Plasmodesmata-associated proteins
Can we see the whole elephant?

Shoko Ueki1,* and Vitaly Citovsky2
1Institute of Plant Science and Resources; Okayama University; Chuo, Kurashiki, Okayama Japan;
2Department of Biochemistry and Cell Biology; State University of New York; Stony Brook, NY USA

**Keywords:** Plasmodesmata, size exclusion limit, symplastic transport, callose, reactive oxygen species

**Abbreviations:** Pd, plasmodesmata; SEL, size exclusion limit; TMV, Tobacco mosaic virus; CMV, Cucumber mosaic virus; GSL, glucan synthase-like; BG, β glucanase; PDCB, plasmodesma callose binding proteins; PDLP, plasmodesma-located proteins; C1RGP, class 1 reversibly glycosylated polypeptides; GnTL, glucosaminyltransferase-like; ROS, reactive oxygen species; TRX-m3, thoredoxin-m3; ISE, increased size exclusion

Encased in rigid cell walls, plant cells have evolved unique channel structures, plasmodesma (Pd), to create a pathway for molecular exchange between adjacent cells. Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

**Overview of Pd structure and composition**

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.

Pd have been implicated in transport of diverse macromolecules, including nucleic acids and proteins, both endogenous and pathogen-derived, and regulation of this Pd-mediated intercellular transport represents an important aspect of plant physiology and development. Recent studies have identified several Pd-associated proteins involved in Pd transport. Furthermore, emerging evidence indicates that even factors that reside in other cellular organelles, such as mitochondria and chloroplasts, can play roles in regulation of traffic through Pd channels. In this minireview, we summarize the current knowledge regarding proteins that associate with Pd and other cellular organelles and likely participate in regulation of Pd transport.
Table 1. Summary of major Pd-associated proteins involved in regulation of Pd permeability.

<table>
<thead>
<tr>
<th>Name</th>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdBG1</td>
<td>23</td>
<td>PdBG1 and PdBG2 are involved in development of lateral root primordia. The biological function of PdBG3 is still unknown.</td>
</tr>
<tr>
<td>PdBG2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PdBG3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BG_pap</td>
<td>24</td>
<td>Loss of function leads to high constitutive accumulation of callose at Pd due to reduced callose degradation, and limited Pd-mediated diffusion of cytoplasmic GFP.</td>
</tr>
<tr>
<td>GSL2/CalS3</td>
<td>22</td>
<td>Involved in regulation of root development by controlling callose levels at Pd.</td>
</tr>
<tr>
<td>PDCBs</td>
<td>25</td>
<td>Contain GPI-anchor motif with X-8 domain. Bind callose in vitro, likely bind and stabilize callose in vivo.</td>
</tr>
<tr>
<td>(^{14}C)RGP1</td>
<td>31, 32</td>
<td>Overexpression increases callose accumulation in source leaves and restricts viral spread.</td>
</tr>
<tr>
<td>PDLPs</td>
<td>26, 30</td>
<td>Type I transmembrane proteins. Negatively regulate Pd-mediated macromolecular diffusion. Serve as anchoring points for viral MP-containing tubule formation.</td>
</tr>
<tr>
<td>PDLP5</td>
<td>28, 29</td>
<td>Also known as HWI1. Expression levels positively correlate with callose accumulation.</td>
</tr>
<tr>
<td>GnTL</td>
<td>37</td>
<td>Interacts with calreticulin, most likely localizes at Pd orifice.</td>
</tr>
<tr>
<td>Remorin</td>
<td>33</td>
<td>Associates with membrane raft-like structures. Expression levels negatively correlate with local and systemic movement of PVX.</td>
</tr>
</tbody>
</table>

these proteins in regulation of Pd-mediated trafficking remain to be discovered.

Pd-associated proteins: gate-keepers for the channel

Although proteomic approaches have identified a relatively large number of potential Pd components, only few Pd-associated proteins have been experimentally shown to participate in or regulate Pd transport (Table 1). One of the best known mechanisms to regulate Pd permeability is through callose deposits, which—with some specific exceptions, such as wounded cells, sieve pores in phloem, and pollen tubes—are found specifically at Pd. Thus, it is not surprising that proteins involved in callose metabolism also localize to Pd and are often involved in Pd transport. Callose accumulation levels are directly determined by 3 classes of cellular factors: callose synthases that produce callose, 1,3-β-D-glucanases that degrade callose, and callose retaining proteins that associate with callose to stabilize it.

