
Article:
Amsbury, S and Benitez Alfonso, Y orcid.org/0000-0001-9779-0413 (2019) Tightening the pores to unload the phloem. Nature Plants, 5 (6). pp. 561-562. ISSN 2055-026X

https://doi.org/10.1038/s41477-019-0450-8

Strapline (i.e broad area): phloem unloading and plasmodesmata regulation

Title: Tightening the pores to unload the phloem

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Standfirst (mini abstract 250c/50w): Root growth depends on the shoot-to-root transport of assimilates via the phloem, which is connected to the meristems by plasmodesmata pores. A mutation in the *Phloem Unloading Modulator* gene is now identified to regulate plasmodesmata internal membrane organisation leading to pores that appear tighter but that are more efficient for transport.

Main Text (800-900):

Phloem loading and unloading, the process by which solutes enter and exit the phloem, is highly important for the transport of photoassimilates from source to sink tissues. This process is controlled by different cellular pathways connecting the phloem and the neighbouring tissues and that provide selectivity while maintaining high solute flow[1]. It has been shown that the predominant unloading pathway is from the phloem Sieve Elements (SE) to the Phloem Pole Pericycle (PPP) although a much-less well characterized bottleneck (restriction) at the interface of the PPP and endodermis has been observed[2]. Concerning this, in a paper published by Nature Plants, Yan et al., reports the identification of Phloem Unloading Modulator (PLM), a protein that regulates the structure of plasmodesmata and phloem transport from the PPP to the endodermis in *Arabidopsis thaliana* roots[3].

Plasmodesmata are channels formed by a portion of the plasma membrane (PM) traversing cell walls[4] (Fig. 1). In the middle they contain an ER tubular structure (desmotubule) connected to the PM by spoke-like tethering elements. The space between the PM and the desmotubule, referred to as the cytoplasmic sleeve, allows for symplastic (cytoplasm-to-cytoplasm) molecular transport between neighbouring cells. Multiple strands of evidence indicate that plasmodesmata play a role in controlling phloem unloading, including the characterization of mutants in callose metabolism[5-7]. Phloem unloading via plasmodesmata is assessed using the reporter p*SUC2*-GFP which is expressed in the phloem companion cells, from where GFP can move by plasmodesmata-mediated diffusion into the neighbouring tissues and throughout the root meristem. Cell wall composition around plasmodesmata (specifically the accumulation of the cell wall polysaccharide callose) and structural states of the pores (simple, branched, etc.) determine molecular flow and thereby symplastic communication[8]. Mutations altering the regulation of Callose Synthase 3 (*cals3-d* and *cals3m*) accumulate callose at plasmodesmata which restricts GFP unloading and, not surprisingly, root growth[7].

Plasmodesmata frequency (number per cell wall area) and, more recently, membrane features such as sterol content and certain type of sphingolipids also appear to play a role in the regulation of symplastic transport[9]. The mechanisms underlying plasmodesmata formation and structural development remain unknown but analysis by electron tomography of Arabidopsis root meristems revealed plasmodesmata that appear 'opaque' (lately named type I) with the PM and the ER/desmotubule membranes in intimate contact and no visible electron-lucent cytoplasmic region or spoke-like elements connecting the two membranes[4] (Fig.1). Since the cytoplasmic sleeve is assumed to be the space available for transport, the observation of 'opaque' plasmodesmata in meristem tissues (which are notable for their high symplastic transport capacity) remains puzzling. This recent collaboration between the E. Bayer and Y. Helariutta teams shed some light on the significance of these observations in the context of phloem unloading[3].

