share some functional similarity with TNTs.

Recently identified PD-localizing proteins support the hypothesis of vesicular PD trafficking via exo-endocytosis or via TNTs. Escobar *et al.* expressed libraries of random, partial cDNAs fused to GFP in tobacco using a tobacco mosaic virus (TMV) vector [34]. One of these partial cDNAs encoded a protein related to a RabGTPase. Rabs play a role in the determination of vesicle transport specificity [35] and might bring cargo to the PD after vesicle packaging. Additional evidence for a role of vesicles in PD trafficking comes from the identification of KNOLLE (target-soluble *N*-ethyl-malleimide-sensitive-factor attachment protein in the syntaxin family), a t-SNARE involved in vesicle targeting, as an interacting partner of the grapevine fanleaf virus MP [36].

Plant transcription factors may move through plasmodesmata by any of the above mechanisms, and there is evidence for both non-selective passive transport [37] and selective transport [10,38••]. The idea that transcription factor movement through PDs occurs by a receptor-mediated mechanism is enticing, as this kind of selective transport mechanism is observed at the nuclear pore. However, no PD targeting signals in transcription factors or PD receptors for transcription factors have been definitively identified. It is possible that the trafficking of some plant transcription factors may occur after

Figure 1



Intercellular connections in animals, higher plants and algae. **(a)** Transmission electron micrograph (TEM) of a TNT between cultured animal PC12 cells shown in longitudinal section. Reprinted with permission from [30••]. Copyright 2004 by the AAAS. **(b)** TEM of transverse sections of *Chara* PD, demonstrating lack of a desmotubule. **(c)** TEM of simple PD in young walls of the algae *Chara*, in longitudinal section. (b) and (c) reprinted with permission from [33]. Copyright 1994 by Springer. **(d)** TEM of a primary or simple PD in longitudinal section, from mature tobacco leaf showing the internal desmotubule (arrowed). Reprinted with permission from [57]. Copyright 1992 by the American Society of Plant Biologists. **(e)** A cartoon depicting the PD shown in (d). CW, cell wall; Dt, desmotubule; ER, endoplasmic reticulum; PM, plasma membrane.

packaging in the Golgi via classical exo- and endocytosis, or via the movement of microvesicles along TNT-like structures in PDs.

Movement of viruses through plasmodesmata

Although microtubules were once thought to be important for viral MP trafficking [18,39], new data dispute this hypothesis. Using DNA shuffling, Gillespie *et al.* found that a TMV MP mutant (MP^{R3}) with limited affinity to microtubules actually showed enhanced trafficking [22], and they suggest that this mutant reveals a role for microtubules in MP degradation, rather than in targeting to PDs. Consistent findings were described by Kragler *et al.*, who used a cytoplasmic two-hybrid screen to identify MPB2C [23••], a microtubule-associated protein that interacts with the TMV MP. Their elegant studies co-localized MPB2C, microtubules and MPs *in vivo*, and functional assays showed that co-expression of MPB2C actually inhibited cell-to cell movement of TMV MP, again suggesting a negative relationship between MP localization to microtubules and cell-to-cell movement. Furthermore, intact TMV viral replication particles traffic cell-to-cell without the involvement of microtubules [32•].

Additional cellular proteins that interact with MPs and may be involved in targeting to PDs have been identified. For example, the TMV MP interacts with pectin methylesterase (PME) [40], an enzyme that modifies the cell wall component pectin. An appealing idea is that this interaction allows the MP to hitch a ride to the cell periphery and to PDs [24]. Insights come from studies of potato virus X, which moves intercellularly using the viral coat protein and triple-gene block (TGB) proteins [41]. A yeast-two hybrid assay with the TGB 12kDa protein identified interacting proteins that themselves interact with β-1,3-glucanase [42], an enzyme involved in callose degradation. As callose is found at the openings of PDs and is involved in regulating pore closure [43,44], it is possible that potato virus X uses this enzyme for targeting to PDs, and also to modify callose to promote viral spread.

