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Review

Plasmodesmata and their role in assimilate translocation

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A B S T R A C T

During multicellularization, plants evolved unique cell-cell connections, the plasmodesmata (PD). PD of angiosperms are complex cellular domains, embedded in the cell wall and consisting of multiple membranes and a large number of proteins. From the beginning, it had been assumed that PD provide passage for a wide range of molecules, from ions to metabolites and hormones, to RNAs and even proteins. In the context of assimilate allocation, it has been hypothesized that sucrose produced in mesophyll cells is transported via PD from cell to cell down a concentration gradient towards the phloem. Entry into the sieve element companion cell complex (SECCC) is then mediated on three potential routes, depending on the species and conditions, – either via diffusion across PD, after conversion to raffinose via PD using a polymer trap mechanism, or via a set of transporters which secrete sucrose from one cell and secondary active uptake into the SECCC. Multiple loading mechanisms can likely coexist. We here review the current knowledge regarding photoassimilate transport across PD between cells as a prerequisite for translocation from leaves to recipient organs, in particular roots and developing seeds. We summarize the state-of-the-art in protein composition, structure, transport mechanism and regulation of PD to apprehend their functions in carbohydrate allocation. Since many aspects of PD biology remain elusive, we highlight areas that require new approaches and technologies to advance our understanding of these enigmatic and important cell-cell connections.

1. Introduction

Assimilates produced in photosynthetically active source organs have to be delivered to supply sink organs that cannot produce their own. Translocation of sugar and organic nitrogen, as well as many other compounds, likely occurs on two routes: (i) transporter-mediated export from one cell, followed by diffusion across the cell wall and subsequent uptake into adjacent cells, or (ii) via cell-cell bridges, the so-called plasmodesmata (PD). During the evolution of multicellular organisms, all kingdoms developed unique cellular connections that serve as efficient pathways of exchanging nutrients and signals. Some fungi have septal pores, animals use gap junctions, and plants evolved PD. Gap junctions are made up of connexin (or pannexin or innexin) oligomers in the membranes of adjacent cells that connect two cells to produce gated channels that allow passage of ions and metabolites with a size exclusion limit (SEL) of 16–20 Å (Skerrett and Williams, 2017). In comparison, PD are far more complex and have to span the cell wall (typically 0.1–2 μm width). PD of early bryophytes and tracheophytes consist of cell walls, multiple membranes (plasma membrane and endoplasmic reticulum) separated by a cytosolic sleeve, and likely hundreds of proteins. PD are thought to mediate the translocation of ions, metabolites, RNAs and proteins; therefore, it has been hypothesized that plants function

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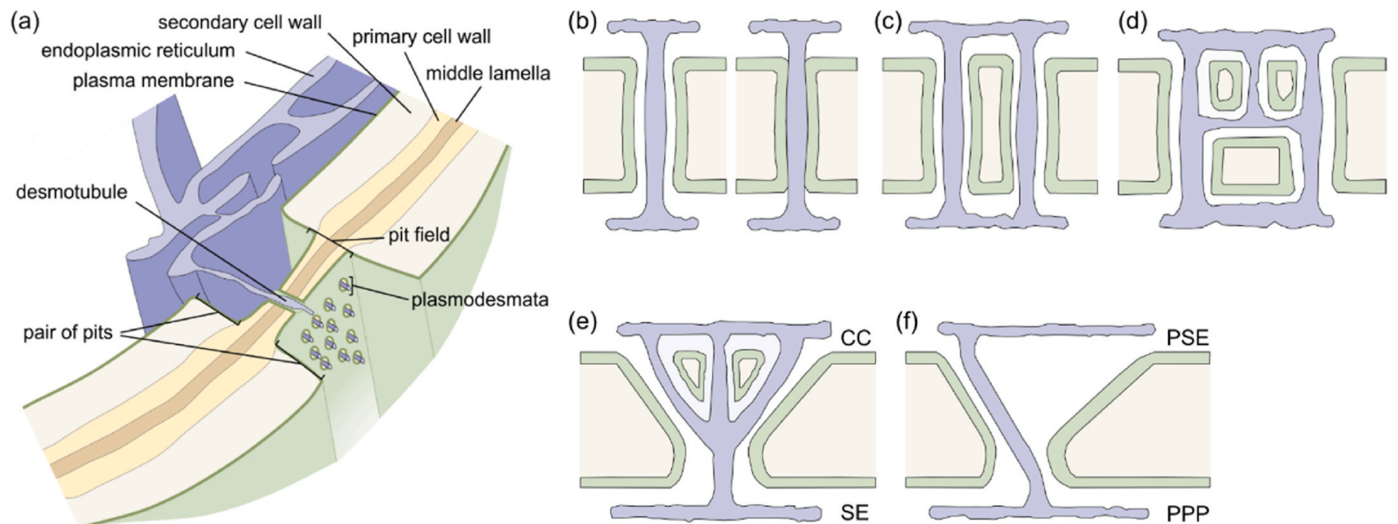

Fig. 1. Diversity of PD organization and morphology. (a) Plasmodesmata clustered into pit fields. PD can be found isolated or grouped in thinner areas of the primary cell wall that are called pit fields. In cells producing secondary wall layers (*e.g.*, sclerenchyma cells), poor cellulose microfibril deposition specifically at both sides of pit fields, might lead to the emergence of depressed area called pits, and primary cell wall located at pit fields might evolve into pit membranes. (b) Simple PD with single cytoplasmic strand (left) and simple PD lacking cytoplasmic sleeves (right); (c) Twinned PD consisting of two closely paired simple PD; (d) Branched PD containing a central cavity; (e) PD pore unit formed between sieve elements and companion cells; (f) Funnel PD formed between proto-sieve element and phloem pole pericycle cell in the root.

essentially as a large synthicium.

2. Types of PD and cell specific roles

Ontogenetically, PD can be classified into primary and secondary PD. Primary PD are produced in the cell plate during cell division, trapping the endoplasmic reticulum (ER) to form the desmotubule (Hepler, 1982). These primary PD develop between mother and daughter cells. Secondary PD are generated *de novo* in existing cell walls requiring cell wall-degrading enzymes and insertion of the endoplasmic reticulum (ER) (Ehlers and Kollmann, 2001). In grafting experiments, it was shown that secondary PD formation involves thinning of the cell wall and tethering of ER and the plasma membrane (PM) (Chambaud et al., 2021). Secondary PD are often generated in close proximity to existing PD, resulting in clusters of PD, called pit fields, located in thinner areas of the primary cell wall (Fig. 1a) (Carr, 1976; Faulkner, 2018). In cells producing secondary wall layers, the primary cell wall located at pit fields may then be converted into pit membranes separated by a pair of pits (Robards, 1976; Peters et al., 2021).

PD are complex and diverse, with morphotypes ranging from unbranched sleeveless PD lacking a sleeve between ER and PM, unbranched simple PD, characterized by a single channel, to complex V-, Y-, X- or H-shaped PD, with branched and twinned structures (Fig. 1b–f). Unique PD types are specifically localized at interfaces between different cell types. PD morphology may be modified during development to acclimate to new requirements. In young and immature organs in embryos, sink leaves, and root meristems, PD are predominantly of the simple type with high basal SEL, allowing for diffusion of large molecules (Oparka et al., 1999). In contrast, branched PD of mature organs such as source leaves often have a decreased SEL (Oparka et al., 1999). However, PD type and SEL do not always correlate; *e.g.*, *ise1* mutants, which have an increased number of branched and twinned PD compared to wild-type, were characterized by a higher SEL (Stonebloom et al., 2009). Notably, PD are not static and exhibit dynamic changes in structure and function: in tobacco (*Nicotiana benthamiana*) and squash (*Cucurbita pepo*) leaves, many simple PD are replaced by complex forms during the sink-source transition indicating the need for altered conductivity and regulation in the 'source' state (Volk et al., 1996; Roberts et al., 2001). Moreover, specific cell types are characterized by unique PD morphologies (Fig. 1b–f), for example, plasmodesmata-pore units (PPU) are specialized PD that connect companion cells (CC) with sieve elements (SE). On the CC-side, PD become highly branched, with a cavity that connects to a wider aperture on the SE side. This asymmetry may imply asymmetry in the permeability of the PD, and, together with the larger size exclusion limit (SEL) of ~70 kDa, likely plays a role in the need of sustaining the enucleate SE by the CC and assimilate translocation (van Bel and Knoblauch, 2000). In roots, funnel-shaped PD are located at the interface between protophloem sieve elements (PSE) and phloem-pole pericycle cells (PPP), with wider apertures at the PSE entrance that are thought to mediate bulk flow for unloading (Ross-Elliott et al., 2017) (Fig. 1f). Sugars and other small solutes may move relatively freely through funnel PD, while macromolecules appear to pass through these pores in discrete pulses, termed batch unloading (Ross-Elliott et al., 2017) (see also section *Contribution of PD to unloading of the phloem*). Recent technical developments in electron tomography have enabled the generation of three-dimensional models of the PD structure in the Arabidopsis (*Arabidopsis thaliana*) root tip columella (Nicolas et al., 2017). Ultrastructural analyses revealed that, while the outermost cell layers of the columella had canonical PD structures (PD type II), mitotically active columella cell initials at the upper layer featured PD without a cytoplasmic sleeve (PD type I) (Nicolas et al., 2017). Remarkably, sleeveless PD type I can traffic synthetic dyes such as carboxyfluorescein diacetate (CFDA) as well as GFP, putting models that predict diffusion of molecules through the cytoplasmic sleeve into question. More recent models that could account for geometric fluctuations in PD shape predicted that particles larger than the diameter of the cytoplasmic sleeve can permeate through the PD pore (Christensen et al., 2021). Of note, calculations of symplasmic permeabilities based on geometrical descriptions of PD suggest that the permeation efficiency does not only depend on the diameter of the cytoplasmic sleeve but also on the length of the PD (and thus cell wall thickness) (Deinum et al., 2019). Larger cytoplasmic sleeves were predicted to be more favorable for permeation efficiency through PD in thicker cell walls (200 nm) (Deinum et al., 2019). This hypothesis is in line with the observations in Arabidopsis columella, where PD type II with a larger cytosolic sleeve mostly appeared in thick cell walls (average 200 nm), and PD type I in thinner cell walls (average 100 nm) (Nicolas et al., 2017).

