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Plasmodesmata dynamics are coordinated by intracellular signaling pathways

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Abstract

Membrane-lined channels called plasmodesmata (PD) connect the cytoplasts of adjacent plant cells across the cell wall, permitting intercellular movement of small molecules, proteins, and RNA. Recent genetic screens for mutants with altered PD transport identified genes suggesting that chloroplasts play crucial roles in coordinating PD transport. Complementing this discovery, studies manipulating expression of PD-localized proteins imply that changes in PD transport strongly impact chloroplast biology. Ongoing efforts to find genes that control root and stomatal development reveal the critical role of PD in enforcing tissue patterning, and newly discovered PD-localized proteins show that PD influence development, intracellular signaling, and defense against pathogens. Together, these studies demonstrate that PD function and formation are tightly integrated with plant physiology.

INTRODUCTION

In multicellular organisms, cells coordinate development, share resources, and communicate physiological and environmental changes. In plants, small membrane-lined channels called plasmodesmata (PD) connect the cytoplasts of adjacent cells across the cell wall, permitting intercellular transport and communication. PD are bound by plasma membrane at their outer limits and contain a central strand of tightly compressed endoplasmic reticulum (the "desmotubule") that almost completely lacks luminal space. Molecules predominantly traffic from cell to cell through the cytosolic sleeve in between the plasma membrane and endoplasmic reticulum (Fig. 1). PD allow exchange of small molecules, such as ions, sugars, and phytohormones, as well as larger molecules, including proteins, RNA, and viruses. Most PD transport occurs via diffusion, but some proteins—viral movement proteins, for example—are targeted to PD and transport more rapidly by mechanisms that remain poorly understood but are currently under investigation [1].

PD research is challenging for several reasons. First, PD are very small (~30–50nm in diameter) and difficult to isolate from other membranes, which has long prevented direct analysis of PD components [2]. Second, PD are essential to plant survival: all land plants have PD, and no mutant lacking PD has ever been isolated [3,4]. Third, PD function is extremely sensitive to manipulation (such as mechanical stress), which complicates efforts to directly assay PD transport [5]. For many years, the PD size exclusion limit (SEL) was believed to be ~1 kDa; we now know that the technique previously used to measure PD

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transport (microinjection of small fluorescent tracers) rapidly triggers PD closure [5], and that the actual SEL is typically at least 27 kDa (the size of GFP), and can often be larger [6].

Despite these difficulties, PD research has made significant progress. Here, we describe (i) insights into the regulation of PD function and formation gained from genetic approaches, (ii) new discoveries pertaining to PD transport of transcription factors, and (iii) our expanding knowledge of PD protein components. These studies support a common theme: PD function and formation are tightly integrated with intracellular signaling pathways and plant physiology.

Regulation of PD Function and Formation: Genetic Insights

In the last decade, three mutant screens were conducted to identify genes regulating PD transport. The first looked for altered movement of fluorescent tracers in embryo-defective mutants, reasoning that any mutant with strong effects on PD transport would exhibit severely disrupted development [3]. From this screen, three mutants have been characterized so far: *increased size exclusion limit 1* and *2* (*ise1* and *ise2*), which have increased PD transport, and *decreased size exclusion limit 1* (*dse1*), which has decreased PD transport [3,7–10]. Two other screens searched for mutants with decreased protein movement (i) from the phloem to surrounding tissues or (ii) from the mesophyll to the epidermis. These efforts led to the characterization of two PD mutants: *gfp arrested trafficking 1* (*gat1*) and *chaperonin containing TCP1 8* (*cct8*) [11–13]. Unexpectedly, none of the genes recovered from these screens encode proteins that localize to PD. ISE1 and ISE2 are RNA helicases that localize to mitochondria and plastids, respectively; DSE1 is a WD40-repeat containing protein found in the cytoplasm and nucleus; GAT1 is a plastid-localized thioredoxin; and CCT8 is a cytoplasmic chaperonin subunit.

