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Organelles-nucleus-plasmodesmata signaling (ONPS): an update on its roles in plant physiology, metabolism and stress responses

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Plasmodesmata allow movement of metabolites and signaling molecules between plant cells and are, therefore, critical players in plant development and physiology, and in responding to environmental signals and stresses. There is emerging evidence that plasmodesmata are controlled by signaling originating from other organelles, primarily the chloroplasts and mitochondria. These signals act in the nucleus to alter expression of genetic pathways that control both trafficking via plasmodesmata and the plasmodesmatal pores themselves. This control circuit was dubbed organelle-nucleus-plasmodesmata signaling (ONPS). Here we discuss how ONPS arose during plant evolution and highlight the discovery of an ONPS-like module for regulating stomata. We also consider recent findings that illuminate details of the ONPS circuit and its roles in plant physiology, metabolism, and defense.

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Introduction

Plasmodesmata (PD) are intercellular membrane-lined cytoplasmic pores that are essential for plant growth, development, and defense [1–3]. PD, while being distinct cellular structures subject to their own regulatory processes, are actually composed of an amalgam of other cellular components: the plasma membrane (PM) delimits the outer boundaries of the pore, the central desmotubule is formed by an appressed endoplasmic reticulum (ER) strand that is continuous between the connected cells, and the cytoplasmic continuum that is the main route for the trafficking of soluble molecules between cells [4]. Subtending all these are the cell walls of the connected cells that surround each pore. Identification of plasmodesmal proteins [5,6], and characterization of the lipid components of PD membranes [7] have contributed significantly to advancing our understanding of PD and how they function to not only traffic solutes such as sugars and signaling molecules including hormones, RNA, and proteins, but also to participate in signaling modules that mediate responses to the environment including during pathogen defense [3].

Despite their importance to plant survival and their myriad functions, PD remain among the least understood structures in plants. PD that form at cell division when strands of the ER are sheathed in the nascent cell wall are primary PD. PD may also form across existing walls in the absence of cell division, and these are called secondary PD. Reflecting their importance to plant function, PD structure and function vary temporally and spatially during plant development, as exemplified by changes to PD during the sink-to-source transition and in response to tissue differentiation [8]. There is accumulating evidence that specialization of PD structure is requisite for specialization of PD function. One recent example comes from the PD in the root pole pericycle that adopt a 'funnel' structure that facilitates phloem unloading [9].

The best characterized mechanism for regulating PD trafficking in response to developmental and environmental signals is the controlled deposition and/or removal of callose, a β-1,3-glucan, in the cell wall surrounding the pore [4]. Callose accumulation effectively constricts the pore, inhibiting intercellular trafficking. During normal development callose is often deposited at PD when it is necessary to sequester a subset of cells from their neighbors to allow the execution of a specialized development program [10–12]. Several proteins responsible for callose accumulation, callose synthases/glucan synthases (CalS/GSLs), have been identified and CalS1, 8 and 10 specifically deposit callose at PD [13]. Removal of callose is mediated by β-1,3-glucanases, and several localize to PD [14]. Perhaps the best studied of these is β-1,3-glucanase putative plasmodesmal associated protein (BG-ppap) that constitutively localizes to PD and mediates callose dynamics during various stresses including virus infection [15]. There are other mechanisms for regulating PD function besides callose. The plant cytoskeleton components actin and myosin can exert influence over PD permeability [16–18]. Phosphorylation of viral proteins and other non-cell-autonomous proteins can also regulate

their transit through PD [19], raising the possibility that PD-resident kinases may also modulate intercellular flux [20,21]. Finally, we and others have discovered a new role for other organelles, particularly chloroplasts and mitochondria, as critical regulators of PD biogenesis and function [22–24]. Here we discuss the model we have developed for organelle regulation of PD, dubbed organelle-nucleus-PD signaling (ONPS). We frame our discussion in the context of how such a system could have evolved, and then consider new evidence that supports the existence of the ONPS module. We also highlight areas for future research expenditure that would not only help test the ONPS hypothesis but could also greatly advance our understanding of the essential PD.