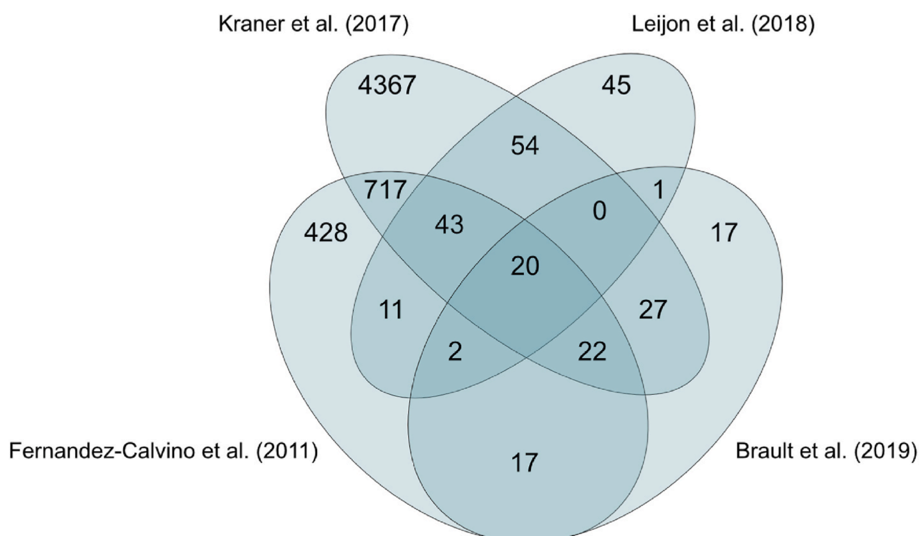

Fig. 2. PD proteome comparison. Venn diagram displaying the number of proteins overlapping between four previously published PD proteomes (Fernandez-Calvino et al., 2011; Kraner et al., 2017b; Leijon et al., 2018; Brault et al., 2019). Twenty core proteins common to all four proteomes were identified. For PD proteins identified from *P. trichocarpa* (Leijon et al., 2018), accessions for the closest Arabidopsis homologs were retrieved before PD proteome comparison. Protein list is available at www.molecular-physiology.hhu.de/summary-of-the-resources/core-plasmodesmatal-proteome.

3. PD protein composition

As PD are embedded in the cell wall, composed of multiple membranes, soluble and membrane proteins, they pose a major challenge in purification for compositional analyses. To date, putative PD proteomes have been established by three principal approaches: by chance through imaging of fluorescently tagged proteins, by proteomics-based approaches using cell wall fractions enriched for PD, and comparative analysis of the proteomes of mutants with reduced PD number (Fernandez-Calvino et al., 2011; Kraner et al., 2017b; Park et al., 2017; Leijon et al., 2018; Brault et al., 2019). Published PD proteomes include 1341 candidate proteins from Arabidopsis, 1113 from *Populus trichocarpa*, and 1070 from tobacco (Fernandez-Calvino et al., 2011; Park et al., 2017; Leijon et al., 2018). However, cell wall fractions are contaminated with non-PD proteins, *e.g.*, derived from ER and plasma membrane. Thus, it is necessary to assess in each case whether the candidates localize to PD. One typical approach is to generate fluorescent protein (FP) fusions and determine the localization by confocal microscopy. Localization studies are often performed by transiently expressing FP fusions driven by strong promoters in heterologous systems such as *N. benthamiana*. However, this experimental system is prone to artefacts as ectopic overexpression can lead to mistargeting or accumulation in the ER and Golgi. Generation of stable transformants with native promoters and all introns followed by analysis in the native cell types is more challenging and time-consuming, but likely essential for reliable validation. Notably, many PD proteins are not specific to PD but localize also to other sites such as PM, ER, etc. In an attempt to obtain a set of candidates, a refined dataset called 'core PD proteome' with 115 candidates from Arabidopsis was established (Brault et al., 2019). This 'core proteome' contains *bona fide* PD components, and does not mean that many of these candidates are PD-associated. Of note, only 22 proteins from the 'core PD proteome' have been validated by localization of FP fusions; moreover, this 'core PD proteome' lacks some of the well-characterized PD receptor-like kinases such as BAM1, CLAVATA1 and CRINKLY4 (Stahl et al., 2013; Rosas-Diaz et al., 2018). To compile a list of putatively PD localized proteins that are commonly found in PD preparations, we compared published PD proteomes and identified twenty common candidates in the four PD proteomes (Fernandez-Calvino et al., 2011; Kraner et al., 2017b; Leijon et al., 2018; Brault et al., 2019) (Fig. 2). Among the 20 candidates were three previously described PD proteins: CALLOSE SYNTHASE 3 (CALS3; AT5G13000), INFLORESCENCE MERISTEM RECEPTOR-LIKE KINASE 2 (IMK2; AT3G51740), MULTIPLE C2 DOMAINS AND TRANSMEMBRANE REGION PROTEIN 3 (MCTP3; AT3G57880). CALS3 co-localized with the RxLR3 effector of *Phytophthora brassicae,* PLAMODESMATA-LOCATED PROTEIN 5 (PDLP5) and callose (Tomczynska et al., 2020). IMK2, present at the plasma membrane, was shown to re-localize to PD in response to osmotic stress (Grison et al., 2019). MCTP3 was shown to be present at PD and interacted with SHOOTMERISTEMLESS (STM), a class I KNOTTED1 (KN1)-like homeobox (KNOX) protein. STM is presumably capable of trafficking through PD (Balkunde et al., 2017; Liu et al., 2018; Brault et al., 2019). Two members of the Tetraspanin family (AT2G23810, AT3G45600), two nucleobase ascorbate transporters (AT2G26510, AT2G27810), and PDLP6 (AT2G01660) were also part of the 'core PD proteome'. An additional twelve proteins were encountered in all four proteomes and have not been described as PD proteins so far (Supplemental Table S1). Notably, this list is not comprehensive, and proteins found in one species but not another may still be *bona fide* PD proteins. Systematic analysis of the localization of all candidates and new technologies such as proximity labeling may help researcher to build an even more comprehensive PD-type-specific parts list and a blueprint.

Different cell types are characterized by unique PD morphologies (*e.g.*, PPU connecting CC-SE). However, little is known about unique composition of distinct PD (Fig. 1). A well-characterized cell-type-specific PD component is MCTP1, which localizes to PD at the CC-SE interface (Liu et al., 2012). MCTP1 interacts with the florigen protein FLOWERING LOCUS T (FT) and is required for the trafficking of FT (Liu et al., 2012; Song et al., 2017). Consistent with these data, a recently published single-cell transcriptome of Arabidopsis leaves showed that *MCTP1* transcripts were specifically enriched in CC (Kim et al., 2021). Among members of the PDLP family, PDLP8 was shown to interact with the PD-located phloem-specific acyl-CoA-binding protein AtACBP6, which might mediate the transport of AtACBP6 from CC to SE (Ye et al., 2017). Additionally, PDLP6-GFP fusions driven by its native promoter, localized in the vasculature of leaves and roots (Li and Aung, 2021, BioRxiv). Overexpression of PDLP6 triggered callose deposition in the vasculature, as well as accumulation of starch granules in the bundle sheath (BS), which might be the result of restrictions of PD permeability between BS and PP (Li and Aung, 2021, BioRxiv). PDLP5 overexpression promoted callose deposition in PD between mesophyll cells. These results intimate a cell-type specific regulation based on the PD composition. In rice (*Oryza sativa*), the remorin protein GRAIN SETTING DEFECT1 (OsGSD1) specifically localized to the plasma membrane and PD in CC. OsGSD1 overexpression led to compromised grain setting and assimilate retention in leaves, possibly caused by reduced PD permeability in CC due to increased callose deposition. Additionally split-FP experiments and coimmunoprecipitation assays provided evidence

that OsGSD1 interacts with the desmotubule associated OsACT1, a protein that may play a role in regulating the CC PD-aperture (Gui et al., 2014). With the increasing number of identified PD proteins and single-cell transcriptome data, it will be possible to identify both proteins common to all PD as well as additional proteins specific for unique PD types (Denyer et al., 2019; Kim et al., 2021; Seyfferth et al., 2021).

4. PD transport mechanisms

Perspectives on the role of PD from Tangl, Pfeffer and Strasburger, to name some of the most prominent scientists who studied PD in the late 18th century, were based on the hypothesis that the 'holes' observed in the cell walls would enable cellular exchange of molecules (Tangl, 1879; von Hanstein, 1880; Strasburger, 1901). This 'hole-model' still dominates the current view. PD-mediated cell-to-cell movement of ions and other molecules is assumed to occur through the cytosolic sleeve of PD and is driven by diffusion, pressure differences between neighboring cells, and/or bulk flow. If movement occurs, it is not clear whether the channels allow free diffusion. Movement was found to be limited by molecule size, an SEL specific to different types of PD. The hypothesis that movement is subject to a SEL is affirmed by tracer movement studies (Goodwin et al., 1990; Barton et al., 2011; Rutschow et al., 2011; Liesche and Schulz, 2012a; Gerlitz et al., 2018; Wang et al., 2020). The SEL was shown to be cell-type specific but also depended on the physiological and developmental states of the tissue (Erwee and Goodwin, 1985; Wolf et al., 1989; Oparka et al., 1999) (see also section *Regulation of PD conductance*). SEL appears to be dynamic, with adaptive capabilities to acclimate to altered flux requirements, for example under stress conditions (Rutschow et al., 2011; O'Lexy et al., 2018). Differences in SEL have been attributed to differences in PD geometry (Deinum et al., 2019; Ostermeyer et al., 2021, BioRxiv).

Alternative models for PD transport assume that SEL and, thus, PD conductivity are more complex and might also depend on other features, in addition to PD geometry. SEL is determined by the Stokes radius of molecules, the flexibility of molecules, the folding state of RNAs and proteins; moreover, it is conceivable that molecules that pass require chaperones or carriers to enable permeation. In the nanometer-sized PD pores, negative electrostatic charges of the plasma membrane throughout the cytosolic sleeve might enable faster permeation for cations and positively charged molecules relative to anions and negatively charged molecules (Peters et al., 2021). Direct quantitative transport studies will be needed to assess permeation rates of differently charged ions to clarify the impact of electrostatic charges on the permeation of ions and molecules.

Some evidence implicated different mechanisms for cell-to-cell movement of small (<1 kDa) and large molecules (>27 kDa). During phloem unloading in Arabidopsis, small molecules (0.34–0.38 Da) permeated from SE into PPP at constant rates, whereas large molecules (27–112 kDa) permeated in discrete pulses, termed 'batch unloading' (Ross-Elliott et al., 2017). Batch unloading of large molecules might depend on thermal motion of structural PD components such as the desmotubule or the spokes (Peters et al., 2021). Peters et al. (2021) recently hypothesized that the desmotubule could be displaced from the center of the pore, thereby creating asymmetry which allows larger molecules to pass. While small molecules would permeate unhindered, independently of the position of the desmotubule, large molecules would be able to permeate only when the desmotubule is in the periphery of the PD. Once having entered the pore, large molecules could lock PD in a wide-open state, thus being cargo-gated (Christensen et al., 2021; Peters et al., 2021).