PLM was identified as a suppressor of the *cals3-1d* mutant allele phenotype[3]. *PLM* encodes a plant-specific enzyme, localized in the ER and involved in sphingolipid synthesis. In *plm*, there was a significant decrease in both ceramides and glycosylinositol phosphoceramides (GIPCs), a lipid class found enriched at plasmodesmata membranes[9]. There were no widespread changes in other polar lipid species indicating that the *plm* mutation specifically disrupts sphingolipid metabolism. In *cals3-d,plm1* double mutant roots expressing p*SUC2*-GFP, phloem unloading and root growth was partially restored but, interestingly, this was not due to a reduction in callose levels or to plasmodesmata frequency. In *plm* loss-of-function, only type I plasmodesmata (with a tight cytoplasmic sleeve) were found in the PPP-endodermis interface whereas in wildtype there was an equal contribution of type II. In spite, the single *plm* mutant display an increase in phloem unloading. These and other results led the authors to conclude that PLM function in regulating the composition of plasmodesmata membranes, which determines, directly or indirectly, the transition from type I to type II plasmodesmata in the PPP-endodermis interface without interfering with plasmodesmata branching, frequency or with cell wall composition (Fig. 1). Importantly, these findings demonstrates, for the first time, that altering type I to type II transition leads to interfaces that are more permeable to symplastic trafficking, despite the narrower or non-existent cytoplasmic sleeve observed in type I plasmodesmata. How and why plasmodesmata lacking a cytoplasmic sleeve are more conductive is still unknown and it is not clear whether this increase in trafficking is equal for both small and large molecules. The lack of visible spokes in type I plasmodesmata could allow larger membrane deformations to accommodate the rapid movement of large molecules (as presented in Fig.1) but it is unclear how the cell wall constraints are overcome for these deformations to occur. We speculate that changes in membrane composition can determine the localization, or delocalization, of proteins that modify plasmodesmata permeability by, for example, changing cell wall architectures or membrane mechanics or acting as chaperons that structurally modify larger proteins for intercellular movement (Fig. 1).

The *plm* mutations demonstrate that plasmodesmata regulation and cell wall/ callose modifications can be unlinked and also suggest that there are unrelated factors controlling ER-PM tethering and plasmodesmata morphology (simple or branched). It also highlights how little we know about these extremely important but yet mysterious structures and open new avenues to target membranes as key components for the localization, and re-localization, at plasmodesmata of membrane signalling proteins that play significant roles in plant development and environmental responses.

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Competing interests

The authors declare no competing financial interests.

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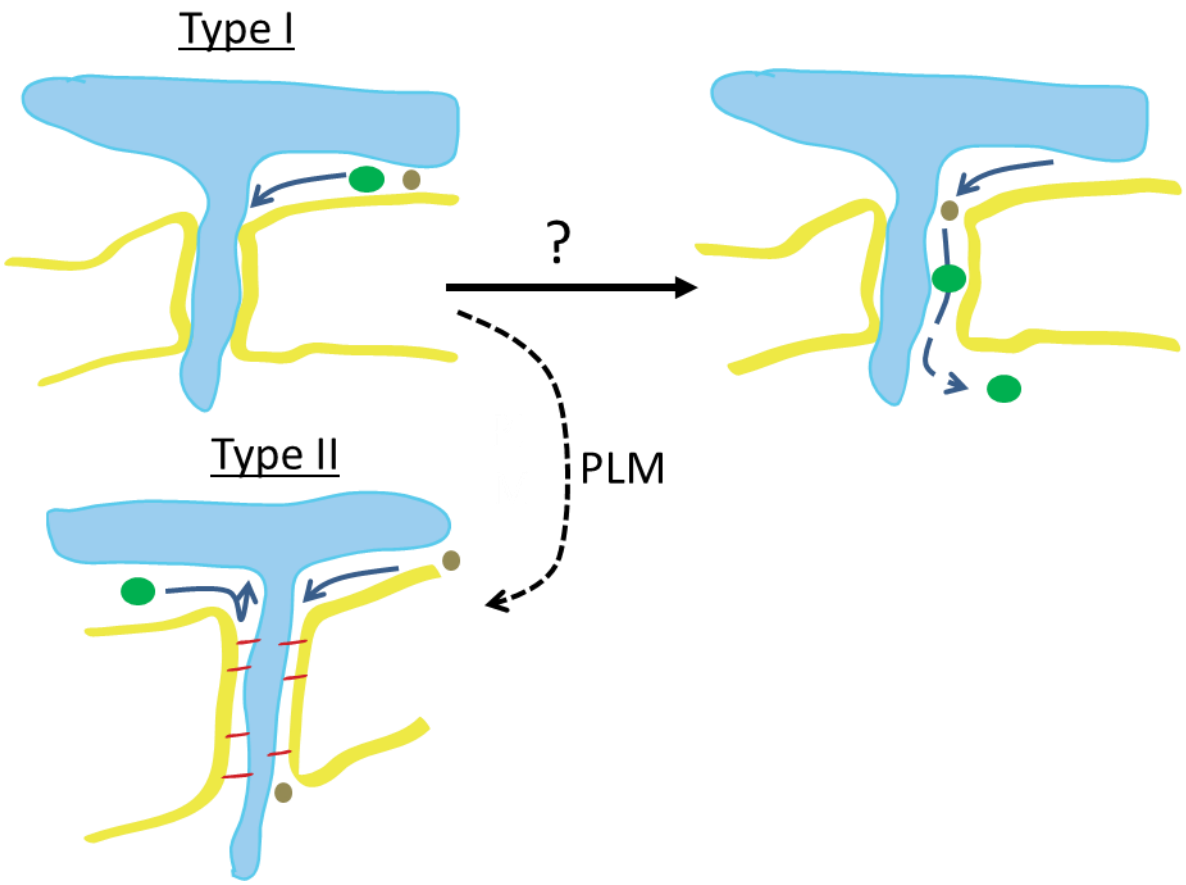
References