Chloroplasts Regulate PD... and PD Regulate Chloroplasts!

Intriguingly, three of these five mutants contribute to a single narrative: PD function and formation are strongly regulated by intracellular signals originating from mitochondria and plastids. Beyond dramatically increasing PD transport and formation [14], loss of either ISE1 or ISE2 affects the expression of ~3,000 genes; genes that encode chloroplast proteins are remarkably overrepresented in both transcriptomes [9]. PD transport is severely reduced in mutants lacking the plastid thioredoxin GAT1, which overproduce hydrogen peroxide (H₂O₂), a known upstream signal impacting intracellular processes and nuclear gene expression [11]. Together, these results demonstrate that there must be extensive signaling among organelles, the nucleus, and PD to coordinate intercellular transport [9]. Physiological studies reinforced this model by showing that changes in the redox states of mitochondria or chloroplasts serve as upstream signals to rapidly alter PD transport [15]. All of these findings have been extensively discussed [16], and are summarized in Figure 2A; here, we focus on studies that lend further support to this model.

Research on viral pathogenesis often provides insights into PD function, since viruses pirate PD for their spread through plant tissues [1,17]. In a stunning convergence of discoveries across different fields, several studies revealed that chloroplast proteins can inhibit or enhance the spread of tobamoviruses, potentially by modifying PD function (Fig. 2C). *Tobacco mosaic virus* (TMV) spreads more rapidly in tobacco plants that have silenced expression of the plastid-localized ATP synthase gamma subunit (*AtpC*) or RuBisCO activase (*RCA*) [18]. On the other hand, silencing the RuBisCO small subunit (*RbcS*) delays the spread of *Tomato mosaic tobamovirus* (ToMV) [19]. These observed changes in pathogenesis could be related to several aspects of viral spread, such as altered viral replication. In light of the mounting evidence that chloroplasts coordinate PD transport, and that loss of ISE1 or ISE2 causes increased PD transport of tobamovirus movement proteins

in particular [14], future studies should also consider that disrupting chloroplast function can have downstream effects on PD transport, and that these changes in PD transport could be partially responsible for the observed abnormal viral spread.

Studies focused on PD proteins have further emphasized the relationship between chloroplasts and PD. Not only do chloroplasts regulate PD—changes in PD transport in turn disrupt chloroplast function (Fig. 2B). First, overexpression of PDL5 (a protein specifically localized at PD; see below), which decreases PD transport [20, 21], leads to chlorosis and overaccumulation of salicylic acid (SA), a phytohormone synthesized in plastids [22]. Second, transgenic expression of the TMV movement protein (P30), which predominantly localizes to PD and promotes viral transport, leads to increased H₂O₂ accumulation, overaccumulation of SA, and transcriptional induction of SA-response and chloroplast ROS-scavenging genes [23]. Older studies showed that transgenic P30 expression also decreases the rate of photosynthetic carbon assimilation [24]. In concert, these discoveries imply that chloroplasts are sensitive to alterations in PD function.

PD Regulate Plant Development: Stomata Formation

Multiple studies over the past few years have underscored the crucial involvement of PD in plant development. By governing movement of biological macromolecules that determine cell fate (such as transcription factors, see below), PD play a prominent role in tissue formation and patterning. Indeed, several screens for mutants defective in developmental processes have found weak mutant alleles of genes that affect PD transport.

In the plant epidermis, stomatal guard cell differentiation is initiated by a transcription factor, SPEECHLESS (SPCH) [25]. Genetic screens for defective stomatal complex formation found two mutants, *chorus* and *kobito1*, both with increased PD movement that allows SPCH to traffic out of a differentiating guard cell and initiate spurious guard cell formation [26,27].