Specific mechanisms for targeting PD components and cargo proteins to PD must exist. One might speculate that N-terminal signal sequences are required, similar as for nuclear or organellar import (Kim and Hwang, 2013). Indeed, some proteins contain N-terminal sequences that are likely involved in PD targeting. The first 50 amino acids of TMV MP, a signal peptide of PDGLP1, and a zinc finger binding motif with the N-terminal part of the nuclear localization sequence (NLS) of INTERCELLULAR TRAFFICKING DOF1 (ITD), were found to be necessary and sufficient for PD targeting of TMV MP, PDGLP1, and ITD, respectively (Ham et al., 2012; Chen et al., 2013; Yuan et al., 2016). By contrast, C-terminal regions such as a transmembrane domain of PDLP1 and a sequence comprising the NLS and the homeodomain of KN1, have been found to be involved in PD targeting of PDLP1 and KN1, respectively (Kim et al., 2005c; Thomas et al., 2008). Yet so far, PD-targeting signal sequences or -peptides have been identified only in this subset of proteins and no universal code for PD targeting could be inferred from the already identified signal sequences (Li et al., 2020). Interestingly, different posttranscriptional modifications, such as glycosylation, phosphorylation and GPI modification seem to be involved in PD trafficking (Taoka et al., 2007; Zavaliev et al., 2016). Alternatively, PD targeting might not only depend on specific peptide sequences but also on less obvious features (*e.g.*, mitochondrial targeting signals contain an alternating pattern of hydrophobic and positively charged amino acids), for example interaction with PD localized proteins.

In summary, evolution generated an energy-efficient and versatile system, whose transport properties can be adjusted to communication and nutritional needs, with specific adjustments depending on the cell type, developmental stage and environmental conditions. No doubt, the systematic identification of PD components and direct transport assays will help to identify common features responsible for PD targeting.

5. Regulation of PD conductance

Principally, two major modes of gating PD have been proposed – rapid gating as observed in response to pressure changes (Oparka and Prior, 1992), and slow modes that involve deposition of callose, a β-1, 3-glucan (Zavaliev et al., 2011). The mechanism for pressure-mediated closure has remained elusive. Callose levels in the neck regions are mainly affected by two groups of PD-localized enzymes, callose synthases and β-1,3-glucanases. PD-LOCALIZED β-1,3-GLUCANASE (PdBG) 1 and 2 are both expressed in lateral root primordia. The *pdbg1,2* double mutant accumulated more callose relative to wild type and displayed reduced cell-to-cell trafficking of macromolecules in roots (Benitez-Alfonso et al., 2013). Overexpression of the PD-associated REVERSIBLY GLYCOSYLATED POLYPEPTIDES 2 (RGP2) protein, led to higher callose levels, likely interfering with conductance since elevated callose levels were correlated with reduced intercellular assimilate transport (Sagi et al., 2005). Members of the CALLOSE SYNTHASE (CALS) family are responsible for callose production at PD orifices. *Cals7* mutants failed to accumulate callose in PD of early sieve plates, resulting in reduced sieve pore number (Xie et al., 2011). Other members, such as CALS3 have similar roles in different cell types, *e.g.*, *cals3s* mutants showed elevated callose levels at PD and impaired GFP movement in Arabidopsis root tips (Vaten et al., 2011). By contrast, PD in maize callose synthase *tie-dyed2* mutants appeared to be unaffected, displaying normal callose deposition (Slewinski et al., 2012). However, *tie-dyed2* mutants hyperaccumulated starch and soluble sugars in leaves due to a defect in the cell-to-cell solute movement at the SE-CC interface. Furthermore, since the TD2 protein did not localize to PD but to the ER, its role is likely indirect (Baker and Braun, 2008; Slewinski et al., 2012). Remorins, were also shown to affect PD conductance. NDR1/HIN1-like26 (NHL26), a vasculature-specific protein that localizes to both PD and ER may be another protein important for PD function (Vilaine et al., 2013). Plants that ectopically overexpressed NHL26 displayed various phenotypes such as delayed flowering and senescence, reduced seed yield and increased fresh weight of rosette leaves (Vilaine et al., 2013). *nhl26* mutants accumulated sugars in mature source leaves which correlated with a reduction in sugar content in phloem sap exudates and seeds. At the same time, the raffinose concentration of phloem sap exudate increased in the mutant. NHL26 may thus play a role in modulating sugar export between CC and SE by affecting PD permeability.

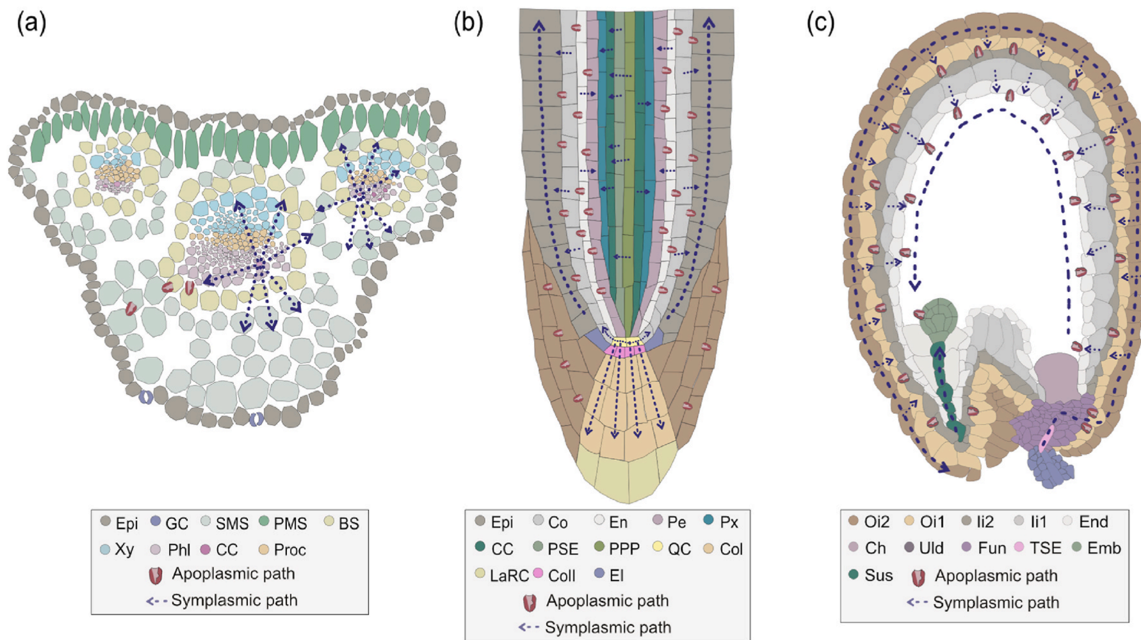

Fig. 3. Overview of the symplasmic domains in Arabidopsis tissues. Symplasmic and apoplasmic domains within the Arabidopsis leaf (a), root (b), and seed at the early globular stage (c). Symplasmic domains were identified by imaging the diffusion of GFP synthesized in specific cell types in studies from different labs. (a) In the leaf, GFP moves out of the companion cell (CC) only during the sink stage (Imlau et al., 1999; Skopelitis et al., 2018). (b) In the root, GFP is efficiently off-loaded from the phloem into primary meristems and from quiescent center (QC) into the columella (Col) (Imlau et al., 1999; Vaten et al., 2011; Liu et al., 2017; Skopelitis et al., 2018). Non-cell-autonomous transcription factors move from the cortex (Co)/endodermis (En) into epidermis (Epi) and pericycle (Pe) (Rim et al., 2011). (c) After fertilization, new branched PD are formed in the unloading zone (Uld) of seeds contributing to the formation of the symplasmically unloading unit (Stadler et al., 2005b; Werner et al., 2011). Efflux transporters are also localized in this zone, indicating the involvement of apoplasmic unloading step from the unloading domain to the integuments (Chen et al., 2015; Müller et al., 2015). The cells of the outer and inner integument of the seed form a symplasmic continuum (Stadler et al., 2005a). Arrows indicate the direction of the cell-to-cell movement in the symplasmic pathway. SE: sieve element. Oi: outer integument, Ii: inner integument. Epi: epidermis, Col: columella, Co: cortex, En: endodermis, Px: protoxylem, PSE: protophloem sieve element, Pe: pericycle, QC: quiescent center, Li: lateral root initials, EI: epidermis initials, LaRC: lateral root cap, ColI: columella initials, PPP: phloem pole pericycle, CC: companion cell, GC: guard cell, MS: mesophyll, PMS: palisade mesophyll, BS: bundle sheath, Xy: xylem, Phl: phloem, Proc: procambium, Pa: parenchyma.

PD are characterized by unique lipid composition. In particular, they share features with detergent-resistant microdomains enriched for sterols and sphingolipids (Grison et al., 2015; Liu et al., 2020). In Arabidopsis, the sphingolipid profiles affected PD permeability (Yan et al., 2019; Iswanto et al., 2020; Liu et al., 2020). Loss-of-function mutants of *PHLOEM UNLOADING MODULATOR* (*PLM*), which affects sphingolipid biosynthesis, caused an increase in phloem unloading at the interface between PPP and root endodermis independently of callose deposition (Yan et al., 2019). In contrast, Arabidopsis double mutants of *SPHINGOID LCB DENATURASE 1* and *2*, which showed a higher content of the long-chain sphingolipid phytosphinganine, displayed reduced PD permeability which correlated with an increase in callose accumulation (Liu et al., 2020). Similarly, Arabidopsis hypocotyls treated with inhibitors of the sphingolipid metabolism displayed reduced PD permeability which was associated with an higher level of callose deposition (Iswanto et al., 2020). A systematic characterization of the PD lipid composition is hampered by the difficulties in purifying PD, but would allow identification of the molecular mechanisms that contribute to lipid-mediated callose deposition and its role in PD transport.

So far, PD conductance was mainly studied by tracking the distribution of fluorescent dyes or by monitoring complex phenomena such as the distribution of ^{14}C-labeled sucrose or accumulation of starch in gain- or loss-of-function mutants. However, in several cases, the differences could be due to indirect effects (*e.g.*, reduction in PD number) rather than PD conductance. For example, maize mutants in *Carbohydrate partitioning defective33* showed decreased sucrose export yet had lower PD numbers at the SE-CC interface (Julius et al., 2018; Tran et al., 2019). The development of direct assays to monitor transport would help boosting our understanding of the mechanisms and regulation of PD conductance.

