Plasmodesmata signaling: many roles, sophisticated statutes

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Recent advances have increased our understanding of plasmodesmata function, their architecture as it relates to signaling capacity, the temporal and spatial regulation of their permeability, and their roles in systemic transport of macromolecules, non-cell autonomous development, and, potentially, plant defense.

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Abbreviations

CP	coat protein
HC-Pro	helper component proteinase
PD	plasmodesmata
PTGS	post-transcriptional gene silencing
SEL	size exclusion limit

Introduction

Plasmodesmata (PD) are channels through the plant cell wall which interconnect individual cells, facilitating symplastic communication. Microinjection studies indicate that only molecules below 1 kDa freely diffuse through plasmodesmata of leaf mesophyll cells [1,2]. This small size exclusion limit (SEL) excludes most cellular components, giving the impression that PD merely function to deliver photosynthate to developing tissues. Viruses expose the dynamic nature of plasmodesmata, however, as they trigger a dramatic increase in PD SEL — great enough to export their genomes, either as virions or protein/nucleic acid complexes, between cells and ultimately into the phloem (reviewed in [3,4•,5]). The speed of viral induced alteration in SEL indicates that viruses usurp an endogenous pathway designed to traffic macromolecules that relay physiological and developmental cues. Evidence is now accumulating that plasmodesmata do indeed traffic macromolecular signals [5–7,8••,9] between cells into the vasculature, and from the vasculature into recipient tissues to regulate growth and survival.

PD architecture: ground plan for dynamic regulation

Despite decades of research, our knowledge of PD remains surprisingly rudimentary. Electron microscopy reveals PD as passages through the cell wall, composed of two sets of membranes — an outer plasma membrane and an inner core of modified endoplasmic reticulum (Figure 1) (reviewed in [3,5]). PD form under two conditions: primary PD form as breaks in the developing cell wall during cytokinesis [10,11]; secondary PD form through existing cell walls to increase the symplastic connection between cells, or to connect cells not clonally related. Formation of secondary PD is critical to achieve communication in three-dimensions — best exemplified in the shoot apical meristem where outer tunica layers divide only anticlinally and must be secondarily connected to underlying corpus cells (Figure 2) [12•]. Evidence that secondary PD may function uniquely finds recent support in the discovery that the cucumber mosaic virus movement protein only traffics through PD when leaves have reached a certain developmental age, corresponding to the presence of secondary PD [13•]. The mechanism of secondary PD formation is unknown, but localization of cellulase reaction product deposits near and within PD coincident with their formation suggests cell wall disruption by hydrolytic enzymes [14]. Understanding the temporal regulation of, and the mechanism of secondary PD formation may elucidate their particular role in cell–cell signaling.

Plasmodesmata selectively transport molecules larger than their SEL would dictate [3,5,7]. PD dilation may occur by an actin/myosin based mechanism, as both actin and myosin localize to PD [15–17]. Actin involvement in PD regulation is further supported by microinjection of cytochalasin D or profilin, which disrupt actin filaments and increase PD SEL from 1 kDa to over 20 kDa [18]. In contrast, phalloidin, which stabilizes actin filaments, prevents movement of a 10 kDa dextran and inhibits PD dilation induced by profilin [18]. Additionally, pre-treatment of tissue with 2,3-butanedione monoxime (BDM), an inhibitor of actin–myosin motility in animals, results in PD with visually constricted necks (Figure 1) [16]. In animal cells BDM stabilizes actin:myosin:ADP:Pi complexes; thus, it may lock a PD actin:myosin complex in a closed configuration. Potentially actin filaments limit the aperture of PD, and their disruption or the inhibition of myosin movement along them, triggers dilation of PD.

Regulation of PD permeability in time and space

As plant cell fate is dependent on positional information, signaling between component cells is critical for proper development [19–21]. Regulating PD permeability provides a simple means to control the quantity and quality of communication between cells. This regulation is illustrated by cell-type specific SELs: 1 kDa for leaf mesophyll and epidermal PD [1,2], 7 kDa for leaf trichomes [22], and 10–25 kDa for PD between sieve elements and companion cells in phloem tissue [23•,24•]. SEL is additionally regulated throughout development, in a temporal and position-dependent manner. Tissue or cell-specific differences in PD function could reflect differences in structure, molecular regulation, frequency, or type (primary versus secondary PD). Moreover, the physiological state of a plant