MPs may also use chaperones to facilitate their trafficking, since the beet yellow virus depends on a virally encoded heat shock protein 70 (HSP70) chaperone homolog [45]. HSP70s facilitate protein transport into organelles [46] and are also involved in protein targeting to nuclei and to other cellular locations (reviewed in [4]). Some MPs also interact with chaperones of the DNAJ class [47], which modulate HSP70 activity. Proteins cross-reacting with HSP70 antibodies were detected in a PD-enriched biochemical fraction. Several of these HSP70s were cloned from a *Curcurbita maxima* stem cDNA library using degenerate primers based on the conserved ATPase domain [4], and the two HSP70s that were present in phloem sap could traffic cell-to-cell and increase the PD SEL. This new subclass of phloem HSP70s may serve as endogenous movement chaperones, stabilizing ribonucleoprotein (RNP) complexes as they pass from the

companion cells into the enucleate sieve tubes of the phloem. Alternatively, they may modulate the PD pore itself by increasing the SEL to facilitate trafficking into the phloem.

Molecular components of plasmodesmata

The molecular components of PDs and their associated trafficking pathways have, until recently, been elusive. It is likely that the actin cytoskeleton plays distinct roles, including regulating the SEL, trafficking cargo to and from the PD [27], and recycling cargo (reviewed in [27]). Centrin [48], a calcium-binding cytoskeletal protein, and calreticulin [49], a calcium-sequestering protein, also localize to PDs and might modulate their function in response to calcium signaling. Indeed, calcium signals rapidly and transiently regulate PD permeability [50], though the mechanisms they use to influence plasmodesmal pore size have yet to be discovered. An insight into a potential mechanism comes from studies of sieve elements, the conductive cells of the phloem, which are reversibly plugged by calcium-sensitive contractile proteins that act as 'cellular stopcocks' [51]. One could imagine calcium regulating the SEL in PD by causing similar conformational changes in proteins.

A new hunt for PD proteins is yielding tantalizing results that demonstrate the complexity of these channels. Two of the candidate PD proteins identified in the viral screen mentioned previously [34] are potentially involved in redox signaling. One of these is a monohydroascorbate reductase homolog. Monohydroascorbate radicals are produced in response to TMV infection [52], and it is possible that the monohydroascorbate reducatase homolog localizes to PDs only transiently, in response to TMV infection. For all of the genes identified in this screen, the logical next step will be to confirm the localization of the native full-length gene products, and to ask if their PD localization is a function of viral infection or whether it plays a role in normal PD function. A second example of environmental regulation of PD composition and function comes from studies of the remarkable freeze-tolerant woody plant, *Cornus sericea*, which is capable of surviving temperatures as low as −269°C. Cold treatment induced the accumulation of a 24 kDa dehydrin-like protein at PDs [53], and it was suggested that this may protect the cell membrane from mechanical damage. We are probably just beginning to understand the dynamic physiological modifications that PDs undergo in response to changing environmental conditions.

