Plasmodesmata in Phloem: Different gateways for different cargoes

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Highlights:

- Plasmodesmata (PD) occurrence and morphology vary greatly within the phloem
- Phloem-mobile molecules likely represent systemic signals and superfluous escapees
- Funnel shaped novel PD direct 'batch unloading' of proteins at the phloem terminus
- Batch unloading may present a novel mechanism for sorting out bona fide signals

Abstract

The long-distance transport of sugars and nutrients through the phloem is essential for the proper function and growth of vascular plants. However, in addition to essential nutrients and sugars, phloem sap also contains small molecules (e.g. hormones) as well as a diverse

population of macromolecules (i.e. proteins small RNAs, and mRNAs), the endogenous functions of which remain largely unknown. Understanding the cellular origins of these mobile macromolecules, their path into and out of the phloem translocation stream, and their fate at their new destination is essential for characterizing their presumptive function. Specialized plasmodesmal connections that regulate phloem entry and exit are central to all of these processes. Here, we highlight new discoveries underscoring plasmodesmal structure and function during unloading of various molecules in the sink, and discuss how these findings shape a new view for the potential function of phloem-mobile macromolecules.

[intro]

Plasmodesmata (PD) are fundamental intercellular structures that allow nutrients and signaling molecules to be transported amongst cells, and within tissues and organ systems. In addition to possessing short-range transport through PD, vascular plants have the ability to perform inter-organ, long-distance transport through the xylem and phloem systems. Xylem mainly conducts water and minerals from roots to shoots along the transpiration stream whereas phloem transports sugars, nutrients, and various macromolecules, including proteins and RNAs from source to sink tissues and organs [1,2] (Fig. 1A). The vasculature is enclosed by one or more layers of compactly arranged cells—bundle sheath in leaves and the pericycle in stems and roots—which together regulate phloem entry and exit of various molecules.

Phloem consists of conducting sieve elements (SEs), as well as non-conducting companion cells (CCs) and phloem parenchyma [3,4]. SEs are the most highly specialized cells in the phloem, having sieve areas and sieve plates: wall areas with pores through which adjoining SE cells are laterally or vertically interconnected, respectively. The sieve plates provide a means to join individual sieve elements end-to-end to form the sieve tube system (STS), the conducting system in the phloem. The sieve plate pores facilitate a mass flow of phloem content along the STS. In the process of differentiation, SEs undergo enucleation and lose some additional organelles, becoming metabolically dependent on associated nucleate CCs. SEs and CCs are connected through distinctively shaped PD and function as a complex, however, only mature SEs serve as true conducting cells of the STS. In actively growing shoot or root tips, protophloem sieve elements (PSE) serve as the phloem terminus where phloem content is released into recipient cells in the sink (Fig. 1B).

At the minor veins of source leaves, solutes can be loaded into the CC-SE complex from surrounding cells, by moving either symplasmically through PD or apoplasmically via specific membrane transporters. However, specialized PD always connect CCs and SEs whether or not phloem loading occurs apoplasmically or symplasmically. Regardless of the loading mechanism adopted by a particular plant species, phloem contents are always unloaded symplasmically from PSE into recipient sink cells through PD (Fig. 1B). Phloem cell interfaces vary broadly in terms of PD frequency and morphology, and this restructuring of PD may be linked to the strategies that each plant species utilizes for phloem-mediated long-distance transport [5]. Here we discuss how structurally distinct PD correlate with the phloem functions of loading, transport, and unloading of mobile macromolecules. Moreover, we highlight recent findings that provide significant new insight into how uniquely shaped PD occurring at the interface between PSE and phloem pole pericycle (PPP) cells may contribute to the unloading of phloem mobile molecules in root tips.