Developing the ONPS hypothesis

Analyses of mutants with defects in intercellular trafficking led to the hypothesis that chloroplasts control PD formation and function. Early work in maize (*Zea mays*) identified the *sxd1* mutant which showed defects in exporting sucrose from the leaf. In this mutant, PD at the interface of bundle sheath (BS) and vascular parenchyma (VP) cells are blocked by callose deposition [25,26]. *SXD1* encodes a chloroplast-localized tocopherol cyclase (*VTE1* homolog) [27], and expression of photosynthesis associated nuclear genes (PhANGs) was disrupted in the *sxd1* mutant [26]. Thus, studies with *sxd1* were the first hint that PD development and function were tied to chloroplast signaling pathways.

Mutations that cause defects in PD and more general intercellular trafficking are expected to result in lethality, an assumption borne out by a dearth of such mutants. A genetic screen of embryonic lethal *Arabidopsis thaliana* mutants in the Zambryski lab identified 14 mutants with defects in intercellular trafficking of a 10-kDa fluorescently labeled dextran [28]. Subsequent mapping and cloning identified two of these mutants, *ise1* and *ise2*, as encoding mitochondrial and chloroplast RNA helicases, respectively [22,29,30]. A separate screen for altered intercellular trafficking in seedling-lethal Arabidopsis mutants by the Jackson lab identified the *gat* series of mutants [23]. *GAT1* encodes a chloroplast thioredoxin m3, and loss of GAT1 led to changes in chloroplast redox status and decreased intercellular trafficking. Other *gat* mutants also show defects in chloroplast oxidative states [31].

Study of gene expression in the mitochondrial *ise1* and chloroplast *ise2* mutants revealed profound alterations in the patterns of expression of chloroplast-related genes, leading to the hypothesis that the observed changes in PD function and intercellular trafficking was caused by defective chloroplast function [22]. We interpreted these findings from *ise1* to mean that defective mitochondria led to either altered mitochondrial-to-nucleus retrograde signaling (Box 1) that impinged on PhANG expression, or that defective mitochondria directly or indirectly perturbed chloroplast development and thence chloroplast-to-nucleus retrograde signaling (Box 1) to disrupt expression of PD associated nuclear genes (PDANGs). Indeed, crosstalk between chloroplast and mitochondrial retrograde signaling is well described [32]. These findings from *ise1* and *ise2*, supported by the independent identification of the maize *sxd1* and Arabidopsis *gat* mutants, led to the hypothesis articulated as organelle-nucleus-PD signaling (ONPS, [22,11]).

Plant evolution and intercellular trafficking

The establishment of the symbiotic relationship between cyanobacteria and the eukaryote host that would give rise to the plant lineage occurred at least 1.9 billion years ago, according to recent estimates [33], (Figure 1). These early photosynthetic eukaryotes then gave rise to several algal lineages including the ancestors of land plants. How multicellular plants evolved from unicellular ancestors continues to be an important question in understanding

Box 1 Organelle retrograde signaling

The cell nucleus controls organelle development through expression of genes that encode organellar proteins which are translated in the cytoplasm and then imported into the organelle, a process called anterograde signaling. Organelles including the chloroplast and mitochondria produce small molecules that act as signals to relay information about their developmental and physiological state to the nucleus to regulate nuclear gene expression. This organelle-to-nucleus signaling is called retrograde signaling. For a comprehensive current review see Ref. [97•].