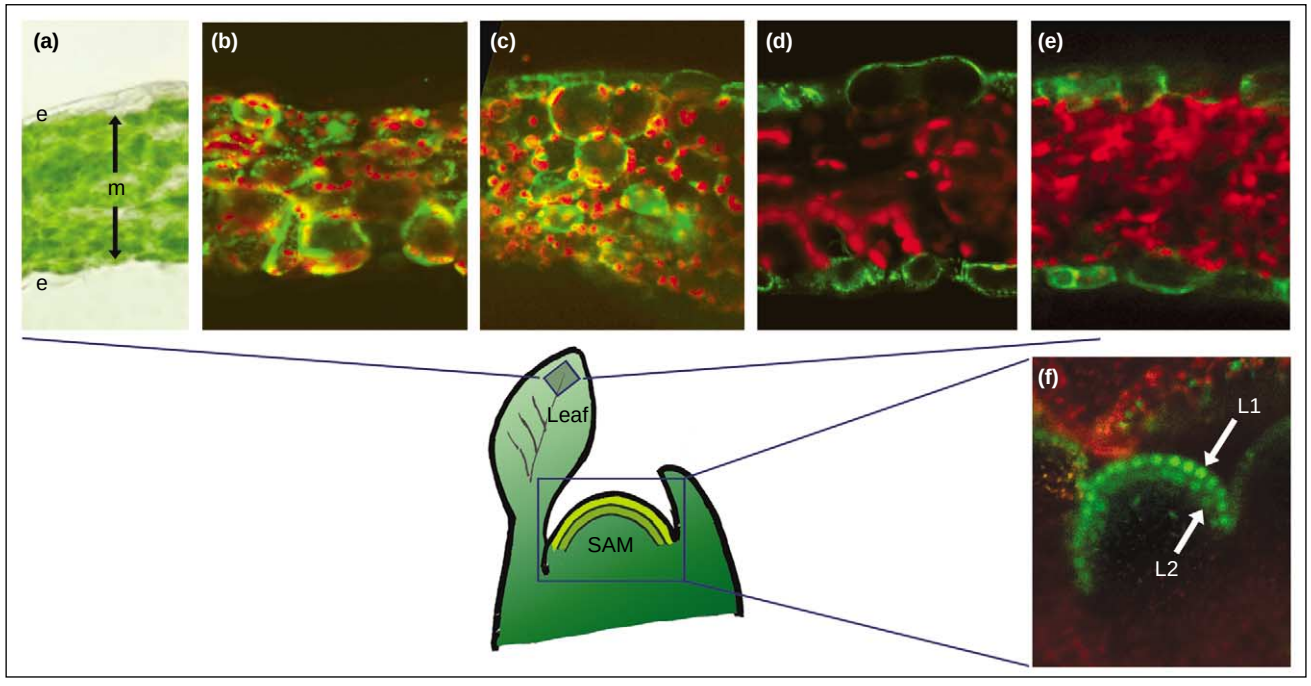
Plasmodesmata and plant development

Details of the mechanisms by which developmental signals traffic through PDs are beginning to emerge. To find host-cell factors involved in trafficking, Lee *et al*. fished for binding partners of a phloem mobile MP paralog, CmPP16 [54••]. They affinity-purified NON-CELL-AUTONOMOUS PATHWAY PROTEIN1 (NCAPP1) from a PD-enriched cell-wall fraction from cultured tobacco cells. Ultrastructural studies localized NtNCAPP1 to the ER, in the vicinity of, but never directly in, PDs, which raises the intriguing question of NtNCAPP1's specific mode of action. Perhaps NCAPP1 is involved in targeting to PDs, rather than contributing directly to the translocation event. In tobacco plants expressing a dominant-negative form of NCAPP1 with an N-terminal deletion, the interactions of both CmPP16 and TMV MP with PDs were blocked. However, trafficking of KN1 or of the cucumber mosaic virus MP were unaffected, suggesting distinct mechanisms of trafficking for these proteins. The NCAPP1 mutant transgenic lines also showed severe developmental defects, including lack of organ symmetry and whorl separation, enlarged terminal flowers, loss of apical dominance, highly asymmetric leaves, dwarfing, and disorganization of cell layers. The authors suggest that the floral phenotype resembles the phenotype caused by overexpression of the tobacco ortholog of LFY, and hypothesize that NCAPP1 may regulate LFY trafficking.

A potential role for NCAPP1 in the regulation of LFY trafficking is disputed by results from Wu *et al*. [37]. From studies of LFY deletion constructs, they conclude that LFY trafficking in the meristem occurs via a non-targeted or passive mechanism. If this is true, why do the dominant-negative NCAPP1 lines show a phenotype reminiscent of LFY overexpression? The involvement of a host-cell factor in LFY movement is at odds with the hypothesis that this protein moves by diffusion. However, if NCAPP1 modifies the shape or charge of the PD pore or of the LFY protein itself, it may change the ability of LFY to move passively through PD. The logical next step to reconcile the data from these groups would be to test the movement of LFY in the dominant-negative NCAPP1 lines.