Remodeling of PD aids phloem function

PD are essential for direct molecular movement within clonal cells and between non-clonal cell layers in almost all plant tissues and organs. The density, morphology, and aperture of PD are dynamic properties that become re-patterned over developmental time and in response to abiotic and biotic challenges [6,7]. These changes in PD patterning correlate with the extent as well as the direction of molecular movement across cellular junctions in most tissues. However, there is no tissue in which this dynamic patterning is illustrated more clearly than in the phloem, wherein PD are precisely remodeled to support all aspects of phloem form and function (Fig. 1B). For example, individual PD formed between CC and SE cells develop into highly specialized asymmetric PD called the pore-plasmodesmata units (PPUs) as the CC-SE complex matures. Also, PD at the end walls of joined individual SE cells become restructured and enlarged to form sieve plate pores (SPPs). Following asymmetric division, SEs undergo a maturation process that involves the restructuring of PD, as well as disintegration of the nucleus and some of the organelles [8]. Loss of these organelles leads to a metabolic reliance of SEs on CCs. This cellular relationship is supported through the development of asymmetrically shaped PPUs that consist of wide and unbranched pores on the SE side and branched pores on the CC side (Fig. 1). PPUs extend across the middle lamella through expanded median cavities. PD also undergo considerable remodeling at the sieve plates, transverse walls joining SEs vertically in the STS; they lose internal structures of PD and become large pores that are sufficiently wide to support heavy flow of phloem exudate, yet small enough to effectively be sealed off when necessary. In addition to these structural modifications of existing PD, PD can be obliterated as well to aid phloem function. For instance, in main veins, PD connecting STS cells (CC-SE complex) and surrounding parenchyma cells are either absent or sealed off, which is thought to aid in minimizing solute leakage along the STS mass transportation pathway [9-11].

Asymmetric PD for macromolecular loading

Each PPU, which are formed at CC-SE junctions, consists of branched PD strands on the CC side that are merged with a large pore on the SE side (see Fig. 1B). The asymmetric morphology of PPUs may impose structural constraints that regulate which macromolecules are able to cross the CC-SE junction. It is also possible that the multiple PD strands on the CC side allows PPU to keep up with the large traffic load, which is enabled by the enlarged pore sizes that are formed along the SE side. PPUs exhibit a large molecular size exclusion limit (SEL) as well, allowing diffusion of macromolecules up to 70 kDa in size [12]. This large SEL might be necessary for the phloem loading and unloading of numerous proteins including transcriptional regulators, RNA-binding proteins, and phloem-resident proteins, as well as various types of RNA species [1,13]. Very little is known regarding the mode by which individual proteins and/or RNA molecules enter into the phloem translocation stream; however, the long-distance signaling molecule, FLOWERING LOCUS T (FT), which moves via active gating of PD in non-phloem cells, was shown to enter via diffusion [14]. Perhaps molecules that are larger than 70 kDa require active gating in order to move through PPUs, while smaller molecules are able to load into the STS via diffusion.

Knowledge regarding phloem-mobile RNAs is based around studies that were performed both in apoplasmic (e.g. Arabidopsis [15]) and symplasmic (e.g. Cucurbits [16]) loaders. In plants that are exclusively apoplasmic in phloem loading, PD connections between pre-phloem cells and the CC-SE complex are typically sparse [17]. Unlike sugars and ions which can cross cell membranes through specific membrane transporters as well as move between cells through PD, macromolecules produced in pre-phloem cells most likely require passing through PD between cells to reach the CC-SE. Therefore, in those plants having few PD connections between the CC-SE and surrounding cells, it is possible that most of the phloem-loaded macromolecules originate from CCs. Furthermore, PPUs allow large free macromolecules (up to 70 kDa in size) to diffuse into the STS; thus, the majority of molecules found in the phloem stream may simply be the product of macromolecular leakage from CCs. This scenario does not exclude the possibility that genuine phloem-mobile molecules carry specific signal(s) that allow them to cross PPUs; however, it is unlikely that these signals are necessary for small macromolecules that are under the PPU SEL to enter into the STS via diffusion. Rather, those may be critical for macromolecules to exit the terminal phloem and move cell to cell in the sink to reach target cells.