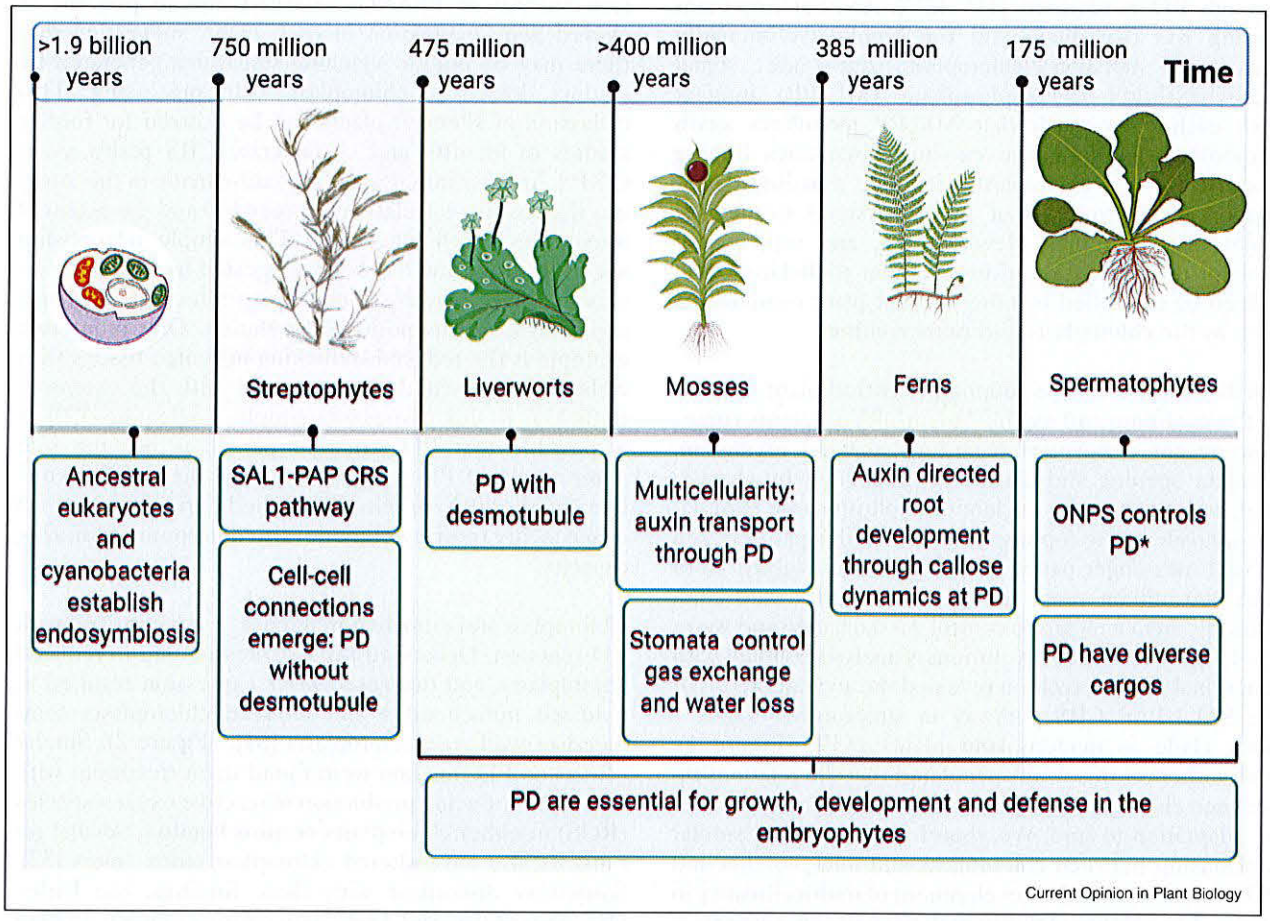
Chloroplast retrograde signaling (CRS)

While chloroplast retrograde signaling (CRS) is traditionally used to described signals that regulate nuclear genes necessary for maintaining chloroplast homeostasis, recent use of the term has expanded to include chloroplast signaling involved in regulating extrachloroplast processes [98]. CRS pathways that impinge on chloroplasts are divided into two types: biogenic and operational signals. Biogenic control signals are generated during chloroplast development and these signals include tetrapyrrole intermediates, heme, and carotenoids. Operational control signals are generated by mature chloroplasts in response to environmental perturbations and include chloroplast metabolites such as phosphoadenosine-5-phosphate (PAP), methylerithrol-cyclodiphosphate (MEcPP), β-cyclocitral (β-CC), tetrapyrroles, and reactive oxygen species (ROS). The nucleotidase/phosphatase SAL1 negatively regulates PAP levels by dephosphorylating PAP into AMP, and PAP accumulates in response to drought and high light stress [99]. MEcPP is a dual-function plastidial metabolite that serves as both an isoprenoid precursor produced by methylerythritol phosphate (MEP) pathway and a stress-specific retrograde signal [100].