Figure 1

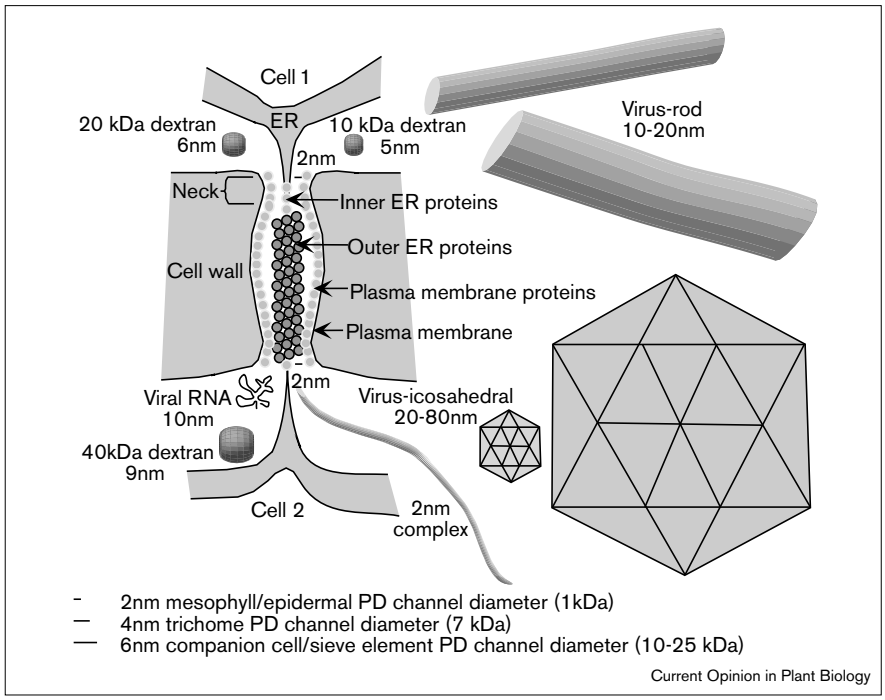


Diagram of plasmodesmata (PD), showing major structural components. The size of the transport channel, 2 nm, is indicated as a short bar close to the neck region. This 2 nm dimension is compared to various macromolecules (drawn to scale), indicating the challenge of cell–cell transport faced by macromolecules and viruses. Virus size ranges, helical viruses (10–20 nm), and icosahedral viruses (20–80 nm) are from [53]. The 2nm complex shown (wavy string-like shape near PD opening in cell 2) represents TMV movement protein bound to viral RNA [54]. Dimensions given are the smallest diameter as measured by electron microscopy or by Stokes radius prediction.

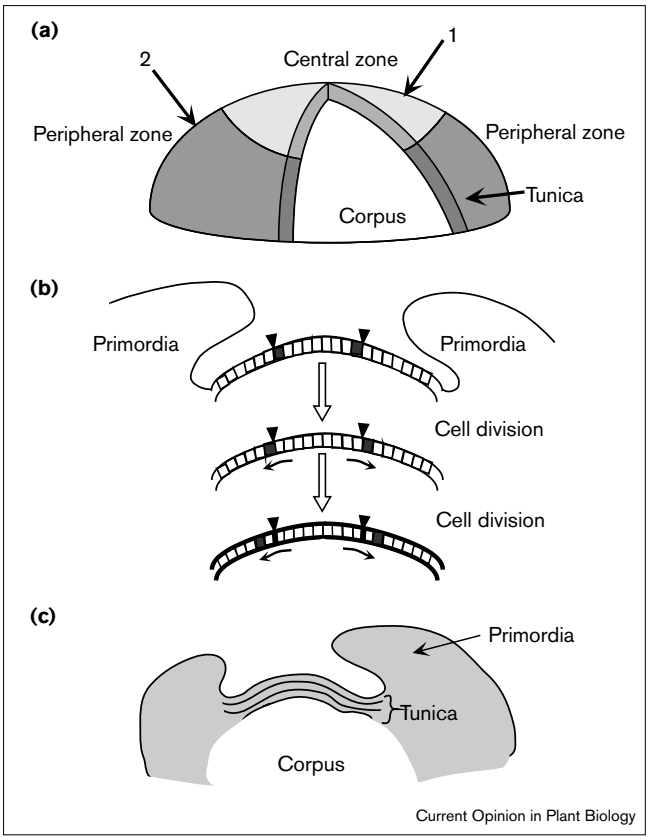
alters PD trafficking capacity (reviewed in [3]). Superimposed on these layers of regulation are signaling macromolecules such as the transcription factor KNOTTED (45 kDa), far larger than the SEL, but capable of manipulating PD, presumably to alter the fate of recipient cells [3,4•,5–7]. The mechanisms and importance of non-cell autonomous regulation through PD are just beginning to be examined.