Recent findings indicate that the phloem is likely carrying a mix of bona fide signals and superfluous or highly abundant RNAs that are loaded into the translocation stream. On one hand, mRNA abundance in source CCs and transcript half-life have been shown to correlate with the long-distance mobility of transcripts in Arabidopsis, supporting a model in which transcripts passively enter the STS through PPUs [18]. On the other hand, RNAs have been shown to move through the phloem translocation stream via selective mechanisms that rely on RNA structures (e.g. tRNA-like structures) and/or sequence specificity to determine transcript mobility [19,20]. Furthermore, these studies show that mobile transcripts can be translated in sink cells, indicating that they maintain molecular integrity during transit and are recognized by the translational machinery in target cells [15,19] (Fig. 2). If the phloem is indeed carrying a mix of signaling and superfluous RNAs, then this would imply that there is a sorting process separating signals from noise during phloem unloading. We discuss one potential mechanism for this process at the end of this review.

Macromolecules crossing graft unions and haustorial interfaces: new insights

It has long since been known that macromolecules (proteins, mRNAs, and sRNAs) move through the phloem translocation stream [1]. However, the recent application of high throughput sequencing to plant heterograft combinations provides new insights into the depth and diversity of long-distance macromolecular transport (RNAs in particular). Heterografting studies in Arabidopsis [15], Cucurbits [16], and grapes [21], as well as parallel studies looking at plant host-parasite trafficking in Cuscuta [22], all demonstrate that thousands of distinct RNA species move over long distances, crossing inter-specific graft unions or host-parasite haustoria [23]. Both the massive scale at which mRNAs are mobilized as well as the consistency of this trend across diverse species raise the question of whether there is a generalized function for these mobilized macromolecules.

While the function for the vast majority of mobile mRNAs remains to be solved, there is accumulating evidence demonstrating that small RNAs are capable of moving and functioning across graft junction and/or haustorial boundaries. A recent study tracking sRNAs moving from grafted shoots into roots, demonstrated that mobile small RNAs are able to influence genome-wide DNA methylation patterns in recipient root cells [24] (Fig. 2). Moreover, these mobile small RNAs were shown to predominately target loci associated with transposable elements in roots, the methylation of which may promote genome stabilization. By investigating RNA mobility between the plant parasite Cuscuta and its hosts, another study demonstrated that Cuscuta generates miRNAs that target and trigger the degradation of host mRNAs, and the knockdown of these mRNAs facilitates parasitic plant success [25] (Fig. 2). Intriguingly, the directionality of miRNA movement in this study goes against the presumed host-parasite source-sink gradient. The potential mechanism that enables RNAs to move against the direction of bulk phloem flow is an active point of discussion [23].

Asymmetric PD for batch unloading

In the root tip, phloem continuity between source and sink tissues is established through the transfer of solutes from the terminal metaphloem SEs (MSEs) laterally into the PSEs [26]. The large number of macromolecules found in the phloem appear to be loaded from CCs into SEs in a non-discriminating manner, in part due to the large SEL of the PPUs (Fig. 1B). Until recently, there were two open questions regarding the fate of these mobile macromolecules: first, whether they are unloaded into the sink tissue via the same path through which they entered (from PSEs into CCs), and second, what becomes of macromolecules that are not transported into surrounding sink cells. New studies now provide surprising insights into these questions. Using GFP-tagged proteins as reporters for protein mobility across graft unions or loading into the STS from CC in Arabidopsis*,* it was found that even organelle-targeted proteins expressed in shoots can escape into the phloem stream, are unloaded into pericycle cells in the root tips, and are subcellularly targeted to their organellar destination in sink cells [27]. In a follow-up study, the authors showed that the majority of macromolecules that are unloaded from PSEs in Arabidopsis roots are laterally diverted into PPP cells, two parenchyma cells outwardly bordering PSE, and that the unloading occurred primarily via the PPP, not CC [11]. Intriguingly, while molecules larger than 40 kDa were trapped in PPP cells and appeared to undergo degradation, those that were smaller than 40 kDa as well as various solutes were spread symplasmically into all cell types in the sink. (Fig. 2). The finding that CCs are not involved in phloem unloading from PSEs raises interesting questions regarding molecular trafficking across PPUs at the CC-PSE interface. Do those PPUs remain open, supporting continuous molecular movement from CCs to PSEs? Or are they only transiently open, thus ceasing functional transport shortly after PSE maturation?