Mitochondrial retrograde regulation (MRR)

Mitochondria transmit signals to nucleus to regulate the gene expression in response to various environmental conditions and its functional states (such as dysfunctional mitochondrial electron transport chain). These signals are called mitochondrial retrograde signals and include mitochondrial reactive oxygen species (mtROS), lipid peroxidation products, calmodulin, cyclic nucleotides, and phospholipases [101].

Figure 1



Evolution of PD and intercellular trafficking during land plant evolution. More than 1.9 billion years ago an ancestral eukaryote established an endosymbiotic association with a photosynthetic cyanobacterium that would evolve into the chloroplast. The SAL1-PAP CRS module evolved in the streptophytes, a group that evolved around 750 million years ago (Mya) and includes the closest algal relatives of extant land plants. Intercellular pores lacking ER but resembling PD evolve in some streptophyte algal lineages. Between 500 and 450 Mya plants moved onto land and these ancestral land plants contained PD that incorporated encased ER, or the desmotubule. PD are found in all embryophytes where they are essential for plant survival. In the mosses, about 400 Mya, the body plan became more complex as auxin gradients were established via PD-mediated trafficking. Also in the mosses, stomata emerged, and they may use SAL1-PAP CRS as a second messenger for regulating these pores and water loss in a dry environment. In the ferns, callose deposition and removal at PD establish auxin gradients for root development. By the time spermatophytes arose, about 175 Mya, PD had evolved for trafficking of numerous types of cargo that allow coordinated responses to myriad intrinsic and extrinsic stimuli. While ONPS operates in spermatophytes the involvement of ONPS in other embryophytes has not yet been investigated.

the processes of plant evolution and development. Recently, the role of PD in the evolution of multicellularity has been evaluated and we refer readers to these excellent discussions [1,34••]. Of note is the work of Newman *et al.* who have conceived of dynamical patterning modules (DPM) that they posit were critical to the evolution of multicellularity [35,36]. DPM theory positions intercellular trafficking and PD as central to the emergence of complex plant body plans through their contributions to cell, tissue and organ polarity and differentiation [34••]. They argue that the presence of intercellular connections is necessary for cell-type specification, a likely prerequisite for multicellularity. Auxin, like PD, is universally deployed in land plants for development and has its origins in the ancestors of land plants [37•]. Benitez *et al.* speculate that PD may have been crucial for establishing auxin as a central player in regulating plant development [34••]. While the transport of auxin by specialized protein carriers is well documented and extensively experimentally supported, there is strong emerging evidence that PD are also critical for establishing auxin gradients. First, in moss, auxin is transported exclusively through PD [38]. Second, the importance of callose dynamics at PD is now known to be indispensable for auxin-directed root development [39,40•]. Third, mathematical modeling of auxin distribution in both

the root tip and in leaves reveals that diffusive movement through PD is required [41••,42••]. Another important finding was that auxin and the highly evolutionarily conserved metabolite/chloroplast retrograde signal methylerythritol cyclodiphosphate (MEcPP) interact with each other, such that MEcPP modulates auxin homeostasis [43•]. These exciting discoveries linking auxin to PD and chloroplast retrograde signaling (CRS) support the coevolution of ancient systems to mediate major events in plant development, and support the concept that regulation of intercellular trafficking could indeed be controlled by other ancient plant components such as the chloroplasts and mitochondria.