In higher plants, all cells of the embryo are connected into a single symplastic unit [25]. As plants develop, however, downregulation of groups of PD gives rise to isolated regions — either permanent symplastic domains, or transient symplastic fields. Mature stomata are an example of permanently, symplastically isolated cells [26,27]. The *Arabidopsis* root similarly illustrates that initially connected cells are symplastically uncoupled as they differentiate, detected by symplastic connectivity in the meristem and elongation zone, but loss of coupling in the zone of differentiation [28–30]. Additionally, the hypocotyl epidermis is uncoupled from the root epidermis, and companion cell/sieve element modules are isolated from non-phloem cells in tissues exporting photosynthate [3,8••,24•,28] Thus, PD SELs are modified to isolate certain cells, and stipulate domains of symplastic connectivity during development. Whether signaling molecules traffic through these 'closed' PD remains to be determined.

Besides permanent symplastic restrictions, 'transient' symplastic fields arise due to temporal and position-dependent regulation of PD, as exhibited by two studies of the shoot apical meristem. A clear illustration of position-dependent modification of PD ensues from microinjection of the small dye, lucifer yellow (LYCH 457 Da) into tunica cells of birch vegetative meristems [31••]. Two symplastic fields in the tunica are revealed: a concentric ring corresponding to the peripheral zone, and a second zone encompassing the central core (Figure 2a). These fields are isolated from each other, as LYCH cannot move between them, and are maintained by a position-dependent process. These fields of connectivity are probably created by regulatory molecules, as callose (often associated with PD occlusion) is not detected. Thus, different fields of meristem activity may exist, possibly functioning in meristem maintenance and primordia formation. Amazingly, plants are able to obstruct PD at specific distances from the meristem center and maintain these sites, even though the cells subject to this regulation are continuously changing spatially due to cell division and lateral displacement (Figure 2b). Furthermore, birch meristems exhibit temporal regulation, resulting in their symplastic isolation during winter dormancy. In this semi-permanent state, symplastic isolation arises from occlusion of the surrounding PD with callose. Two pathways of closure then emerge, a more permanent modification resulting from occlusion of PD with callose, and temporary alterations presumably resulting from molecular regulation.

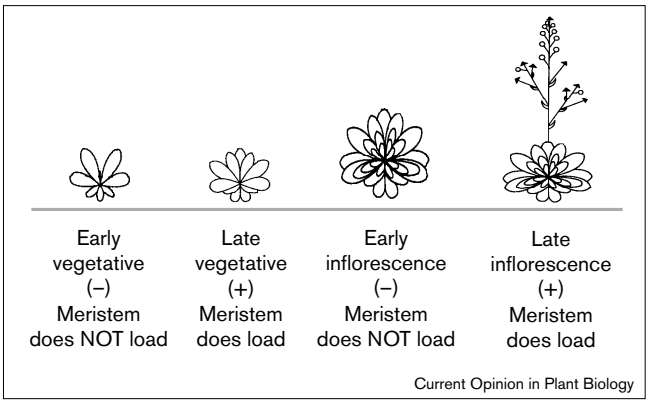
A second study illustrates the dynamics of temporal PD regulation by using confocal microscopy to trace the phloem unloading of the fluorescent dye HPTS (520 Da) in *Arabidopsis* vegetative and inflorescence meristems [32••]. Dye does not enter young vegetative meristems, whereas

Figure 2



Symplastic movement of tracer in apical meristem of birch and *Arabidopsis*. **(a)** shows the result of lucifer yellow microinjection into two regions of the birch vegetative meristem. When injected into the center (1), dye moves locally within the central zone (light gray region). When injected lower on the flanks of the meristem (2), dye moves only within the lower peripheral zone (darker gray region). Thus, a boundary region, indicated by the dark black band, prohibits symplastic movement from the central zone to the peripheral zone, and vice versa. **(b)** Longitudinal view of symplastic fields in birch meristem illustrating that as cells (gray) just inside the boundary (top view), are displaced laterally (subsequent two views) following anticlinal cell divisions, PD aperture must be modified (open or closed), depending on their localization relative to the boundary region. **(c)** Diagram of longitudinal section through *Arabidopsis* inflorescence meristem revealing spatial restriction of the fluorescent tracer, HPTS (8-hydroxypyrene 1,3,6 trisulfonic acid). HPTS is found in the tunica layers and primordia (light gray regions), but is absent in the underlying corpus (white). Note that the *Arabidopsis* inflorescence tunica contains three layers of cells, in contrast to only one in birch. Both the birch [12•,31••] and *Arabidopsis* [32••] studies are consistent in revealing the symplastic isolation of the tunica from the corpus.