chorus is a weak recessive allele of *GLUCAN SYNTHASE-LIKE 8 (GSL8)*, a gene that encodes a putative callose synthase [26,28,29]. Callose is a cell wall polysaccharide that restricts intercellular transport when deposited just outside PD (Fig. 1), and is also deposited at the cell plate during cytokinesis [29]. Plants with a strong loss-of-function allele of *gsl8* are defective in cell division and have an extremely disorganized epidermis, including aggregation of guard cells, possibly due to incomplete cytokinesis or due to changes in PD transport of developmental signals [28]. In *chorus* mutants, which do not show strong defects in cell division, callose does not accumulate to wild-type levels at PD, and accordingly, PD transport increases [26]. Moreover, whereas SPCH-YFP does not move from cell to cell in wild-type plants, SPCH-YFP readily diffuses via PD in *chorus* mutants [26]. These findings support the conclusion that callose-mediated restriction of PD transport is required for epidermal patterning.

Similar to *chorus*, plants with a recessive allele of *KOBITO1* form clusters of guard cells and have increased PD transport permitting diffusion of SPCH [27]. Unlike *chorus*, however, *kobito1* mutants do not show defects in callose deposition at PD, and may even overproduce callose. KOBITO1 is a putative glycosyltransferase-like protein of unknown function previously implicated in cellulose biosynthesis [30,31]. Mutants defective in cellulose biosynthesis do not show *kob1*-like phenotypes, however, suggesting that KOBITO1 functions in another, as yet uncharacterized pathway restricting PD movement.

PD Regulate Plant Development: Root Tissue Differentiation

A screen for *Arabidopsis* mutants with aberrant root development found that callose deposition at PD plays a significant regulatory role in root patterning [32]. A dominant

mutation (*cals3-d*) in *GSL12*, which like *GSL8* encodes a putative callose synthase, causes increased callose deposition specifically in the cell wall surrounding PD. This decreases PD transport and prevents intercellular movement of the SHORT-ROOT (SHR) transcription factor and a regulatory small RNA, miRNA156. This is a clear demonstration that SHR indeed transports between cells through PD, a final confirmation after more than a decade of research on the mode of SHR movement [33,34].

Callose-mediated regulation of PD transport is also controlled by enzymes that degrade callose at PD, thereby increasing PD transport [29]. A recent study found that misexpression of these enzymes, called PD-localized α -1,3 glucanases (PDBGs), impacts lateral root formation [35]. During lateral root primordia formation, PD transport in the primordia becomes more and more restricted as callose accumulates at PD. Correspondingly, loss of PDBG function—increasing levels of callose at PD—causes ectopic lateral root formation, and overexpression of PDBGs decreases lateral root formation [35]. Future work is needed to identify the mobile signals regulated by callose-mediated restriction of PD transport to promote lateral root formation. These signals may include transcription factors, small RNAs, or phytohormones (such as auxin, known to accumulate at sites of lateral root formation) [36].

Transcription Factors Frequently Traffic Through Plasmodesmata

Early work on leaf, root, and meristem development uncovered a handful of key transcription factors that move from cell to cell. Currently, transcription factor movement appears to be the rule rather than the exception. Several reviews have described advances in our knowledge of the movement of transcription factors, small RNAs, and viruses through PD [1,17,33,34,37,38]; here we briefly summarize a few notable new reports.

Transcription factors move via PD through one of two broad pathways: either the protein moves diffusively through PD, analogous to GFP transport [6,39], or movement is facilitated by another molecule (such as a PD protein) [33,34]. Some transcription factors contain small domains or even specific residues required for their intercellular transport, such as the “intercellular trafficking motif” found in Dof family transcription factors that promotes PD transport [40]. It remains unclear if this domain interacts with a PD protein that facilitates transport or promotes movement through another mechanism.

Generally, transcription factor movement in roots strongly correlates with protein size, suggesting that diffusion is the typical mechanism for transcription factor movement [41]. Since many transcription factors are small enough to freely diffuse through PD, and several families of larger transcription factors include domains that promote PD trafficking, we can conclude that PD-mediated transcription factor movement is widespread. Indeed, when 76 transcription factors were tagged with mCherry and ectopically expressed in the root cortex and endodermis, 22 trafficked through PD [41]. This probably considerably underestimates transcription factor movement, since tagging proteins with the fluorophore mCherry (29 kDa) likely restricts the movement of larger proteins. Extrapolating from the results of these reports [33,34,40–42], hundreds of the ~2,000 transcription factors in *Arabidopsis* likely traffic through PD.