Further support for the coupling of critical plant features in the evolution and establishment of land plants comes from recent discoveries regarding stomatal regulation. Stomata opening and closure are regulated by abscisic acid, and involves the nucleotidase/phosphatase (SAL1)-phosphoadenosine-5-phosphate (PAP) CRS pathway as a second messenger pathway [44]. Stomata evolved more than 400 million years ago as plants moved onto land, allowing them a means to control gas exchange and water loss (Figure 1). Recent evolutionary analysis coupled with functional characterization revealed the ancient origins of the SAL1-PAP CRS pathway in streptophyte algae, a sister clade to modern land plants [45•], (Figure 1). The authors of this work speculated that the relationship between chloroplasts and stomata is ancient and essential for adaptation to land. We, therefore, posit that a similar relationship between chloroplasts and mitochondria and PD evolved during the development of multicellularity in plants. The validity of this hypothesis will only come from the elucidation of the retrograde signaling pathways and PDANGs of ONPS.

Chloroplast retrograde signaling in ONPS

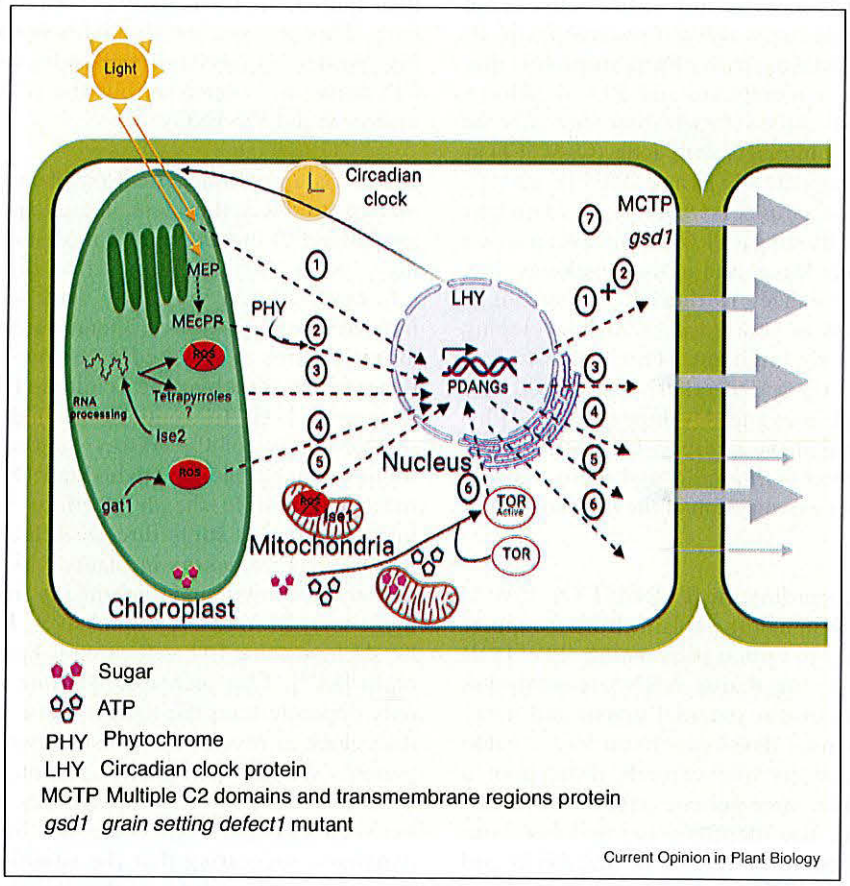
Recent efforts have focused on elucidating the mechanism of ONPS. Knockdown of the gene encoding the chloroplast ribosomal protein uL15c led to defective chloroplast translation and increased PD-mediated trafficking [46], supporting previous observations that chloroplast translation impinged on PD function [47]. In a more comprehensive approach to understanding the relationship between chloroplast signaling and PD function, several nuclear genes encoding chloroplast RNA-processing proteins (*RH39, RH22, RH3, ISE2, IPI1, RNaseJ, PNPase*) were silenced in *Nicotiana benthamiana* (Figure 2). Reduced expression of *RH22* and one of the two *N. benthamiana PNPase* homologs, *PNPaseA*, resulted in increased intercellular trafficking of GFP [48•]. In contrast, knockdown of *RNaseJ*, IPI*1*, *RH3* or both *PNPase* homologs led to decreased intercellular trafficking. One important finding from this study is that there is no correlation between leaf chlorosis and intercellular trafficking, as plants with similar levels of chlorosis can display dramatic differences in PD flux, as illustrated by silencing different *PNPase* homeologs [48•]. Expression analysis of PhANGs revealed unique patterns of altered gene expression in each plant, suggesting that there may be unique signaling signatures generated by distinct defects in chloroplast RNA processing. This collection of silenced plants can be utilized for further studies to identify and characterize CRS pathways in ONPS. Another important observation made in this study was the positive correlation between PD and the extent of intercellular trafficking [48•]. This simple relationship seems intuitive and had been suggested by earlier observations [49]. However, other experimental evidence argues against this positive correlation. One prominent example is the reduced trafficking in source tissues with elaborately branched PD compared with the extensive trafficking in sink tissues with simple, single pores [50]. It is possible that PD structure per se is not the sole determinant of PD permeability, and that other factors like whether PD are primary (formed during cytokinesis) or secondary (post-cytokinetic) also determine trafficking capacity.