6. Viral movement proteins affect carbon allocation

A group of proteins that are not native plant components but target PD are the viral movement proteins (MPs), which are required for cell-to-cell movement of viral genomes and virus. MPs accumulate at PD where they gate PD at the infection front, increasing the SEL and enabling the spread of infectious components (Wolf et al., 1989; Benitez-Alfonso et al., 2010). Notably, MPs impact also carbohydrate metabolism and allocation. For instance, transgenic tobacco plants expressing the tobacco mosaic virus (TMV) MP under constitutive or phloem-restricted promoters caused a reduction in assimilate translocation to roots and an accumulation of starch in source leaves (Lucas et al., 1993; Balachandran et al., 1995; Olesinski et al., 1995). In contrast, expression of TMV MP under a green tissue-specific promoter in potato resulted in lower carbohydrate content and higher sucrose export (Olesinski et al., 1996). Tobacco plants expressing a MP from the phloem-limited potato leafroll virus (PLRV MP17) accumulated more sugars in source leaves (Herbers et al., 1997). Starch accumulation depended on MP17 dosage; low levels of MP17 caused a decrease in sugar content, possibly due to increased PD SEL, while carbohydrate excess seemed to be a consequence of indirect effects of high MP17 levels (Hofius et al., 2001). Deletion mutants in an MP domain of TMV that affected the SEL indicated that the effects on carbohydrates were SEL-independent (Balachandran et al., 1995). These findings imply complex roles that may be tissue- or even species-specific and an emerging model where carbon allocation might be regulated by MPs in subtle manners by interference of MPs endogenous signals involved in supracellular communication (Balachandran et al., 1995; Lucas and Wolf, 1999). This effect could be simply a side effect of alterations of the PD to allow the virus to spread, or may serve in providing zones that

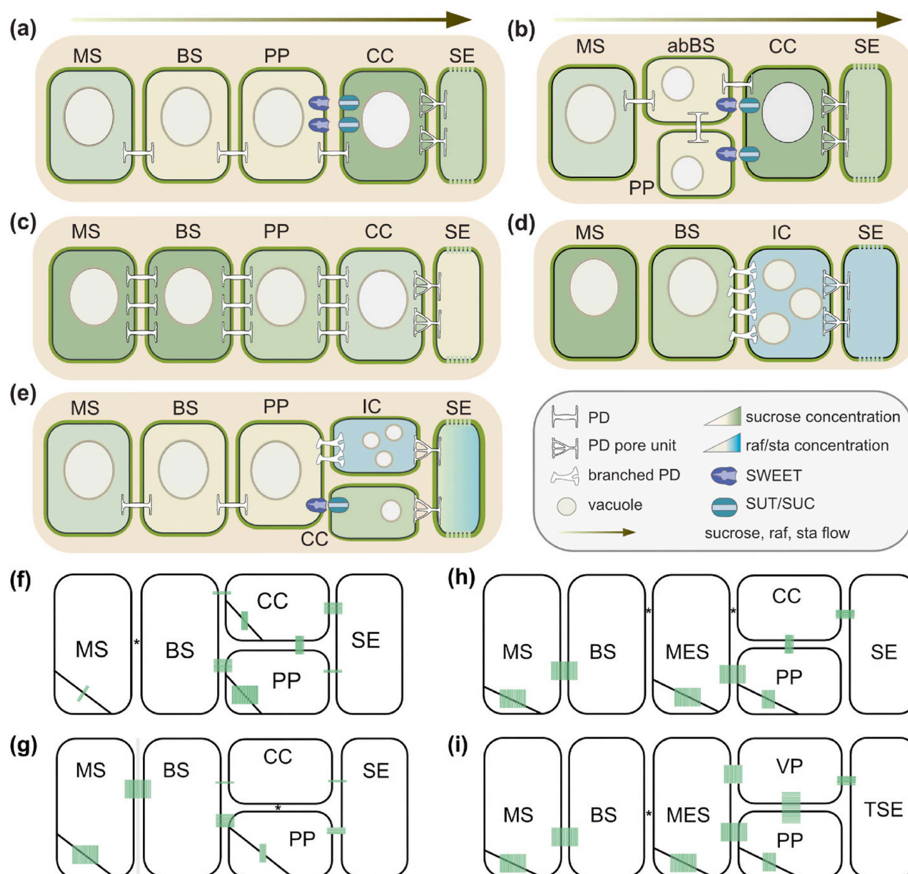

Fig. 4. Plasmodesmatal connectivity in relation to phloem loading. (a–d) Sucrose produced in mesophyll cells flows towards sieve elements for phloem loading by distinct mechanisms. Arrows indicate the direction of flow of sucrose and raffinose/stachyose from MS to SE as indicated in the legend of the schematics (right panel of Fig. 4e). Note that relative cell size depicted in the schematics does not reflect actual sizes. (a) Apoplasmic phloem loading in Arabidopsis. (b) Apoplasmic phloem loading in maize (c) Passive symplasmic loading. (d) Polymer trap symplasmic loading. (e) Mixed loading. (f–i) Plasmodesmograms indicating the density of plasmodesmata at the cellular interfaces of leaves in Arabidopsis (f), maize (g), and rice (h, i). Likely, different mechanisms may coexist in a single species and contribution may depend on environmental condition or developmental state. Two potential routes for phloem loading in rice: an apoplasmic (h) and a symplasmic route (i). Asterisks indicate data not found in the corresponding interface. The number of green lines denotes PD density at cellular interfaces. MS: mesophyll cell, BS: bundle sheath cell, PP: phloem parenchyma, CC: companion cell, SE: sieve element, IC: intermediary cell, VP: vascular parenchyma, MES: mestome sheath. raf: raffinose, sta: stachyose. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

have sufficient energy to allow for effective virus replication. No doubt, MPs are highly useful tools to study PD function and their contribution to carbon allocation. In summary, a better understanding of PD composition and function would be important also for developing new ways to engineer virus resistance.

7. Symplasmic domains may enable exchange of communication and sugars in zones

During differentiation, dynamic control over PD distribution and permeability leads to changes in the formation of “symplasmic domains”, where exchange occurs through PD between specific cell groups while being restricted in others (Rinne and van der Schoot, 1998). These functional domains allow specific developmental programs to take place in restricted areas. Although not equivalent to native components of plant cells, GFP and dye loading have been used as a proxy for symplasmic translocation of small molecules (Duckett et al., 1994; Oparka et al., 1999; Kim et al., 2005b; Stadler et al., 2005a). By tracing GFP expression driven by the phloem specific *SUC2* promoter, different sink tissues such as the seed coat, the anther connective tissue, cells of the root tip and sink leaf mesophyll cells were identified as symplasmic domains connected to the phloem (Imlau et al., 1999). Free GFP expressed from the *SUC2* promoter diffused throughout sink leaves (Fig. 3a), while it was restricted to the vasculature after maturation (Imlau et al., 1999; Skopelitis et al., 2018). Notably, microRNAs like miRGFP could move across sink and mature source leaves (Skopelitis et al., 2018).

Symplasmic domains can be regulated in a spatio-temporal manner. For instance, during the vegetative phase, Arabidopsis shoot apices are symplasmically isolated in the meristematic corpus while shortly before and during the floral transition the symplasmic domain is extended throughout the whole shoot apex (Gisel et al., 1999). In poplar and birch, the shoot apical meristem is symplasmically isolated during dormancy (Rinne et al., 2001, 2011). In Arabidopsis, root hair cells gradually become symplasmically isolated during differentiation (Duckett et al., 1994), while cotton fiber cells are temporarily symplasmically isolated during fiber cell elongation (Ruan et al., 2001).

In the Arabidopsis root apex, mCherry expressed from cortical epidermal cells moved throughout all root tissues (Rim et al., 2011). By using differently sized mCherry reporters *i.e.,* 2xmCherry or mCherry-NLS, high SELs (~60 kDa) between the stellar-endodermal and the cortical-epidermal cell boundaries were observed, while the SEL between QC and cortex cells to root cap cells was lower (27 and 54 kDa, respectively) (Fig. 3b). Notably, several non-cell-autonomous transcription factor FP-fusions were shown to move across the stellar-endodermal and cortical-epidermal cell borders, despite surpassing the proposed SEL of 60 kDa, possibly implying other mechanisms (Rim et al., 2011).

During stomatal development, the stomata identity factor SPEECHLESS (SPCH) is symplasmically restricted to stomatal initials. After differentiation, stomata become fully symplasmically isolated by losing their PD (Lee et al., 2005). Callose synthase GLUCAN SYNTHASE-LIKE 8 mutants failed to accumulate callose in PD. As the result, stomatal initials were symplasmically connected and formed clustered stomata (Guseman et al., 2010).

During seed filling Arabidopsis seeds are provided with assimilates through the funicular phloem, which is symplasmically extended by the outer integument of the seed, while transport to the inner integument, a separate symplasmic domain, occurs apoplasmically (see also section *Contribution of PD to unloading of the phloem*). The suspensor, which connects embryo with maternal tissues and endosperm, forms a symplasmic domain with the embryo in early embryogenesis (Fig. 3c). At later stages of embryogenesis, the suspensor becomes symplasmically isolated and degenerates (Stadler et al., 2005a; Lafon-Placette and

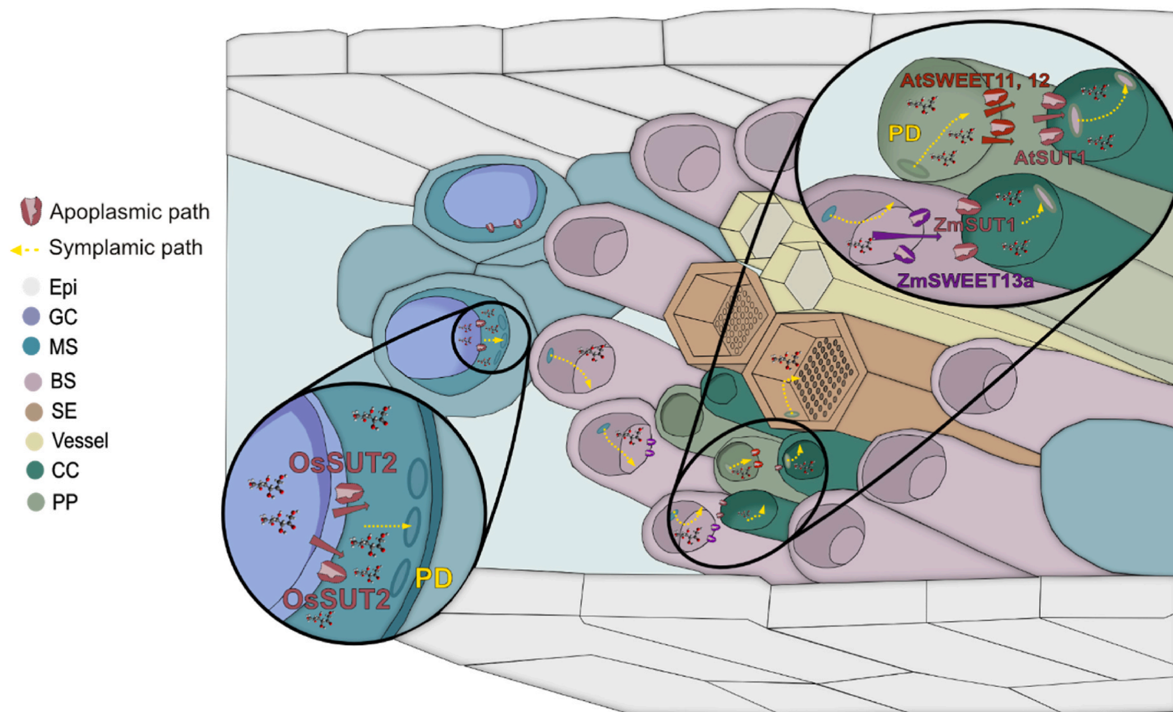

Fig. 5. A hypothetical model for phloem loading combining mechanisms identified in Arabidopsis, maize, and rice. Sucrose is primarily synthesized in leaf mesophyll cells. In Arabidopsis, sucrose is thought to move cell to cell via PD down a concentration gradient until it reaches the PP. At the PP-SECC interface, AtSWEET11 and AtSWEET12 mediate the efflux of sucrose into the apoplasm of Arabidopsis leaves, from where it is actively loaded into the SECC via SUT1 sucrose/H^+ symporters. In maize, ZmSWEET13s are found in the abaxial bundle sheath cells of minor veins where they mediate the efflux of sucrose towards the phloem, then following a symplasmic path to the SECCC. In rice, high levels of sucrose are stored in vacuoles of mesophyll cells where the vacuolar sucrose/H^+ symporter OsSUT2 mediates its export to the cytosol against a concentration gradient. OsSUT2-mediated transport, as a consequence of the large cytosol/vacuolar proton gradient, should lead to sucrose concentrations in the cytosol that exceed that of phloem sap (600 mM) to enable passive phloem loading via PD down the concentration gradient. Epi: epidermis, GC: guard cell, MS: mesophyll, BS: bundle sheath, SE: sieve element, CC: companion cell, PP: phloem parenchyma. Illustration modified from an unpublished cartoon developed by Guido Grossmann (HHU, Düsseldorf).