Figure 3



Temporal oscillation of symplastic transport to the meristem during vegetative and floral development in *Arabidopsis* grown under short day (SD) conditions (8 hr light, 16 hr dark). The fluorescent tracer, HPTS, is loaded through cut petioles into the phloem and unloads at distal sites, such as the meristem. Vegetative plants with 11 or less leaves (28 SD) do not traffic tracer to the meristem, whereas older plants with greater than 12 leaves (29 SD) do. Traffic to the shoot apical meristem continues during the vegetative phase, but as soon as plants commit to flowering (42 SD), as demonstrated by the appearance of floral primordia, traffic to the meristem abruptly stops. Traffic to the inflorescence meristem recommences only after secondary inflorescence shoots appear. Interpreted form results in [32••].

older, vegetative plants import HPTS into the tunica layers of their meristems, but not into the underlying corpus (Figures 2c and 3). That the tunica functions differently than the corpus is intriguing, suggesting that primary PD, between tunica cells, and secondary PD, between tunica and corpus, may be differentially regulated. Surprisingly, apices abruptly stop traffic of HPTS coincident with the commitment to flowering. Dye traffic to the apex resumes only after secondary inflorescences and mature siliques appear (Figure 3). These oscillating states of connectivity may correspond to the traffic of important developmental signals such as those regulating the induction of flowering. These symplastic fields highlight that beyond the transcriptional activation of genes involved in morphogenesis, supracellular signaling may regulate developmental transitions. Furthermore, while it is implicit that PD are (somehow) opening and closing, the actual molecules involved in this regulation are yet to be determined.

Systemic macromolecular transport

Tissue-specific transport capacities in plants are clearly exposed by viruses as they manipulate a complex and ever-changing host to replicate, move locally, and invade the phloem. Entry into, and exit from, the vasculature presents a major traffic checkpoint for many viruses, and a selective restriction for endogenous molecules [4•,7,33••] This restriction is surprising as PD between companion cells and their dependent sieve elements, the component cells of phloem tissue, have a large SEL [24]. A unequivocal view of this SEL is provided by the unimpeded movement of the green fluorescent protein (GFP, 27 kDa) into and out of the phloem in transgenic plants expressing this heterologous protein under the control of a companion cell specific promoter [23•]. Unloading of GFP occurs in non-phloem recipient tissue as well, reflecting a significantly larger PD permeability than expected for these tissues. Despite this large SEL, many viruses are either not capable of invading or escaping the phloem, suggesting that special requirements exist. Indeed, an endogenous protein, CmPP16, might function as a

phloem-specific chaperone to overcome these limitations by selectively loading RNAs and proteins into sieve elements for vascular transit [9].

The prerequisite for facilitating factors enabling phloem entry is mirrored by viruses as they require different components for long-distance transport than for cell–cell transit [33••,34]. Viruses have evolved different ways of overcoming the challenge of phloem entry, but, with few exceptions, viral coat proteins (CP) are required; thus, viral genomes either traffic as virions in the phloem or CPs perform a unique, nonstructural (i.e. non virion) role in enabling vascular entry. Some viruses encode nonstructural proteins that enable phloem entry, for example 2 b of cucumoviruses, HC-Pro of potyviruses, and p19 of tomato bushy stunt virus [35–37]. Both HC-Pro and 2b may act indirectly in vascular transport by interfering with plant defenses (see next section). Another class of vascular transporter, ORF3 of groundnut rosette umbravirus, can functionally replace the coat protein of a unrelated virus, tobacco mosaic virus (TMV), in enabling vascular spread [38]. ORF3 is a nonstructural protein, which does not affect viral pathogenicity. Interestingly, umbraviruses do not encode coat proteins and thus must transport RNA in a nonvirion form, suggesting that TMV CP may perform a similar nonstructural function in vascular transit. The phytovirus, beet necrotic yellow vein virus, utilizes an intriguing derivative mechanism whereby its third genomic RNA, and not its protein products, mediates viral movement into the phloem [39]. Additionally, mutations in host components that control long-distance transport of viruses may contribute to identifying the regulatory components of vascular PD transport [40•–42•].

PD Involvement in plant defense?