Plasmodesmal Proteins Coordinate Plant Defense Responses

Early studies of PD using transmission electron microscopy recognized that PD contain electron-dense components likely representing PD proteins. Until recently, however, very few PD-localized proteins had been identified, and their functions at PD remained elusive. Following years of concerted efforts to isolate PD for proteomic analysis, Fernández-Calvino *et al.* published the first draft of an *Arabidopsis* PD proteome representing 1,260

genes [43]. This list likely includes many false positives (one-quarter of these proteins have predicted mitochondrial and/or plastid transit peptides), and the full array of PD proteins are not represented (PD were isolated from only one cell type, excluding PD proteins that may be expressed under different conditions). Nonetheless, this PD proteome is an excellent starting point to guide future efforts.

Table 1 lists newly identified PD proteins (for previously identified PD proteins, see [16]). Here we highlight a few recently discovered PD proteins that have clear implications in plant responses to pathogens. PD-LOCALIZED PROTEIN 5 (PDLP5) is one of an eight-member family of proteins with strong homology to PDLP1 [20,44,45]. Like PDLP1, PDLP5 is a membrane-anchored protein that contains two Gnk2-homologous domains with unknown molecular function. *PDLP5* was first identified as one of a small number of genes induced by infection with the plant pathogen *Pseudomonas syringae* [46,47]. PDLP5 overexpression causes decreased PD transport, likely by promoting callose deposition at PD in an SA-dependent pathway [20,21]. Native PDLP5 expression is associated with increased defense against *Pseudomonas syringae* and TMV, presumably due to increased production of ROS and SA in chloroplasts and decreased PD transport limiting TMV spread [48,49].

Another recently identified protein that preferentially localizes to PD, LYSIN MOTIF DOMAIN-CONTAINING GPI-ANCHORED PROTEIN (LYM2), is a receptor-like protein that triggers restriction of PD movement in response to chitin, a fungal pathogen-associated molecular pattern [50]. Knockout *lym2* mutants do not decrease PD transport in the presence of chitin, and they are indeed more susceptible to fungal pathogens. The discoveries of PDLP5 and LYM2, proteins that confer resistance to plant pathogens, emphasize the crucial role of PD in plant defense [51].

Unanswered Questions

These advances in our knowledge of PD biology raise several new questions. We now have very strong evidence that callose accumulation at PD is a major mechanism restricting PD movement, and that aberrant callose deposition can cause myriad developmental defects. The discovery of *dse1* demonstrates that other mechanisms can reduce PD transport, since *dse1* has restricted intercellular movement without callose deposition near PD. Future studies on the signaling pathway connecting DSE1 with PD function and formation may provide new insights into PD regulation.

Most efforts to understand the relationship between PD transport and plant development have focused on transcription factor and small RNA movement. Phytohormones, however, can also transport from cell to cell via PD, and likely move more rapidly via diffusion through PD than other pathways, such as import/export through transporters at the plasma membrane [52]. How, then, are phytohormone concentration gradients maintained when phytohormones can rapidly diffuse through tissues via PD? The growing set of genetic tools for modulating PD function should allow for more direct studies on the relationship between phytohormone signaling and PD transport.

We still know very little about how PD form after cell division (“secondary PD”) [16,53]. The cell wall needs to allow new PD to be inserted between neighboring cells in a process distinct from PD formation at the cell plate during division, but how this happens remains unclear. New techniques to rapidly quantify the number of PD connecting cells will greatly benefit attempts to identify conditions or genetic alterations that impact PD formation [54]. The increased formation of secondary PD in leaves with silenced *ISE1* or *ISE2* expression may present a model system for future analyses [14,16]. Further studies of the putative cell wall enzyme KOBITO1 (*kobito1* has increased PD transport, see above) and other cell wall modifying proteins on PD formation may provide answers.