Köhler, 2014). Throughout embryogenesis the embryo's PD permeability is downregulated, to establish four symplasmic subdomains. These subdomains align with the axial body pattern: cotyledons, shoot apex, hypocotyl, and roots (Kim et al., 2005b). Symplasmic domains also are formed in certain plant-microbe interactions, *e.g.*, during nodule development in rhizobia-colonized tissues of *Medicago truncatula*, symplasmic domains are created while PD deposited callose levels decrease. The ß-glucanase MtBG2 seems to play an active role in callose degradation during nodule development (Gaudioso-Pedraza et al., 2018).

While symplasmic isolation is one option for controlling trafficking during development, multiple lines of evidence implicate other transport mechanisms, such as unidirectional PD flow in trichomes (Christensen et al., 2009), dilation of PD during protein trafficking across PD (Wolf et al., 1989; Xoconostle-Cázares et al., 1999), and cargo-gating dependent transport mechanism (Peters et al., 2021) (see also *PD transport mechanism*).

8. A focus on cell-to-cell translocation of carbohydrates

The translocation of carbohydrates between organs occurs via the phloem. The angiosperm leaf phloem is made up of three major cell types: the phloem parenchyma, CC and SE (van Bel and Knoblauch, 2000; Kim et al., 2021). SEs are the actual conduits, but since mature SE does not contain a nucleus and are assumed to not participate in major ways in metabolism of assimilates, these must be supplied from the neighboring CC via PD. It is generally assumed that sucrose, produced in the mesophyll, moves via PD towards the phloem (Fig. 4). The subsequent path then involves PD in the bundle sheath, phloem parenchyma and in what has been defined as symplasmic species, even into the SECCC (described in the *Symplasmic phloem loading* section below). Plasmodesmograms were generated to map PD frequencies in route between mesophyll and sieve tubes in different species (van Bel et al., 1992) (Fig. 4f–i). However, the presence of high frequencies of PD does not fully reflect the existence of a symplasmic communication, even more for a specific compound since PD could be obstructed or at least non-permeable for the studied compounds. Direct evidence for the contribution of the symplasmic pathway to carbohydrate transport is still awaited. In apoplasmic species, there is a sparsity of PD between PP and the SECCC, requiring apoplasmic steps that make use of plasma membrane sucrose transporter pairs (described in the *Apoplasmic phloem loading* section below). Notably, mixed translocation may occur. Unloading of carbohydrates in sink tissues is also achieved either symplasmic or apoplasmically, sometimes requiring transporters at post-phloem unloading steps.

9. Apoplasmic and symplasmic pathways for phloem loading

The phloem sap distributes a wide range of ions, metabolites, RNAs and proteins between plant organs. According to Münch's hypothesis, the flow of the phloem sap is generated by osmotically driven pressure gradients between the loading sites (typically leaves, also defined as source organs, high pressure) to the unloading sites (diverse organs like roots, flowers or developing seeds, together defined as sink organs; lower pressure) (Knoblauch and Peters, 2010). In many plant species, sucrose is the dominant osmolyte contributing to the osmotic driving force for phloem translocation. The sucrose concentration in the phloem sap varies between species. The SECCC of sugar beet leaves contains 80% of the total leaf sucrose (45 µg cm^{-2} leaf blade) and has a sucrose concentration of 0.8 M (Geiger et al., 1974). Sucrose concentrations of 0.8, 1.0 and 1.0 M have been measured in spinach, barley and maize

leaves, respectively (Lohaus et al., 1994). In maize, the data on sucrose concentration in the phloem sap vary between 0.9 and 1.4 M (Ohshima et al., 1990; Weiner et al., 1991). By comparison, the phloem sap of rice contains 0.5–0.6 M sucrose (Kawabe et al., 1980; Hayashi and Chino, 1986). Lower concentrations of sucrose of ~250 mM have been measured in the phloem sap of wheat. Interestingly, in wheat, sucrose does not appear to be the dominant osmolyte since the phloem sap contains higher levels of K^+ (~300 mM) and amino acids (~260 mM) (Hayashi and Chino, 1986).

Besides the role in generating the osmotic driving force for phloem transport, sucrose serves as the primary carbon and energy source for diverse sink organs. To be exported from the source leaf, sucrose must be translocated from the mesophyll where it is produced, across several cell types before it can be loaded into the SE (Figs. 4 and 5). Thus, appropriate sucrose gradients are required for translocation. Results from incipient plasmolysis support the existence of such gradients in a variety of species (Geiger et al., 1974) (Fig. 4a–e). The export rate for sucrose per leaf has been estimated to $\sim 5 \times 10^{-13}$ mole sec^{-1} mm^{-2} and the average flux of sucrose per plasmodesma to 7×10^{-19} mole sec^{-1} for mesophyll cells, 2.4×10^{-19} mole sec^{-1} for bundle sheath, and 8.1×10^{-19} mole sec^{-1} for the SE-CC interface (Gunning, 1976). More recently, mathematical models based on structural features of specific PD have been generated to predict solute flux through PD (Deinum et al., 2019). The frequency of PD along the route from mesophyll and sieve tubes varies between different species, as illustrated in plasmodesmograms (van Bel et al., 1992) (Fig. 4f–i). For instance, C_4 species were shown to have twice the number of PD per pit field area compared with their C_3 counterparts, and the pit field area per mesophyll-bundle sheath interface area is five times higher in C_4 species (Danila et al., 2016). However, as the density of PD at cell wall interfaces alone does not fully represent the actual symplasmic transport capacity or symplasmic continuity, how far these differences in density influence symplasmic flux remains to be elucidated (Liesche et al., 2019).

So far, two major modes of phloem loading have been described based on the plasmodesmatal connectivity and presence of transporters: apoplasmic loading, mediated by membrane transporters, and symplasmic loading via the PD. The symplasmic loading pathway can occur in an active or passive manner. Some species adopt a mixture of both apoplasmic and symplasmic pathways and are therefore referred to as mixed loaders. Various substrates are loaded into the phloem other than assimilates (*i.e.*, amino acids and secondary metabolites), yet here we focus on the process of phloem loading of sucrose. A detailed description of the phloem loading process of other substrates than sucrose can be found elsewhere (Eom et al., 2015; Tegeder and Hammes, 2018; Kim et al., 2021).

10. Phloem loading with the help of an apoplasmic step

Based on the connectivity of CC with the adjacent phloem parenchyma, plant species were categorized into different 'loading types' by counting the relative frequency of PD at the PP-CC interface using electron microscopy or confocal microscopy of fluorescently tagged PD. Type 1 (or "open phloem") plants are characterized by a high, Type 1–2a with intermediate, and Type 2 (or "closed phloem") by low PD abundance at the PP-CC interfaces (Turgeon and Webb, 1976; Gamalei, 1989, 1991). This classification was further refined as Type 1, Type 1–2a, Type 2a, Type 2b (transfer cells), Type 2c (Kranz anatomy) and a new type called "intermediary cells" (Turgeon et al., 2001). Species with "closed phloem" are assumed to load sucrose from the apoplasm. in Type 2 species, a pair of sucrose transporters is required for phloem loading: (i) SWEET-uniporters for efflux of sucrose from PP into the apoplasm, and (ii) SUT/SUC H^+/sucrose symporters for energized import into the SECCC. The mechanism of apoplasmic phloem loading has been studied most extensively in Arabidopsis and maize (Fig. 4a and b). In Arabidopsis, SWEET11, SWEET12 (and possibly SWEET13) mediate cellular efflux of sucrose into the apoplasm inside the phloem that had been synthesized in mesophyll cells and was translocated to the phloem parenchyma via the PD (Chen et al., 2012). Sucrose from the apoplasm is then taken up into the adjacent SECCC by H^+/sucrose symporters (SUT/SUC) (Figs. 4a and 5). In maize, a similar pair of transporters is required for transfer to the sieve elements, yet SWEETs are present in a distinct cell type. *Zm*SWEET13a,b,c are specifically produced in the two abaxial BS cells of minor veins (rank-2 intermediate veins) from where they efflux sucrose into the general apoplasmic space of the phloem. The SECCC then uses the H^+/sucrose symporter SUT1 (different clade than SUT1 in dicots) to actively import sucrose (Figs. 4b and 5). Notably, none of the sucrose transport mutants characterized today fully blocked sucrose delivery, indicating that there are parallel pathways that may differ in their relative importance (see section *Mixed Loading Mechanisms*).

11. Phloem loading along symplasmic paths

It has been suggested that symplasmic phloem loaders are independent of plasma membrane SWEET and SUT activities by movement of sugars exclusively in the symplasm. Notably however, the SWEET and SUT gene families are present in all genomes, implying that they may be required under certain conditions also in these species. Two types of symplasmic phloem loading mechanisms have been described: passive symplasmic loading and active symplasmic loading, also known as the polymer trap mechanism.

1. Passive symplasmic loading. As postulated by the pressure-flow hypothesis (Münch, 1930), passive loading is only thermodynamically feasible if the sucrose concentration in mesophyll cells is higher relative to that in the phloem sap. Indeed, in many woody and several herbaceous species, the concentration of sucrose in the cytosol of mesophyll cells was found to be higher as compared to the concentration in the phloem sap (Turgeon and Medville, 1998; Fu et al., 2011) (Fig. 4c). These species accumulated less starch in minor veins when treated with exogenous sucrose compared with plants that load sucrose actively and were rich in PD along the path from mesophyll to the SE (Turgeon and Ayre, 2005; Reidel et al., 2009; Rennie and Turgeon, 2009; Fu et al., 2011). However, although these factors fulfill the prerequisites for passive loading, there was a fundamental question of whether the pressure generated in passive loaders is sufficient to drive long-distance transport in large plants, such as tress. Modeling studies using anatomical data and experimental observations using synthetic tree-on-a-chip system indicated that small pressure differences may be sufficient to drive long-distance sugar translocation in tress (Jensen et al., 2012; Comtet et al., 2017). Furthermore, anatomical studies of the sieve tube and sieve plate in trees revealed a shift in SE structure that scales the sieve tube resistance along the path from leaf to root and, thus reduces the pressure required for sugar transport (Savage et al., 2017). These results indicate that tall trees can also rely on pressure-flow for symplasmic sugar transport.