An aspect of cell to cell and long distance trafficking under intense investigation is post-transcriptional gene silencing (PTGS), whereby accumulation of specific RNAs is diminished [43]. Following introduction of transgenes or viruses, PTGS silences genes *in trans* in a sequence homology-dependent manner. Gene silencing is an efficient strategy whereby plants protect themselves from invading foreign nucleic acids, either transgenes, plant viruses, or possibly transposons. Locally triggered PTGS results in systemic silencing, indicating that a signal is trafficked locally and throughout the plant, in a manner similar to virus spread. This system of silencing may be achieved through sequence specific RNA degradation [43]. Indeed the sequence specificity of PTGS suggests that the signaling molecule may itself be an RNA, capable of PD transport to track and silence 'aberrant' transcripts in distant cells.

Evidence that the silencing signal moves derives from experiments where grafting a non silenced scion onto a silenced plant results in silencing in the originally non silenced graft [44]. Furthermore, constructs with homology to integrated and actively expressed transgenes can be bombarded into single cells and induce silencing of the transgene at local and distant sites [45•,46•]. Both types of studies suggest the silencing signal moves cell to cell via plasmodesmata and long distance via the vascular system. Interestingly, the shoot apical meristem, subject to symplastically isolation at times in development, appears resistant to gene silencing [47•]. Symplastic isolation may protect meristems from invading viruses and differentiation signals, requiring that PTGS be re-initiated in each new primordia.

There are likely to be three stages of PTGS: initiation, spread, and maintenance [45•,46•]. Recently, local silencing was separated from systemic silencing in a transgenic tobacco population [46•]. Interestingly, the extent of silencing was determined by the amount of DNA; a low level of DNA transiently expressed in leaf tissue was sufficient only to induce local silencing, whereas a larger quantity of DNA apparently breached a threshold and induced systemic gene silencing. Thus, the quantity of induction is important to trigger either transport of a signaling molecule to recipient tissue, or re-initiation of PTGS in distant tissues. How the specificity of gene silencing is controlled and propagated within a cell and throughout the plant in unclear. Future research will determine the nature of the signaling molecule, how it is induced, recognized, and transported, and if PD are directly implicated in such transport.

Further insight into PTGS results from the discovery of viruses able to combat the 'immunological response' of gene silencing. Potyviruses combat PTGS by encoding the protein HC-Pro (helper component proteinase), which is a pathogenicity determinant, a stimulator of genome amplification, and a factor required for long-distance transport of the virus [34,48,49••,50•,51•]. PTGS does not occur in the presence of HC-Pro. Notably, if PTGS against a potyvirus is already initiated in plants at the time of viral infection, then HC-Pro is noneffective [49••]. Cucumoviruses encode a protein, which similarly combats gene silencing [47•,51•]. Intriguingly, the 2b protein of cucumber mosaic virus (CMV) is effective against PTGS even if viral infection occurs after PTGS has been mounted. The 2b protein prevents silencing in newly emergent tissues, indicating it prevents systemic signaling or re-initiation of PTGS, whereas HC-Pro presumably prevents signal initiation or maintenance. Further, a CMV 2b homologue can be involved in two different defense pathways, underscoring the interplay between virus and host. Dependent on the virus genome context and host plant involved, this single protein either combats PTGS, or mimics an avirulence gene in inducing gene-for-gene resistance [52••]. These results convey the complexity of plant signaling cascades and the potential interplay between defense pathways.

Future directions

To comprehend the roles of PD in signaling, it becomes imperative to determine which developmental and physiological programs are regulated in a non cell autonomous manner. This requires identification of the signaling mole-

cules involved and the PD components controlling their movement. To date, known molecules which traffic between cells utilizing PD are predominately limited to pathogens. Many proteins and RNAs are present in sieve elements of the phloem, undoubtedly imported through PD from the connecting companion cells [7,8••,33••]. Whether these molecules function in sieve element maintenance or as signals to distant recipient tissues is unknown. Although indirect evidence suggests that PD are manipulated by non-cell autonomous transcription factors that alter gene expression in target cells as part of a supracellular program, and by silencing signals that function to track and destroy 'invading' nucleic acids, the identity of most endogenous molecules that depend on PD to carry out their functions is lacking. We must further undertake the task of dissecting the developmental, spatial, temporal, and physiological layers of PD regulation. Additionally, it is critical to understand the requirement of cells to isolate themselves from, or integrate themselves with their neighbors into fields or domains, and to establish if such patterns are representative of actual signaling molecules. Such studies will expose how dynamic networks of cells achieve morphogenesis, respond to the environment, and defend themselves.

Acknowledgements

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