The special role of the PPP-PSE junction for unloading large molecules appears to be strongly correlated with the finding that PD at the PSE-PPP junction exhibit a previously unrecognized asymmetric morphology. The PD at this junction form a funnel shape that has a wide opening (~150 nm) at the PSE side and tapers towards the PPP [11] (see Fig. 1B). Mathematical modeling demonstrates that this funnel-shaped PD morphology can support efficient phloem unloading into the PSE at a lower pressure differential than what was previously assumed to be required for unloading through PD with simple forms. The presence of this novel mode of unloading, termed ‘batch unloading’ (i.e., that this occurs in pulses, rather than a continuous outflow), suggests that the majority of macromolecules that are loaded into the phloem stream may exit the terminal PSE but not necessarily be unloaded into the surrounding sink tissue. Once discharged into the PPP, those molecules may be resorted and re-mobilized into new sink cells or degraded into their basic building blocks (Fig. 2). Here, a series of tantalizing questions arise – How do signaling macromolecules evade degradation in the PPP? Do they follow the batch unloading path and enter PPP before they reach their target cells within the sink, or do they take an alternative route (e.g. via CCs) and evade potential destruction in PPP? Do they have specific structures/motifs protecting them from degradation in the PPP if they follow the batch unloading path?

Future perspectives

Why do plants invest so much energy and resources into long-distance macromolecular transport? One possibility is that some mobile macromolecules are true long-distance signals while others function to meet the metabolic needs of the actively developing sink, essentially serving as building blocks for transcription and translation (see Fig. 2). A nutritional role for superfluous macromolecules is logical, as it is much more efficient to recycle RNAs and proteins supplied by source tissues into essential building blocks in the active sink than to synthesize amino acids and ribonucleotides *de novo*. Another potential benefit of transporting superfluous macromolecules through the STS is that their presence in the phloem may aid in the bulk transport, protection, and delivery of signaling molecules. The batch unloading process at the phloem terminus can then function as a site for sorting out superfluous nutritive macromolecules from true signals. In this view, RNAs and proteins that are not destined to serve as signaling molecules may be utilized as building blocks in sink cells. Future experiments that track the origin, transport, and destination of mobile macromolecules will be crucial for deciphering the putative functions of these enigmatic RNAs and proteins. Moreover, manipulations of PD morphology and size at the PSE-PPP boundary will help in determining the role of batch unloading in sorting macromolecular signals from “noise”.