1. Milne, R.J., Grof, C.P., Patrick, J.W. Mechanisms of phloem unloading: shaped by cellular pathways, their conductances and sink function. *Curr Opin Plant Biol*, **43**, 8-15 (2018).

2 Ross-Elliott, T. J. *et al.* Phloem unloading in Arabidopsis roots is convective and regulated by the phloem-pole pericycle. *Elife* **6**, doi:10.7554/eLife.24125 (2017).
3 Yan, D. *et al.* PLM modulates phloem unloading through sphingolipid biosynthesis and plasmodesmal ultrastructure. *Nat Plants* **X**, X-X (2019).
4 Nicolas, W. J. *et al.* Architecture and permeability of post-cytokinesis plasmodesmata lacking cytoplasmic sleeves. *Nat Plants* **3**, 17082, doi:10.1038/nplants.2017.82 (2017).
5 Benitez-Alfonso, Y. *et al.* Control of Arabidopsis meristem development by thioredoxin-dependent regulation of intercellular transport. *Proc Natl Acad Sci U S A*. **106**(9), 3615-20 (2009).
6 Barratt, D.H.P. *et al.* Callose Synthase GSL7 Is Necessary for Normal Phloem Transport and Inflorescence Growth in Arabidopsis. *Plant Physiology* **155**, 328-341 (2011).
7 Vaten, A. *et al.* Callose Biosynthesis Regulates Symplastic Trafficking during Root Development. *Developmental Cell* **21**, 1144-1155 (2011).
8 Amsbury, S., Kirk, P., Benitez-Alfonso, Y. Emerging models on the regulation of intercellular transport by plasmodesmata-associated callose. *J Exp Bot*. **69**(1), 105-115 (2017).
9 Grison, M. S. *et al.* Specific membrane lipid composition is important for plasmodesmata function in Arabidopsis. *Plant Cell* **27**, 1228-1250, doi:10.1105/tpc.114.135731 (2015).

Figure 1: Type I and Type II plasmodesmata differ in symplastic conductivity. The model shows simple plasmodesmata resembling those observed using electron-tomography by Yan et al, 2019 in the Phloem Pole Pericyle (PPP)- endodermis interface of *Arabidopsis thaliana* roots. Plasma membrane (PM) is drawn in yellow, desmotubule in blue and spokes-like elements connecting the two are shown in red. In type I plasmodesmata there is not a visible cytoplasmic space available for transport between the desmotubule and the PM, whereas in type II the space is available but appears interrupted by spoke-like elements. We propose that, at least in the PPP- endodermis interface, Type I plasmodesmata are more efficient for the transport of small and large molecules because their membrane composition allows either larger deformations or the localization of proteins that actively modify channel aperture, for example, by interfering with the physical constriction of cell walls. Transition to Type II, partially determined by Phloem Unloading Modulator (PLM), restricts the mobility of large molecules such as GFP, perhaps due to interference by the spoke like elements or to other factors associated with changes in membrane and/or cell wall structure and mechanical properties.


Type I
?
Type II
PLM