2. Active symplasmic loading. The 'polymer trap' hypothesis for active symplasmic loading was based by the observation that species with specialized companion cells (referred to as intermediary cells) translocate raffinose family oligosaccharides (RFOs; raffinose; stachyose (Turgeon and Gowan, 1990). According to the 'polymer trap' model, sucrose (342 Da) diffuses down the concentration gradient across PD from the mesophyll across bundle sheath into intermediary cells. Intermediary cells convert a portion of sucrose into raffinose (594 Da) and stachyose (667 Da). The SEL of PDs at the interface is predicted to be so small, that it only allows sucrose to enter intermediary cells, while RFOs are too large to diffuse back (Fisher, 1986; Turgeon et al., 1993) (Fig. 4d). This way RFOs are trapped in the intermediary cell (polymer trap) (Fisher, 1986; Turgeon and Medville, 1998; Botha and Murugan, 2021). Thus, symplasmic translocation is energized by the enzymatic conversion of sucrose to raffinose by galactinol synthases in the intermediary cells (McCaskill and Turgeon, 2007). At present, it is not yet clear how much the cytoplasmic sleeve space would have to be reduced

to prevent back leakage of sucrose from the intermediary cells to the bundle sheath cells. Assuming that the hydrodynamic radius governs the mobility of sugars through PD (Terry and Robards, 1987), the cytoplasmic sleeve space in the plasmodesmata within the IC-side needs to be reduced to < 10.4 Å (hydrodynamic diameter of sucrose) to prevent leakage of sucrose back to the bundle sheath (Botha et al., 1993; Botha, 2005; Botha and Murugan, 2021). Recent high-resolution TEM analyses in *T. capensis* leaf veins revealed that the radius of the open cytoplasmic sleeve within the sphincter zone (physical constriction) on the IC side of the bundle sheath-intermediary cell PD was reduced to 1.5 nm (Botha et al., 1993; Botha, 2005; Botha and Murugan, 2021). If these PD have such a small SEL, then likely larger molecules such as RNAS and proteins, should not be able to move across this interface. We note, however, that formally, neither the transport of sucrose through PD, nor an SEL that allows sucrose to pass, - but prevents raffinose to back-diffuse through PD, have experimentally been demonstrated. As direct evidence is still lacking, there are still controversial views on whether additional factors are involved for active symplasmic loading (Dölger et al., 2014).

12. Mixed loading mechanisms

Species were generally classified as falling into one of the phloem loading strategies. However, multiple lines of evidence indicate that a single species can use more than one strategy, or mixed loading. All species, no matter how they have been classified maintain the SWEET and SUT sucrose transporter genes. Thus, parallel use of multiple phloem loading strategies may be beneficial under specific environmental conditions. In line with this notion, an 'ecophysiological hypothesis' has been proposed. According to this hypothesis, the relative activity of apoplasmic/symplasmic modes depends on environmental conditions and this heterogeneity allows plants to rapidly adapt to biotic and abiotic stresses (van Bel and Gamalei, 1992). Whether all species are generally mixed loaders, and/or whether there is an unexplored mechanism that species use to load sugars (*i.e.*, regulation of PD during symplasmic loading) remains to be elucidated.

Indirect evidence for mixed loading comes from anatomical features of polymer trap species. In various polymer trap species, such as cucurbits, CC have few symplasmic connections to surrounding cells (resembling ordinary companion cells of apoplasmic loading species) in addition to intermediary cells (Turgeon and Webb, 1976) (Fig. 4e). In other species such as *Asarina scandens* and *Acanthus mollis*, cell wall ingrowths were observed in the companion cells (van Bel and Gamalei, 1992; Rennie and Turgeon, 2009). Cell wall ingrowths are typical features of cells involved in apoplasmic loading that are thought to magnify the plasma membrane surface area, allowing deployment of more transporters and effectively increasing the flux. Analyses in polymer trapping species such as *Alonsoa meridionalis* (*A. meridionalis*) showed that the sucrose concentration in the phloem sap can be as high as in mesophyll cells, indicating the absence of the required sucrose gradient. Furthermore, *Am*SUT1 was shown to be present in the plasma membrane of SE and CC supporting the claim that apoplasmic loading through ordinary CC is also a mechanism that *A. meridionalis* uses for phloem loading (Knop et al., 2004; Voitsekhovskaja et al., 2006).

Other evidence for the coexistence of phloem loading mechanism can be derived from the analysis of mutants that either disrupt key pathways critical for polymer trapping or apoplasmic sucrose transporters. The majority of mutants with impaired RFO production in species using active symplasmic loading strategies did not display severe phenotypic symptoms or still transported sucrose (McCaskill and Turgeon, 2007). Furthermore, mutants of sucrose transporters in species considered as apoplasmic loaders, including *sut1/suc2* or *sweet11;12* in Arabidopsis or *zmsweet13a,b,c* and *sut1* in maize, were able to produced seeds, indicative of a remnant alternative loading mechanism (Riesmeier et al., 1994; Gottwald et al., 2000; Slewinski et al., 2009; Srivastava et al., 2009; Chen et al., 2012; Bezrutczyk et al., 2018). Although we can neither rule out that the mutants are not null, nor that compensatory mechanisms exist, these observations are consistent with mixed loading (McCaskill and Turgeon, 2007).

Notably, rice, which like maize belongs to the *Poaceae*, seems to use a symplasmic loading pathway, despite the high concentration of sucrose in the phloem sap. The presence of numerous PD connections between the CC and the parenchyma cells and the movement of the low molecular weight dye CFDA led to an assumption that sucrose loading occurs symplasmically (Kanako et al., 1980; Chonan et al., 1981; Scofield et al., 2007). Later, multiple members of OsSUT and SWEET families and proton pumping pyrophosphatases (H^+-PPases) were shown to be expressed in the phloem (Scofield et al., 2007; Regmi et al., 2016; Wang et al., 2021). More recently, the OsDOF11 transcription factor was shown to affect phloem loading by promoting the expression of OsSUTs and OsSWEETs (Wu et al., 2018). Together, these results served as strong evidence for claiming that rice employs apoplasmic loading as a major phloem loading strategy. However, *sweet13,14* double mutants had no obvious defect in phloem loading, and *ossut1* mutants and RNAi lines grown in growth chambers did not show measurable defects, yet field-grown mutants showed a mild decrease in height (Ishimaru et al., 2001; Scofield et al., 2002; Eom et al., 2012). By contrast mutants in the vacuolar SUT2, present in mesophyll cells, were lethal, possibly providing a mechanism for generating a steep sucrose gradient between the mesophyll cytosol and the phloem via active sucrose efflux from the vacuole into the mesophyll cytosol (Eom et al., 2012, 2019).

13. Contribution of PD to unloading of the phloem

Following phloem loading, assimilates are distributed through the sieve tubes to sink organs. The transfer of assimilates from the phloem cells to sink cells is called phloem unloading. The most widely accepted model describing phloem unloading is the high-pressure manifold model (Fisher et al., 2000). Based on this model, a high hydrostatic pressure is maintained throughout the flow in the sieve tube with low-pressure gradients from source to sink, but high-pressure gradients between the SE and the neighboring cells where the unloading occurs (unloading zone). Recent turgor measurements in some species such as morning glory however, did not support this model as low pressures were generated for flow but high-pressure differentials sufficient for unloading were not maintained in the unloading zone (Christensen et al., 2009; Knoblauch et al., 2016; Milne et al., 2018). These observations raised the possibility that the PD conductance in the unloading zone is high or/and an alternative strategy, such as active unloading processes, are being used. Indeed, as for phloem loading, phloem unloading can occur along sym- or apoplasmic pathways, and the relative contribution seems to vary depending on species, sink organs, and the sugars that are transported (sucrose, RFOs, or sugar alcohols).

In various species and tissues such as sink leaves of dicots, tubers, stem zones of herbaceous crop plants, developing fruit and seeds, phloem unloading occurs predominantly via the symplasmic route (Oparka et al., 1994; Ruan and Patrick, 1995; Wright and Oparka, 1997; Imlau et al., 1999; Imlau et al., 1999, 1999; Fisher and Cash-Clark, 2000; Patrick and Offler, 2001; Ruan et al., 2001; Viola et al., 2001; Wu et al., 2004; Kim and Zambryski, 2005; Rae et al., 2005; Nardozza et al., 2013; Milne et al., 2017; Skopelitis et al., 2018; Mehdi et al., 2019; Ko et al., 2021; Pan et al., 2021) (Fig. 3). For symplasmic unloading to occur, functional PD connections between the phloem cell types and neighboring cells must be present. Indeed, numerous PD are observed between SECCC and neighboring cells at sink organs as described in the section *Symplasmic domains may enable exchange of communication and sugars in zones*.

Phloem unloading has been studied most extensively in roots and seeds of Arabidopsis (Fig. 3b and c). A recent study that combined microscopy and mathematical modeling provided evidence for a mechanism in which small solutes (0.34–0.38 kDa) (suitable for sucrose translocation) freely move across PD, and are unloaded from the SE into

the PPP by funnel-shaped PD by a combination of mass flow and diffusion (Ross-Elliott et al., 2017) (Fig. 3b) (see also *PD transport mechanisms*). SUT sucrose transporters (*e.g.*, AtSUC1) may function in sucrose retrieval to maintain high turgor pressure necessary for symplasmic unloading by bulk flow (Milne et al., 2017, 2018). At the PPP, PLM, a putative enzyme necessary for sphingolipid metabolism was shown to play a role in symplasmic unloading by affecting PD ultrastructure at the interface between the PPP and endodermis (Yan et al., 2019). Similar as in Arabidopsis, phloem unloading in cassava (Manihot esculenta) was shown to occur via the symplasmic pathway into the phloem parenchyma cells of tuberous roots (Mehdi et al., 2019).