To date, 12 putative callose synthases (CalS, or more often termed glucan-synthase-like, GSL, proteins) have been identified in Arabidopsis based on sequence homology. Among them, GSL7,18-20 GSL8,21 and GSL1222 have been demonstrated to be involved in regulation of callose at Pd, while the actual Pd localization was only demonstrated for GSL12.22

On the other hand, the Arabidopsis family of callose-hydrolyzing enzymes, 1,3-β-D-glucanases, comprises more than 50 members, and some of them, including PdBG1/2/3,23 have been shown to target to Pd. In addition, another Pd-associating 1,3-β-D-glucanase with a GPI anchor, BG_pap, was shown to be involved in Pd regulation;24 its loss leads to high constitutive accumulation of callose at Pd, due to reduced degradation, and restricts Pd-mediated intercellular diffusion of cytoplasmic GFP.24

Besides callose-hydrolyzing enzymes, other Pd-targeted proteins are linked to callose regulation. For example, a group of proteins with a GPI-anchor, which bind to and likely stabilize callose, have been demonstrated to be targeted to Pd, and, therefore, were designated Pd callose binding proteins (PDCBs).25 Pd-located proteins (PDLPs) represent another example of Pd-associated cellular factors;26 these belong to the family of type I transmembrane proteins with the transmembrane domain proposed to contain a Pd targeting sequence. Among 11 Arabidopsis homologs identified based on the protein sequence similarity, 8 were shown to localize at Pd. Presumably, because of the redundant expression of several PDLPs in leaf tissues,26,27 single mutants in individual PDLPs did not affect symplastic transport. On the other hand, GFP cell-to-cell diffusion in leaf tissue was markedly increased in double knockout lines that carried deletions in 2 of the 3 PDLPs which are predominantly expressed in the wild type leaves, suggesting that at least some, if not all, PDLPs may act as negative regulators for macromolecular diffusion through Pd. This notion is also supported by the observation that overexpression of PDLP1 leads to a striking decrease in GFP diffusion via Pd. A later study provided additional insights into the PDLP action, showing that overexpression of one of leaf PDLPs, PDLP5 (also known as HWI128), leads to callose accumulation at Pd, while its suppression reduces Pd callose levels, suggesting that PDLPs function by elevating callose deposits, which, in turn, restrict macromolecular diffusion through Pd.29 Interestingly, PDLPs also play a crucial role in movement of Grape fan leaf virus (GFLV) and Cauliflower mosaic virus (CaMV).30 MPs of these viruses form tubular structures that span Pd, and all 8 PDLPs interact with GFLV MP.30 Thus, these viruses may utilize PDLPs as anchor points for initiation of the tubule assembly, while inhibiting the activity of PDLPs as callose upregulators, to facilitate tubule formation through the channel.

Class I reversibly glycosylated polypeptides (C1RGP) are yet another type of Pd-targeted proteins that are delivered to the plasma membrane of Pd through the ER/Golgi pathway.31 In C1RGP2 overexpressing tobacco plants, callose accumulation is increased in source leaves while the flow of photoassimilates and viral spread are impaired.32 These observations implicate C1RGP in callose regulation, and, in turn, in Pd-mediated macromolecular traffic between cells. The molecular mechanism by which C1RGP2 achieves these effects, however, remains unknown.

Another protein that resides in the plasma membrane in close proximity to Pd is remorin.33 Remorin molecules cluster in a specific membrane domain, similar to lipid rafts, within the cytosolic leaflet of the plasma membrane.33 Whether this
protein participates in regulation of Pd is still unclear; however, its expression levels negatively correlate with cell-to-cell movement of Potato virus X (PVX) and with phloem unloading in the inoculated leaves, suggesting involvement of remorin in the process of viral movement between cells.33

Several other membrane-associated proteins have been found to target to Pd, but their functions in Pd are still obscure. For example, calreticulin, an ER resident protein, has been shown to accumulate at Pd,34 most likely within the trans-Pd ER. In the cellular ER, calreticulin is involved mainly in Ca2+ sequestration and signaling and in chaperone activity,35 whereas the role that calreticulin might play at Pd awaits further studies. Interestingly and consistent with its Pd accumulation, calreticulin was shown to interact with TMV MP,36 suggesting that it may be involved in viral movement through Pd. Recent data indicate that, in Arabidopsis, calreticulin also interacts with a glucosaminyl transferase-like (GnTL), which itself associates with Pd.37 Interestingly, GnTL colocalized with PDCB, but not with TMV MP. Within Pd, TMV MP often accumulates in the central cavity38 whereas PDCB colocalizes with callose at the neck, sphincter region of Pd,35 suggesting that GnTL also localizes at Pd collar region, for as yet unknown function.