Chloroplast and mitochondrial redox status correlate with PD function. Decreased *ISE2* expression led to reduced chloroplasts, and decreased *ISE1* expression resulted in oxidized mitochondria and reduced chloroplasts compared to wild-type chloroplasts [51], (Figure 2). Similar effects on PD function were found upon treatment with chemicals inducing production of reactive oxygen species (ROS) in either chloroplasts or mitochondria. Several *gat* mutants also have altered chloroplast redox states [52]. Somewhat discordant with these findings, the barley (*Hordeus vulgare)* and *Arabidopsis chlorina* mutants lacking *chlorophyllide-a-oxygenase (cao)* and chlorophyll b have increased chloroplast ROS levels, increased intercellular trafficking and enhanced PD formation with more PD connecting leaf mesophyll cells or the L1 layer of the shoot apical meristem [53]. Thus, different ROS in varying quantities and combinations could act as signals to control PD formation and function [54], via retrograde signaling or as direct signals to the PD, as may happen with the callose-modulating protein, PDLP5 [24]. However, the situation with chloroplast oxidation may not be straightforward as ROS signaling since changes in NADPH and ATP production are associated with ROS production. Silencing expression of a subunit of the chloroplast ATPase, *AtpC*, by VIGS resulted in increased cell-to-cell movement of GFP [55••] and also plant viruses [56]. Further critical examination of ATP and NADPH metabolism in mutants already known to have defects in PD will help clarify the importance of ROS in ONPS and may identify new components of ONPS.

ONPS in plant physiology and metabolism

At the core of ONPS is the idea that the balance between carbon supply from photosynthesis and PD development/function is regulated by chloroplasts. For carbon

Figure 2



ONPS in plant physiology and metabolism. (1) Intercellular trafficking is higher in the day than night, and this is under circadian control (LHY) but likely does not involve callose metabolism. (2) Light regulation of PD may also involve of the MEcPP CRS pathway and the phytochrome (PHY) photoreceptors. (3) Defects in chloroplast processing, exemplified by the *ise2* mutation, lead to CRS possibly via tetrapyrroles and other pathways and increases PD formation and intercellular trafficking. Other RNA processing mutants can decrease intercellular trafficking. (4) The *gat1* mutation oxidizes the chloroplasts by increasing ROS production, and these ROS may themselves act as CRS or trigger other CRS pathways to decrease intercellular trafficking. (5) The *ise1* mutation leads to more reduced mitochondria and generates signals that trigger CRS or MRR that change PDANG expression to increase intercellular trafficking. (6) The balance of ATP and sugars from the chloroplasts and mitochondria activate the TOR kinase, and under conditions of abundance; this leads to decreased intercellular trafficking. (7) An MCTP protein localized to PD is important for PD formation, and controlling intercellular trafficking. GSD1 also localizes to PD and the *gsd1* mutant shows increased intercellular trafficking.