In seeds of Arabidopsis, phloem unloading takes place in the so-called unloading domain (ULD). The ULD emerges after fertilization when secondary PDs are formed *de novo* between the terminal SE and surrounding cells (Fig. 3c). Studies using fluorescent tracers revealed symplasmic continuity between terminal SE and the neighboring cells in the ULD, which extends to the outer integument (Patrick and Offler, 2001; Werner et al., 2011; Müller et al., 2015). SWEET efflux transporters were not detected at the ULD, in line with the notion that symplasmic routes are the dominant paths for unloading sugars in Arabidopsis seeds. After sugars have been unloaded into the ULD, post-phloem transport occurs likely both on sym- and apoplasmic paths. Multiple SWEETs (AtSWEET11, 12, and 15) are positioned at different cell layers to mediate a cascade of sucrose transport from the maternal tissue to the developing seed (Chen et al., 2015). So far, the mechanism of how sucrose enters the embryo is not resolved, Two possible routes have been proposed. The first route involves sucrose uptake in the suspensor (likely mediated by SWEET12) (Chen et al., 2015). The suspensor and the embryo are symplasmically connected at the globular stage. PD connecting the suspensor and embryo were shown to have a SEL of >2.6 nm (Stadler et al., 2005a). The second route requires the export of sucrose from the endothelium and uptake in the endosperm. Hexoses are the most abundant sugars accumulating in the endosperm during the early embryogenesis stage (Baud et al., 2002; Morley-Smith et al., 2008). Therefore, hexose transporters and invertases might play a major role in this path. Following import into the embryo, sugars likely move symplasmically throughout the embryo (Kim et al., 2005a, 2005b). As in Arabidopsis, phloem unloading and post-phloem transport occur via both sym- and apoplasmic routes in various species (Gisel et al., 2002; Kim et al., 2002a). In rice, sucrose is unloaded into the parenchyma cells and then to the nucellar layers symplasmically, while at later stages (export from the nucellar layer and import into the endosperm) occurs on an apoplasmic path (Oparka and Gates, 1981; Oparka and Gates, 1981; Yang et al., 2018). Here SWEETs and SUTs likely play roles at different places in the nucellar projection and at the interface to the endosperm (Yang et al., 2018).

In some species, phloem unloading pathways change during development. Unloading processes shift in Arabidopsis (Chen et al., 2015). In several legumes, apoplasmic pathways change during seed development by switching from a mechanism also involving invertases and hexose importers during the cell division phase to a sucrose transport pathway during the storage phase (Patrick and Offler, 2001; Borisjuk et al., 2002). In tobacco leaves, dye coupling from CC to PP it discontinued in the transition from sink to source, which correlates with dynamic changes from simple to complex PD (Oparka et al., 1999; Roberts et al., 2001). A correlation between onset of flowering and decreased leaf-to-shoot trafficking of symplasmic tracers was observed, which suggests a selective transport mechanism of signaling molecules during floral induction (Gisel et al., 1999, 2002; Ruan et al., 2001).

Due to the importance of fruit development in food production, phloem unloading during fruit development were studied in much detail. In some species, there is a shift from the apoplasmic to the symplasmic pathway. For instance, in potato plants (*Solanum tuberosum*), the apoplasmic unloading pathway is considered to be the major route for sugar transport in stolon undergoing extension growth while symplasmic unloading becomes the dominant path after tuberization (Viola et al., 2001). There are also species in which the process is different. In grape *Vitis vinifera* berries, symplasmic post-phloem unloading predominates in the early and middle stages in fruit development as shown with symplasmic tracers that were released from the phloem strands. However during ripening, the movement of symplasmic tracers was blocked, indicating a transition to an apoplasmic path (Zhang et al., 2006). TEM micrographs showed a higher electron-density in the PD at the late stage of fruit development which might be the cause of the blocking of the PD and the shift in the unloading pathways (Zhang et al., 2006).

Similar to grape berries, at the early stage of tomato (*Solanum lycopersium*) fruit development, photoassimilates are predominantly unloaded from the SECCC to surrounding parenchyma cells via PD. Yet, at later stages, apoplasmic routes seem to be the dominant path for sucrose unloading, indicted by a reduction in the mobility of symplasmic tracers and reduced abundance of PD connections (Ruan and Patrick, 1995). A recently identified sucrose transporter SlSSWEET15 accumulated in vascular tissues of developing fruits. Fruits of *slsweet15* mutants were smaller in size, implicating SlSWEET15 in mediating apoplasmic sucrose unloading during fruit development (Ruan and Patrick, 1995; Ko et al., 2021). Understanding phloem unloading mechanisms in fruits may allow scientists to develop strategies to improve fruit quality and yield.

14. Dynamic regulation of symplasmic connectivity

Although primary and secondary PD differ in their ontogeny, to our knowledge, no structural and functional differences have been reported yet. In contrast, simple and branched PD have been considerably investigated with the aim to identify differences in functions during plant development. Their obvious structural features allows identification and determination of spatial and temporal distribution; for instance in the sink-source transition of *Nicotiana tabacum* leaves (Roberts et al., 2001). The frequency of simple PD declines substantially during the sink-to-source transition between all examined cell types. At the same time branched PD increase in number (Oparka et al., 1999). As shown by analyses of the cell-to-cell movement of GFP fusions, the rise of branched PD frequency is associated with a substantial decrease of the PD SEL (Oparka et al., 1999; Crawford and Zambryski, 2001). In immature sink tissues, the steady state SEL of PD was shown to be around 50 kDa, allowing basal transport of large components such as proteins (average molecular mass of Arabidopsis proteins: 43 kDa) (Oparka et al., 1999; Tiessen et al., 2012). By contrast, in mature source tissues of tobacco, the SEL was determined to lie between 0.7 and 4 kDa, excluding the possibility of transport for the majority proteins, provided that the SEL determined is relevant for the transport of native proteins (Wolf et al., 1989). Since cellular differentiation requires the exchange of positional information with neighboring cells, in particular via non-cell-autonomous proteins and RNA (Marzec and Kurczynska, 2014; Otero et al., 2016), this decrease in SEL and/or PD branching could be a mechanism to restrict signaling to a specific cell cluster, without impairing the transport of sucrose (Ehlers and Kollmann, 2001). Roberts et al., also highlighted that branched PD appear asynchronously in the different leaf cell layers (Roberts et al., 2001). Branched PD develop first at the junction between epidermal cells and trichomes, between stalk cells of the trichome and in vascular tissues. They then emerge in sequential order in the upper epidermis, the lower epidermis, the spongy mesophyll and the anticlinal walls between palisade cells. At the end of the leaf development, only glandular cells of trichomes retain simple PD. Interestingly, this sequence of emergence coincides with that of tobacco leaf development (Poethig and Sussex, 1985). This is consistent with the general observation that immature tissues typically contain predominantly simple PD (Burch-Smith et al., 2011b), and indicates that PD branching is somehow linked to tissue differentiation. The sink-source transition was also found to be associated with an increase in leaf thickness by 40–50%, and the formation of large intercellular spaces in the mesophyll, leading to a decrease in shared-cell-wall interface area (Roberts et al., 2001). It has been suggested that during cell enlargement

and separation occurring during the sink-source transition, many simple PD are ripped apart, and that others are converted into branched PD. Although the roles of branched PD are still unclear, it has been hypothesized that they could represent solid junctions aiming at preserving cell adhesion, thereby contributing to cell shape formation and cell positioning during cell expansion and differentiation (Roberts et al., 2001).

15. Tools for studying and modifying PD transport

Our current understanding of PD conductance is based mainly on the use of tools that track cell-to-cell movement of molecules (Table 1) and our limited ability to manipulate PD gating and the analysis of the resulting alterations in distribution of the molecules, *e.g.*, accumulation or depletion in defined zones (Table 2). Actual translocation via PD has rarely been shown (Table 1). For instance, there are no data on the actual transport of K^+, Ca^+, glucose, and limited data regarding RNAs, proteins and sucrose. Most of the approaches undertaken with these tools are based on the assumption that cell-to-cell movement occurred through PD, and provide only indirect evidence since apoplasmic pathways could not be excluded. Unambiguous evidence can only come from studies that monitor the actual translocation through PD. One such method applicable to proteins is single molecule tracking, which has

Table 1
Tools available to study PD permeability.

TOOLS		MAIN STRENGTHS	MAIN WEAKNESSES	EXAMPLES	REF.
Indirect	**Dyes**	- Fast and easy to implement - High spatial specificity by photobleaching or photoactivation	- Only relative to the employed dye; do not provide information regarding endogenous molecules - No direct evidence	Assaying PD permeability using CFDA and the Drop-And-See (DANS) quantitative approach in Arabidopsis leaves	Cui et al. (2015)
				3D photoactivation microscopy using CMNB-caged fluorescein to study PD permeability between single cells in *Cucurbita maxima* mesophyll	Liesche and Schulz (2012b)
				Fluorescence loss in photobleaching using CFDA to study PD permeability along the phloem loading pathway in nine species	Liesche et al. (2019)
	Fluorescent proteins (FP)	- High spatial specificity using tissue-specific promoters, photobleaching, bombardment, or using photoactivable FP - To study PD SEL by fusion to *FP* sequence - For specific non-cell-autonomous proteins/RNA by fusion to *FP* sequence	- Fusion to *FP* sequence might affect permeability of non-cell-autonomous molecules - No direct evidence	Expression of GFP 1X, 2X and 3X under *STM* promoter to study cell-to-cell communication and SEL in Arabidopsis embryos	Kim and Zambryski (2005)
				Bombardment with vectors encoding GFP in Arabidopsis epidermal cells to compare PD permeability between WT and β-1,3-glucanase deficient mutant	Levy et al. (2007)
				DRONPA-s photoactivable FP to compare symplasmic connection of single cells in Arabidopsis roots	Gerlitz et al. (2018)
				Fusion of maize KN1 to *GFP* to study the non-cell-autonomous protein KN1 specific PD diffusion in Arabidopsis leaves	Kim et al. (2002b)
				RNA-SL/MS2-GFP system to track *FT* mRNA in *N. benthamiana* leaves	Luo et al. (2018)
	Sucrose analogue	Real-time imaging of sucrose analogue in living plant cells	- No direct evidence	Alkyne-tagged compound as a sucrose analogue is recognized by sucrose transporters and allow to visualize real-time uptake of sucrose	de Moliner et al. (2021)
	RNA interference	- For non-tagged RNA - High spatial specificity using tissue-specific promoters	- Possible off-targets - No direct evidence	miRGFP sensor system to study miRNA mobility in Arabidopsis	Skopelitis et al. (2018)
				GFP sensor system in which *GFP* sequence is fused to miR171 target sequence to study miR171 mobility in *N. benthamiana*	Parizotto et al. (2004)
				Expression of *SUL* inverted repeat sequence under *SUC2* promoter to study RNAi spreading in Arabidopsis	Rosas-Diaz et al. (2018)
	Sensors	- For endogenous molecules - In native conditions	- Specific sensors with compatible affinities are rarely available - Might affect the homeostasis of investigated molecules - No direct evidence	GCaMP3 fluorescent protein cytosolic calcium sensor to monitor calcium propagation in response to wounding in Arabidopsis	Toyota et al. (2018)
				Cameleon (NES-YC3.6) fluorescent protein cytosolic calcium sensor to study the calcium signal emerging at the root tip in response to a water potential gradient in Arabidopsis	Shkolnik et al. (2018)
	Electro-physiology	- Inducible by wounding - Endogenous signal - In native conditions	- Chemical nature of the current is unknown - No direct	Propagation of slow wave potentials depending on GLRs in response to wounding in Arabidopsis	Mousavi et al. (2013); Moe-Lange et al. (2021)
Direct	**Single molecule microscopy**	- Direct evidence of transport through PD	- Requires fusion to FP which might affect permeability	Single molecule tracking of mobile transcription factors in roots	Clark et al. (2016)

SEL: size exclusion limit; CFDA: 5-(and-6)-carboxyfluorescein diacetate; STM: SHOOT MERISTEMLESS; CMNB-caged fluorescein: Fluorescein bis-(5-Carboxymethoxy-2-Nitrobenzyl) ether, dipotassium salt; WT: wild type; KN1: KNOTTED1; MS2: a bacteriophage coat protein; SL: RNA stem loop; FT: Flowering locus T; SUC2: SUCROSE TRANSPORTER2; SUL: SULFUR; GLR: GLUTAMATE RECEPTOR-LIKE.