**Non-Pd regulators of pd**

Traditionally, studies of Pd regulation have focused on factors associated with these channels or located in their vicinity. Defying this apparent logic, several factors that associate with cell organelles distant from Pd have been suggested to participate in regulation of Pd transport.99-102 One of such non-Pd factors is a chloroplastic protein thioredoxin-m3 (TRX-m3) that may participate in modulation of callose depositions by regulating intracellular levels of reactive oxygen species (ROS).103 A loss of function mutation in the TRX-m3 gene, originally designated gat1 (gat1), leads to reduced diffusion of GFP through Pd from the companion cells in the root phloem; the mutant lines accumulate high levels of ROS, and, importantly, exhibit higher levels of callose in their root tips, indicating a functional link between ROS and callose at Pd.104

The effect of ROS on Pd permeability is complex because, in another instance, higher ROS levels in fact increase the Pd-mediated macromolecular diffusion. Specifically, mutations in the Arabidopsis DEAD-box RNA helicase INCREASED SIZE EXCLUSION (ISE1) gene elevate ROS levels and increased Pd permeability in embryos at the mid-torpedo stage.105,106 ISE1 is a mitochondrial protein, and silencing its expression results in more oxidized mitochondria, but more reduced plastids, as determined using redox-sensitive GFP reporter targeted to the mitochondria or plastids.107 ISE2, later identified as a putative DEVH-box RNA helicase, is another example of a non-Pd protein that modulates Pd permeability.108 ISE2 is a chloroplast protein,109 and similar to ISE1-silenced tissues, silencing of ISE2 resulted in more reduced plastids and elevated Pd permeability.110 Furthermore, pharmacological studies revealed that, when oxidative shift in plastids was induced, Pd permeability was also reduced even when the same treatment induced oxidation of mitochondria, suggesting that oxidative shift in plastids overrides the positive effect of oxidized mitochondria on Pd permeability. Collectively, these studies indicated that oxidation status of plastids and mitochondria and their crosstalk affect Pd permeability, potentially acting as regulators of intercellular transport. One of the possible mechanisms of this regulatory effect could be via structural modification of Pd due to the altered redox status during Pd development. Increased diffusion through Pd in the ise1 or ise2 mid-torpedo embryos was accompanied by increased incidence of morphologically altered Pd, specifically, winned and branched channels.111-113 Transcriptome analysis revealed that expression levels of callose metabolism-related genes are altered in these mutants, however, these changes did not parallel changes in Pd permeability; thus, ISE1 and ISE2 may exert their regulatory effects on Pd permeability by a callose sphincter-independent mechanism. However, the same study revealed that expression of many other genes belonging to different functional categories was also altered in the mutant lines, suggesting that the mutations in these genes significantly affected the plant homeostasis. Thus, the precise mechanism of regulation of Pd by ISE1 and ISE2 remains undefined.

**Concluding Remarks**

Identification of Pd-associated proteins has always been one of the major stumbling blocks to the studies of Pd function and regulation. Development of sensitive proteomic approaches along with refined Pd fractionation techniques allowed identification of increasing numbers of Pd-associated factors.13,14 Many of them are annotated as membrane-associated proteins, including receptor-like proteins. In many cases, however, these breakthroughs in Pd protein discovery have not been followed by equally successful insights into the detailed functions of the discovered factors within Pd yet. From those factors the function of which is known, most act in the callose-mediated Pd regulation pathway. In contrast, the protein machinery of the actin-mediated Pd regulatory pathway, the second major regulatory mechanism of Pd transport, is virtually unknown; this pathway will likely represent a focus for future studies, especially since the highly dynamic nature of actin microfilaments makes it a good candidate for fine tuning of and quick changes in Pd permeability.

In addition to these Pd-associated regulatory machineries, several lines of evidence demonstrated the existence of a non-Pd system, originating from plastids and mitochondria, that regulates Pd permeability. Because organized cell-to-cell communication is essential to orchestrate the whole-body physiology of higher plants, the existence of complex crosstalk regulatory pathways for Pd permeability to respond to environmental and developmental stimuli makes biological sense. Thus, most likely we have not yet discovered the entire complement of Pd components, structural or functional. Identification of these components and understanding their activity will represent the focus of the future research.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
The V.C. group is supported by grants from USDA/NIFA, NIH, NSF, BARD, and BSF. The S.U. group is supported by grants from Nagase Science and Technology Foundation, Casio Science Promotion Foundation, and Ohara Foundation for Agricultural Research.

References


7. Ueki S, Citovski V. To gate, or not to gate: regulatory mechanisms for intercellular protein transport and virus movement in plants. Mol Plant 2011; 4:782-93; PMID:21764703; http://dx.doi.org/10.1093/mp/sss060


43. Stonebloom S, Burch-Smith T, Kim I, Meinke D, Mindrinos M, Zambryski P. Loss of the plant DEAD-box protein ISE1 leads to defective mitochondria and increased cell-to-cell transport via plasmodesmata. Proc Natl Acad Sci U S A 2009; 106:17229-34; PMID:19805190; http://dx.doi.org/10.1073/pnas.0909229106