partitioning in a plant to be effective carbon fixation and transport must be coordinated [57,58]. The cell-to-cell trafficking of this fixed carbon is largely via PD, and when PD function is perturbed or insufficient carbohydrates may accumulate in leaves, leading to feed-back inhibition of photosynthesis, as described in Section 'Developing the ONPS hypothesis' for the *sxd1* mutant. In contrast, if too great a proportion of a plant's carbon resources is allocated to transport structures, carbon use efficiency is compromised. Both scenarios can lead to adverse outcomes in plant growth, development and yield [59,60]. Besides *sxd1*, four other maize mutants with defects in carbohydrate partitioning have been identified [61•]. In the *tie-dyed(tdy)1* [62] and *tdy2* mutants [63], and the *carbohydrate partitioning defective(cpd)1* [64] and *cpd33* [61•] mutants sucrose export to the phloem is reduced. The carbohydrate export defect was due to callose accumulation at PD at the mesophyll-phloem interface [25] or at sieve pores in the phloem (*cpd1* [64]), or by defects in PD formation (*cpd33*) [61•], although no obvious defects in PD were observed in the *tdy* mutants [62].

Callose dynamics at the PD are not the only means for controlling carbohydrate partitioning. *CPD33* encodes a Multiple C2 domains and transmembrane region (MCTP) protein [61•]. MCTPs are broadly distributed across eukaryotes and tether the ER to the PM. In Arabidopsis, MCTPs are also found to localize to PD and to control intercellular trafficking [65]. PM proteins like the MCTPs may, therefore, be important for PD

formation, as revealed by the reduced numbers of PD in *cpd33* [61•], (Figure 2). Phloem loading was defective in the rice (*Oryza sativa) grain setting defect1-Dominant (*gsd*1-D)* mutant [66]. *GSD1* encodes a putative Remorin protein that localizes to the plasma membrane and PD of phloem companion cells, and correct localization depends on S-acylation [67]. GSD1 interacts with actin (OsACT1) at the PD to negatively regulate PD permeability (Figure 2). Another rice mutant, *sugar accumulation 1 (ossac1)* mutant has impaired sugar partitioning [68]. *OsSAC1* was expressed at the bottoms of young leaves and in the developing leaf sheath and OsSAC1 localizes to the ER. Interestingly, OsGSD1 was detected in young leaves and developing leaf sheaths of the *ossac1* mutant. This suggests that OsSAC1 and GSD1 may act together to regulate sugar partitioning in young leaves and developing leaf sheaths. These examples highlight the interplay between PD and photosynthesis in carbon partitioning, and these mutants may be used for further examination of the mechanisms of ONPS.