Table 2
Tools available to manipulate PD permeability.

TOOLS	EXAMPLES	MAIN STRENGTHS	MAIN WEAKNESSES	REF.
Related to callose metabolism	*iCALS3m* system spatiotemporally regulates the expression of a recombinant AtCALS3. Its induction allowed to inhibit SHR and *MIR165a* movement of from the stele to the endodermis and from the ground tissue to the vascular tissues, respectively.	- Decrease the SEL - Inducible - Reversible	- Pleiotropic effects *e.g.,* callose may cover cell wall and thus may affect apoplasmic pathways	Zuo et al. (2000); Vaten et al. (2011)
Related to other PD proteins	Overexpression of *AtPDLP5* is associated to callose accumulation at PD and a limitation of the PD permeability for several molecules including Ca^{2+} in Arabidopsis.	- Decrease the SEL - Inducible - Reversible	- Pleiotropic effects *e.g.,* callose may cover cell wall and thus may affect apoplasmic pathways	Lee et al. (2011); Toyota et al. (2018)
Related to non-PD proteins	*CHER1* encodes for a TGN located choline transporter. Its corresponding defective mutant exhibits a reduced pore density and an altered pore structure in sieve areas.	Enable to study PD protein composition by comparison with WT	- Defect in both PD number and structure - Pleiotropic effects *e.g.,* starch excess	Dettmer et al. (2014); Kraner et al. (2017a)
	ISE1 or *ISE2* encode for mitochondria and chloroplast RNA helicases, respectively. Silencing of one of them increases secondary PD formation in immature sink leaves and MP30-2xGFP cell-to-cell movement in *N. benthamiana* leaves.	Inducible	Pleiotropic effects, *e.g.,* - Alteration of organelle redox state - Defect in plastid structure (ISE2)	Stonebloom et al. (2009); Burch-Smith and Zambryski (2010); Burch-Smith et al. (2011a)
	GAT1 encodes for a plastid thioredoxin. Its ectopic expression increases GFP diffusion through PD while its corresponding mutant is affected in GFP unloading from the phloem into the meristem in Arabidopsis.	- Inducible - Increase/decrease the SEL	Pleiotropic effects *e.g.,* ROS accumulation in *gat1* mutant	Benitez-Alfonso et al. (2009)
	Maize KN1 is a non-cell-autonomous protein involved in development. Its expression in tobacco mesophyll cells increases PD SEL leading to its diffusion complexed with its corresponding anti-sense RNA.	- Increase the SEL - Inducible - Reversible	Pleiotropic effects *e.g.,* abnormal plant development	Lucas et al. (1995); Kragler et al. (1998)
Related to viral MPs	Expression of some viral MPs or of their plant orthologs increases PD SEL at least in part by regulating callose deposition at PD, leading to MP-RNA complexes diffusion.	- Increase the SEL - Can be inducible	No reversibility	Xoconostle-Cázares et al. (1999); Epel (2009); Zavaliev et al. (2011)
Obstruction or alteration of PD structure	Viral MP protein or non-cell-autonomous proteins such as KN1 complexed to 15nm gold particles prevent the increase of the PD SEL in *N. benthamiana* mesophyll cells.	Enable to study - PD permeability at steady-state SEL - SEL increase mechanism	- Need to generate gold-protein complexes - Microinjection is invasive	Kragler et al. (1998)
Pressure changes	The increase of turgor pressure differential between two cells impedes PD permeability: differentials of 200 kPa are sufficient to block PD permeability for LYCH (444 Da) in trichome cells of *Nicotiana clevelandii.*	- Instantaneous - Reversible - Almost absolute closure	- Microinjection is invasive - Pleiotropic effects *e.g.,* wounding response	Oparka and Prior (1992); Park et al. (2019)
ABA	ABA treatment triggers restriction of DENDRA2 (26 kDa) trafficking by decreasing PD SEL in *Physcomitrium patens.* Traffic of macromolecules increased in *P. patens* ABA-synthesis defective mutant, confirming the role of ABA in PD aperture, even at endogenous level.	- Fast (within 1hr) - Reversible (within 24 hrs) - Decrease/increase the SEL	ABA treatment might lead to pleiotropic effects, *e.g.,* stomata closure	Kitagawa et al. (2019)

CALS: CALLOSE SYNTHASE; PDLP: PLASMODESMAL-LOCALIZED PROTEIN; SHR: SHORT-ROOT; CHER1: CHOLINE TRANSPORTER-LIKE1; TGN: trans-Golgi network; *ISE: INCREASED SIZE EXCLUSION LIMIT*; MP: movement protein; GAT1: GFP ARRESTED TRAFFICKING; KN1: KNOTTED 1; LYCH: Lucifer yellow CH; ABA: Abscisic acid.

successfully been used to track movement of mobile transcription factors in roots (Clark et al., 2016). Recently, a minimally modified alkyne-tagged sucrose analog was shown to diffuse between BY2 cells (de Moliner et al., 2021). The synthesis of minimally modified sucrose analogs that can be used in Raman microscopy represents a major advance. However, since sucrose transporters mediate cellular uptake of the analogs it will be challenging to differentiate between flux through transporters versus PD. While it is conceivable that the observed differences in the timing of accumulation of the analog could be due to differences in PD connectivity between adjacent cells, more work will be required to evaluate this hypothesis (de Moliner et al., 2021). There is thus a need to develop new tools that allow tracking of cell-to-cell movement directly at the PD. Targeting of sensors to PD, for example sensors for sucrose, might be a potential means to detect sucrose movement across PD (Sadoine et al., 2021).

Investigating PD function and PD permeability requires approaches for manipulation (Table 2). Not surprisingly, mutants for PD located proteins or more generally for proteins involved in PD permeability often exhibit strong phenotypes and are embryolethal (Benitez-Alfonso et al., 2009; Stonebloom et al., 2009; Saatian et al., 2018). Although, several tools to spatiotemporally control the decrease of PD permeability by pressure changes or by callose deposition exist (Table 2), they require invasive approaches such as microinjection or the use of tissue specific inducible systems, which are not available for all species and tissues. Thus, the current adaptation of optogenetic tools to plants to control promoters of ion fluxes by light with a high spatiotemporal resolution offers new perspectives (Papanatsiou et al., 2019; Ochoa-Fernandez et al., 2020; Zhou et al., 2021).

Box 1
Nomenclature: Plasm or Plast

There are various forms used for components of cells and for cellular domains. Most will likely agree that a protoplast is a plant cell from which the cell wall has been removed by cell wall digesting enzymes. Similarly, when cell walls of yeast cells are removed, we use the term spheroplast. The protoplast contains the protoplasm (protoplasma; 1839 Johannes Evangelista Purkinje; 1787–1869; gelatinous fluid in living tissue; *Urschleim* in German), also called cytoplasm. These terms are over 100 years old (von Hanstein, 1880). Thus, unitary entities such as a plasma membrane-encapsulated unit rend on a 't', as in protoplast. Chloroplasts are entities ending on 't'. The cytoplasm consists of the cytosol, which had also been called hyaloplasm, plus the particles (named Kleinkörperchen by von Hanstein, 1880) or microsomata (microsomes, or better cell organelles and vesicles). PD connect the cytoplasm of most plant cells, creating a symplasm, as opposed to the continuous extracellular space (cell walls, xylem), which forms the apoplasm, in both cases not entities but both a plasm(a), a continuous liquid domain either inside or outside of plant cells. There is also some confusion regarding the term tonoplast. According to the definitions above, the vacuole is equivalent to the tonoplast, while its content would be a -plasm, *i.e.*, the vacuolar protoplasm. However, the term tonoplast has, already early on, also been used for the membrane that encapsulates the vacuole (Weber, 1932).

Eduard Tangl is considered the first to recognize the importance of the structures underlying the cell-cell connections that we now call PD. Tangl had named them Protoplasmafortsätze (protoplasm extensions), Eduard Strasburger originally used the German term *Plasmafäden* (plasma threads). In 1901, he coined the German term *Plasmodesmen* (Strasburger, 1901). Based on the ethymology, a single one is called plasmodesma (sometimes plasmodesmos), plural plasmodesmata.

Note that much of the original literature was in German, nevertheless you will frequently find that the word symplast and apoplast are used, also historically.

16. Summary and outlook

While extensive progress has been made regarding composition, structure and function of PD, our understanding of most aspects including composition of PD is in its beginnings, a blueprint is missing, and we have no reliable structural model of PD. Also, the transport mechanisms remain elusive, in part since the assays often just identify accumulation of a substrate in an adjacent cell, not an actual passage through PD. Thus there is a lot to be learned with novel approaches and technologies; single cell transcriptomes may help to target PD more specifically (Kim et al., 2021), base editing may enable finer tuning of PD activities with the help of *knock down* approaches (Monsur et al., 2020), biosensors may help to measure transport through PD in a more direct way (Okumoto, 2012) and cryo-electron tomography as used for example for resolving the structure of nuclear pores is among the most promising tools (Beck et al., 2007; Mosalaganti et al., 2018).

Greek (and New Latin) etymology (www.etymonline.com/)

protos: first
plásma: moldable substance, the liquid part of blood, etc., as distinguished from the corpuscles
hyalos: glass, clear alabaster, crystal lens used as a burning glass (as in hyaloplasma)
kytos: a hollow, receptacle, basket (as in cytoplasm)
desmá: band, bond, ligament (as in plasmodesmata)
apo-: word-forming element meaning "from, away from; after; in descent from" (apoplasm)
syn-; sym-: word-forming element meaning "together with, jointly; alike; at the same time' (symplasm)

CRediT authorship contribution statement

Manuel Miras: conceived of the review, wrote and edited large sections. **Mathieu Pottier:** analyzed literature, wrote and edited sections. **T. Moritz Schladt:** analyzed literature, wrote and edited sections. **J. Obinna Ejike:** analyzed literature, wrote and edited sections. **Laura Redzich:** performed the analysis of the PD proteome and generated the database. **Wolf B. Frommer:** conceived of the review, wrote and edited large sections. **Ji-Yun Kim:** conceived of the review, wrote and edited large sections. MM, JYK and WBF did most of the editing during the revision process. All authors contributed to the generation of the Tables and Figures.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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