Lastly, it is now clear that PD function and formation are tightly integrated with cellular physiology, with a particularly essential connection between PD and chloroplasts. Chloroplasts regulate PD function, as shown in several mutants and by physiological studies. Moreover, PD regulate chloroplast function, as demonstrated by the multiple effects on chloroplasts caused by altering expression of PD-localized proteins (P30 and PDLP5). Future PD research will continue to better characterize the mechanisms underlying these regulatory pathways that are central to plant development, defense, and vitality.

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cause pleiotropic phenotypes: *pdglp1* mutants have waxy stems lacking trichomes and decreased seed production [59], while *35S::PDGLP* plants have shortened primary roots, lengthened lateral roots, and disorganized root meristems.

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HIGHLIGHTS

- Many proteins important for plasmodesmal transport do not localize to plasmodesmata.
- Signals from chloroplasts regulate plasmodesmata function and formation
- Changes in plasmodesmal transport disrupt chloroplast function.
- Hundreds of plant transcription factors likely move cell-to-cell via plasmodesmata.
- Plasmodesmata play a prominent role in plant development and defense.

Figure 1.

Scaled cartoon model of a plasmodesma, following Tilsner *et al.* [55]. Plasmodesmata are plasma membrane-lined channels that cross the cell wall, containing cytosol and a central, tightly compressed strand of endoplasmic reticulum called the desmotubule (purple). The cytosol is continuous between adjacent cells, allowing small and large molecules to move from one cell to the next. Here, GFP (green) is moving via diffusion through PD. Callose deposition bordering PD (light blue areas) negatively regulates PD transport, and is mediated by callose synthases (blue hexagons, left). Degradation of callose bordering PD positively regulates PD transport, and is mediated by β -1,3-glucanases (dark blue hexagons with membrane-spanning tails, right). Some PD proteins affect PD transport through unclear mechanisms, such as PDLP5 (purple circles with membrane-spanning tails, left), which here is shown inhibiting GFP transport, possibly by promoting local callose synthesis.

Figure 2.

(A) Plasmodesmata function and formation are coordinated by changes in chloroplast (green) and mitochondrial (red) function. These changes alter expression of nuclear genes (blue) through organelle retrograde signaling pathways (black arrows). Loss of organelle RNA helicases (ISE1 or ISE2) causes dramatic changes in nuclear gene expression and increases PD transport and biogenesis. ROS produced specifically in the mitochondria also positively regulates PD function. On the other hand, superoxide formation in the chloroplast or loss of either GAT1 or Sxd1 [56,57] (which also leads to oxidation of the chloroplast redox state) negatively regulate PD function. (B) PD function regulates chloroplast physiology: PDLP5 or P30 expression cause increased ROS formation, SA biosynthesis, and decreased photosynthesis. (C) Various chloroplast proteins also impact tobamoviral spread, possibly through effects on PD function.

Table 1

Newly Identified Plasmodesmal Localized Proteins *

Protein Name	Protein Function	Reference
GLUCAN SYNTHASE-LIKE 12 (GSL12) *	Putative callose synthase, negatively regulates PD transport	[29,32]
LYSIN MOTIF DOMAIN-CONTAINING GPI-ANCHORED PROTEIN 2 (LYM2) *	Receptor-like protein, negatively regulates PD transport in response to chitin	[45]
PD GERMIN-LIKE PROTEIN 1, 2 (PDGLP1,2)	Positively regulate PD transport	[58]
NETWORKED 1A (NET1A)	Actin-binding protein	[60]
CLAVATA1 (CLV1)	Receptor-like kinase, regulates stem cell identity, forms multimeric complexes with ARABIDOPSIS CRINKLY 4 (ACR4) at PD	[61]
-1,6-N-ACETYLGLUCOSAMINYL TRANSFERASE-LIKE ENZYME (GnTL)	Glycosyltransferase-like protein	[62]

* See text for more details on select proteins.