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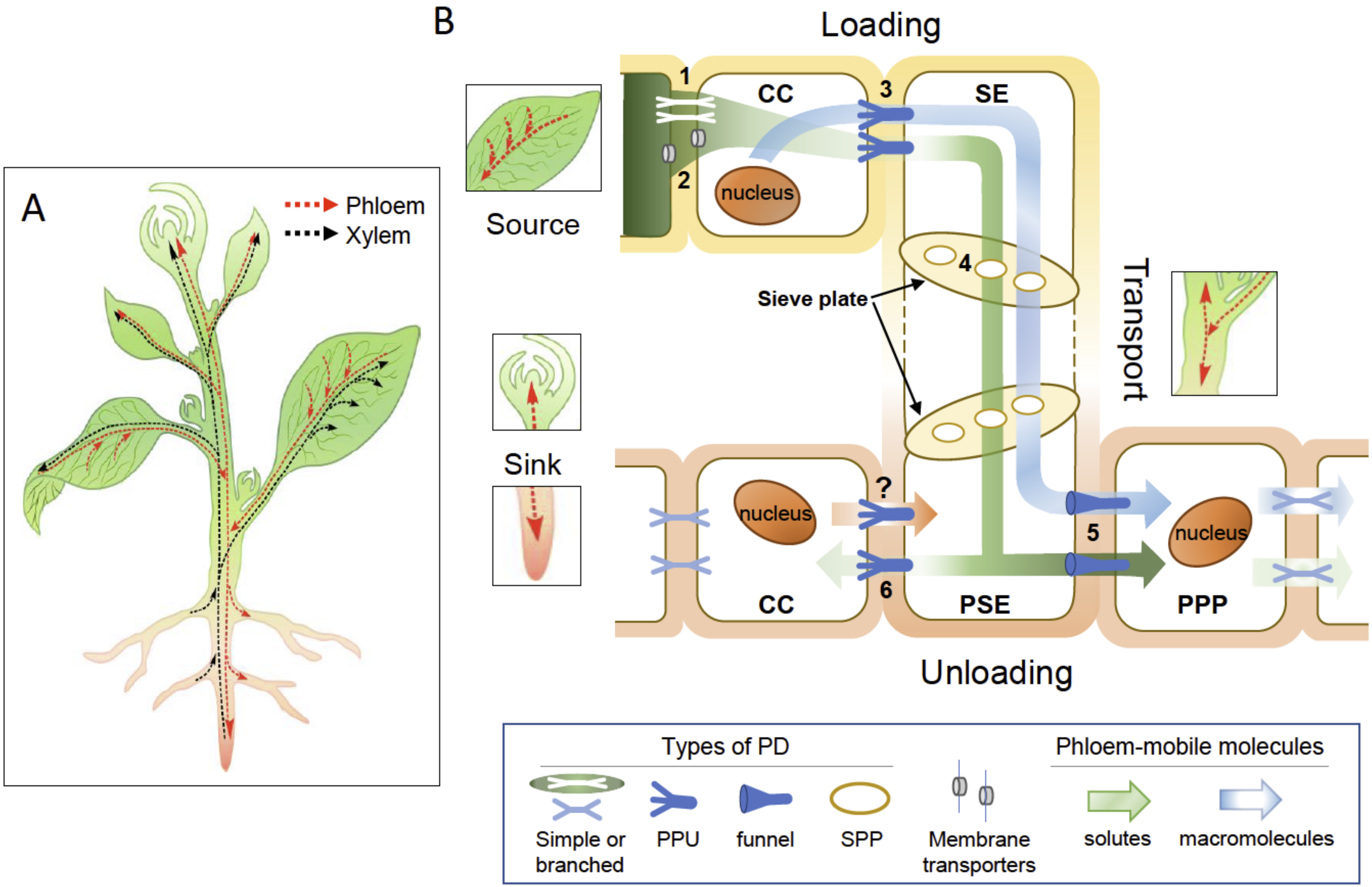
* Fluorescently tagged proteins that are targeted to organelles escape into the phloem translocation stream

Figure legends

Figure 1. Phloem loading, transport, and unloading processes are supported by functionally diverse PD. A. An overview of phloem (red) and xylem (black) transport paths for a typical flowering plant. **B.** An in-depth illustration of the cellular pathways that feed into phloem loading (from source leaves), long-distance transport through the main veins, and unloading (at sink tissues). Loading of solutes into the phloem translocation stream can occur either symplasmically through PD **(1)** or apoplasmically via membrane transporters **(2)** (green-shaded arrow). In source tissues, solutes (green arrow) and macromolecules (blue arrow) that are present in companion cells (CCs) can freely diffuse through pore-plasmodesmata units (PPUs) to enter into sieve elements (SEs) for long-distance transport **(3).** Following mass transport through SEs, which are connected end-to-end with specialized sieve plate pores (SPPs) derived from PD **(4),** phloem contents are unloaded into sink cells mainly through phloem pole pericycle (PPP) cells **(5).** Large macromolecules and small solutes are unloaded into PPP through funnel-shaped PD positioned along the protophloem sieve element (PSE)-PPP interface **(5).** Small amounts of solutes may also be unloaded into CC through PPUs **(6);** whether molecular traffic can move from CC into PSE through PPUs remains to be determined.

Figure 2. A model for macromolecular sorting at the phloem terminus. At the phloem terminus, mobile macromolecules that are transported through the sieve tube system (STS) exit into lateral PSEs. Large (>40 kDa) and small (<40 kDa) macromolecules (RNAs and proteins) enter the PPP through funnel-shaped PD (Step 1). Once unloaded into PPP, proteins and transcripts that are not protected signaling molecules are degraded into free nucleotides and amino acids. These “building blocks” can diffuse into neighboring sink cells (Step 2), where they are recycled into new RNAs and proteins (Step 3). Mobile small RNAs may be protected from degradation in PPP and move cell-to-cell via PD to reach target sink cells where they act to direct DNA-methylation of target loci in the nucleus (N). In addition to unloading into PPP, small amounts of solutes and macromolecules (potentially, signaling molecules moving via active gating of PD) may be unloaded into CC through PPUs. In CC, the latter may not undergo degradation process but instead, move as intact molecules through local PD connections into target cells.