In recent studies of KN1 homeodomain protein trafficking in the model plant *Arabidopsis thaliana*, Kim *et al*. reported tissue-specific regulation of trafficking [38••]. A GFP–KN1 fusion was able to traffic from the inner layers of the mature leaf to the outer layer, the epidermis — but strikingly not in the opposite direction (Figure 2). However, in the shoot meristem, where cells are in a relatively undifferentiated state, the GFP-KN1 fusion was able to traffic out of the epidermal (L1) layer. These results, taken together with early studies of *Chara* PDs (reviewed in [55]) suggesting that symplastic isolation (PD closure) plays a role in differentiation, provide an insight into why plasmodesmata change their function during differentiation. The changes in PD during development may regulate the trafficking of factors involved in cell-fate determination and cell-cycle regulation. Genetic screens using KN1 trafficking as a tool to dissect the mechanisms of PD regulation should be enlightening in this regard.

Figure 2



Trafficking of KN1 in *Arabidopsis* is under tissue-specific and developmental regulation. **(a)** Light micrograph of a leaf in cross-section showing the mesophyll (m) and epidermal (e) cell layers. **(b)** A mesophyll-specific promoter driving expression of the cell-autonomous ER localized GFP. **(c)** The same promoter driving a GFP–KN1 fusion shows fluorescence in epidermal cells caused by trafficking of the fusion protein. **(d)** An epidermal-specific promoter driving the cell autonomous GFP. **(e)** The same promoter driving GFP-KN1expression; note the lack of movement from epidermis to underlying cell layers. **(f)** An epidermal-specific promoter driving GFP-SHOOTMERISTEMLESS (an *Arabidopsis* homolog of maize KN1) in the shoot meristem. Note that in this case trafficking out of the epidermal layer does occur into underlying L2 cells (arrowed). The red color in (b–f) is due to chlorophyll auto-fluorescence. Reprinted with permission from [38••]. Copyright 2003 by Development and Company of Biologists LTD. The cartoon below depicts a plant whose organs are shown in (a–f).

In a novel screen to identify *Arabidopsis* mutants defective in regulating the PD SEL, Kim *et al*. isolated a mutant called *increased size exclusion limit 1* or *ise1* or [56]. The SEL is down-regulated at the torpedo stage of embryo development, and this transition does not occur in *ise1* mutant embryos. One of the remarkable phenotypes of *ise1* mutants is that all root epidermal cells make hairs (specialized cells in the root to increase surface area for nutrient and water uptake from soil), whereas in the wild-type, rows of hair cells are separated by rows of non-hair cells. The *ise1* root phenotype is mimicked by transgenic plants constitutively over-expressing CPC, a positive regulator of root hair development [12]. Interestingly, the CPC protein itself normally traffics from root-hair cells to non-root-hair cells, where it represses GLABRA2, a negative regulator of hair-cell fate. Might *ise1* mutants affect the trafficking of CPC or other factors required for root hair development? An interesting experiment would be to test for the presence of CPC and other root-hair developmental proteins in the *ise1* mutants.

Conclusions

PDs are proving to be complex, but the studies discussed here contribute significantly to our understanding of how trafficking occurs between plant cells. PDs probably use multiple trafficking pathways to regulate physiological processes, and distinct mechanisms of transport through simple and branched PDs probably afford developmental flexibility. The discovery of a cell-to-cell transport mechanism based on membrane continuity in animal cells should encourage plant researchers to use the tools and knowledge gained from this system. Experiments designed to uncover the mechanisms controlling the selective permeability of PDs to passive and active transport will certainly guide research over the next few years. Plant viruses and endogenous movement proteins such as transcription factors or phloem proteins will continue to be useful tools in the elucidation of the different modes of active transport, and analysis of cell-to-cell trafficking mutants should reveal how PDs control plant-cell biology and orchestrate development.

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This paper and [37] clearly demonstrate that even passive transport through PD may be selective. Wu *et al.* [37] show data using LFY~GFP deletions that movement of LFY in the meristem is consistent with diffusion. However Lee *et al.* have identified a cell factor that may affect LFY trafficking in the meristem. These groups should design experiments together to test the specific way in which NCAPP1 affects LFY trafficking.

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