Figure 1


A
Phloem
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Simple or branched
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Membrane transporters
Phloem-mobile molecules
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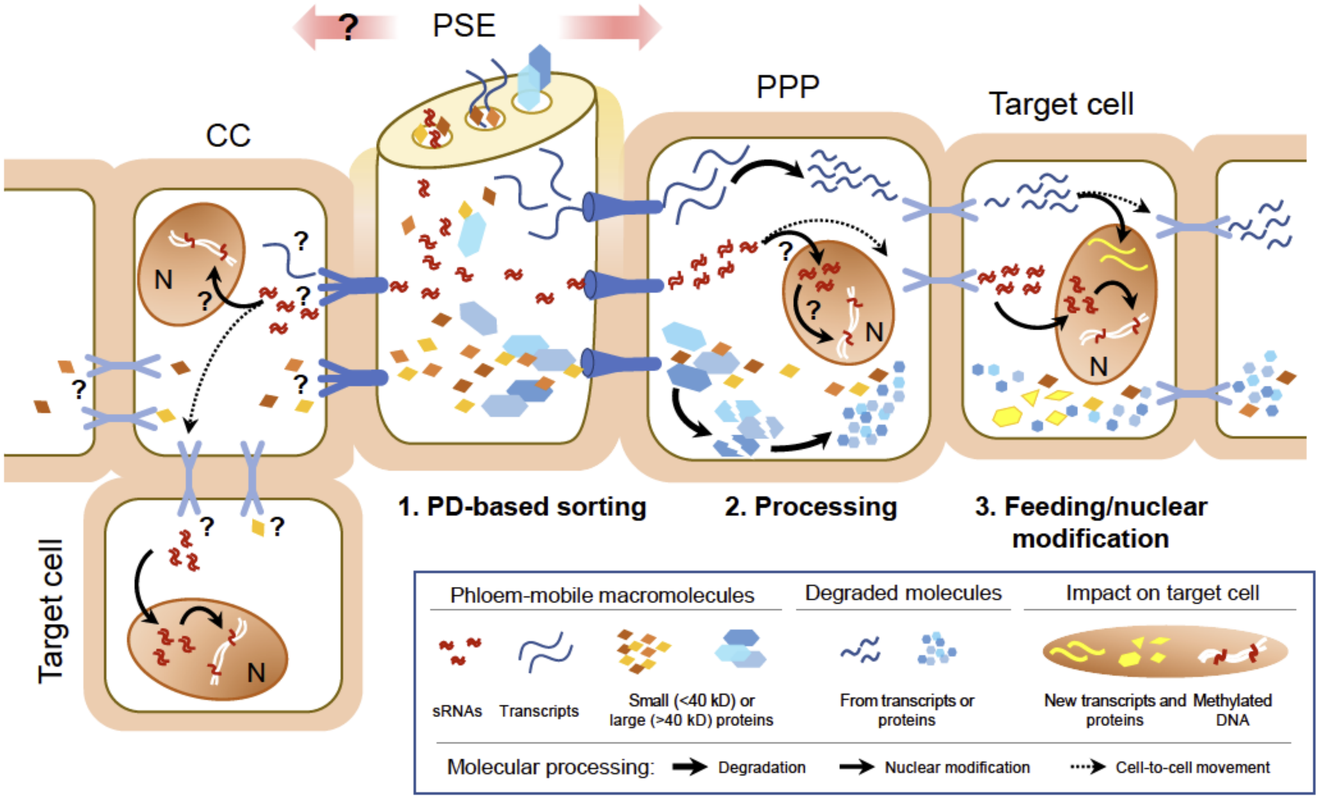
Figure 2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1. PD-based sorting
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3. Feeding/nuclear modification
Phloem-mobile macromolecules
Degraded molecules
Impact on target cell
sRNAs
Transcripts
Small (<40 kD) or large (>40 kD) proteins
From transcripts or proteins
New transcripts and proteins
Methylated DNA
Molecular processing:
Degradation
Nuclear modification
Cell-to-cell movement