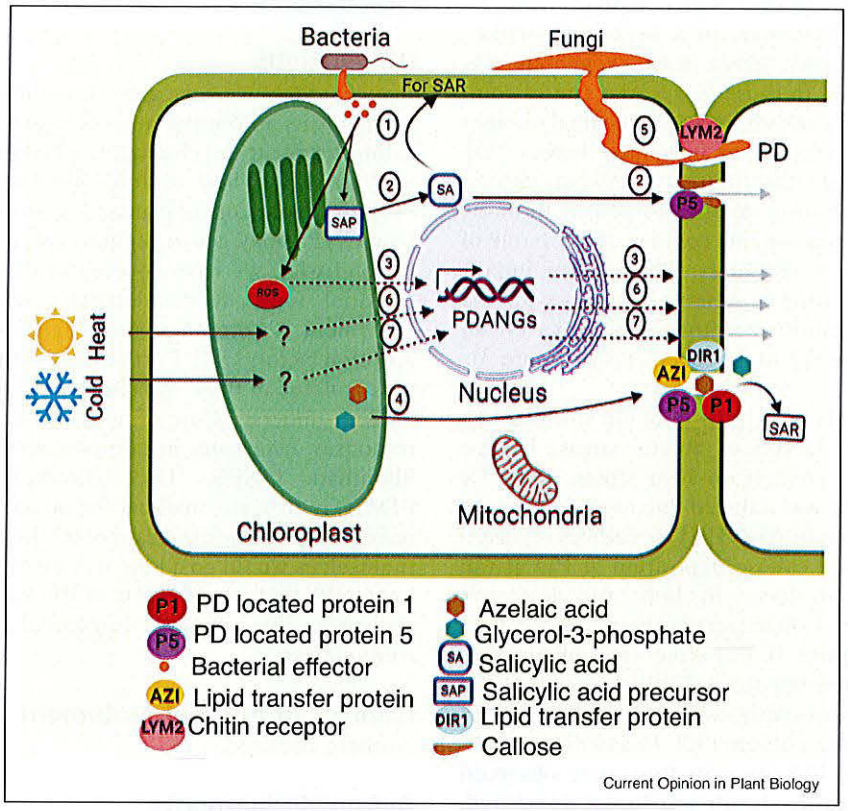
Exciting recent data regarding the TARGET OF RAPAMYCIN (TOR) kinase signaling in plants further support that ONPS contributes to carbon partitioning. The TOR kinase complex acts by regulating mRNA transcription and translation in response to external signals and nutrient availability to promote development under favorable growth conditions, and its role extends throughout a plant's life [69–71]. The same genetic screen that identified *ise1* and *ise2* [28], also identified *ise3* and *ise4*, later cloned and determined to be alleles of *At2g25570* and *At5g67630*, respectively [72••]. *At5g67630* encodes Reptin, a chaperone that is a component of the R2TP ATPase complex that contributes to TOR activation [72••,73]. Through a comprehensive approach involving forward and reverse genetics as well as drug treatments, Brunkard *et al.* demonstrate that active TOR signaling negatively regulates intercellular trafficking [72••]. In addition, through use of chemical inhibitors, TOR signaling was revealed to promote the sink-to-source transition in leaves. TOR is significantly more active in mature leaves that produce excess sugars than in young growing leaves that must import sugars, and this increase in TOR activity in mature leaves correlates with decreased rates of PD transport (Figure 2). Another interesting finding from this study was that the mitochondrial SEL1-like repeat containing (SLR) protein encoded by *At2g25570/ise3*, putatively involved in ATP synthesis during oxidative phosphorylation, is necessary for TOR activity. This supports the involvement of mitochondria in regulating PD-mediated intercellular signaling, previously described for *ise1* [30], (Figure 2). Comparative gene expression analysis in the *ise3*, *ise4* and characterized *tor* mutants confirmed that defects in TOR signaling pathways were the primary defect in all the mutants. But an intriguing observation was that while changes in transcript levels of PDANGs were observed, the patterns of expression could not always be correlated with observed changes in intercellular trafficking, particularly as related to callose metabolism. This observation highlights not only the probable involvement of mechanisms besides callose in regulating PD during development, but there likely remain many undiscovered PDANGs.

Light has a profound effect on plant growth and development, and it is, therefore, not unexpected that light can modulate PD function and symplastic transport. Surprisingly, there are few studies that directly examine the effects of light on PD. Early work reported that white-light irradiation inhibited lateral transport from the stele into the cortex in etiolated maize mesocotyls, suggesting that as chloroplast biogenesis initiated, PD trafficking was decreased [74]. In contrast, at high light intensities, several species of C4 grasses showed higher photosynthetic efficiency along with higher PD frequency at Kranz mesophyll/bundle sheath interface in leaves [75]. An important recent study directly addressed the effects of light on PD trafficking in plants. Using differing photoperiods for growth and a minimally invasive GFP movement assay in *N. benthamiana* leaves, Brunkard and Zambryski found that PD trafficking is higher in the day than night [55••]. This increased daytime trafficking was not only dependent on the light but also involved the circadian clock as revealed by knockdown of the clock component *LHY* [55••], (Figure 2). An interesting observation of this study was that differences in PD trafficking between day and night were not mediated by callose dynamics, suggesting that the situation is not always as clear cut as described in the literature, where callose deposition limits PD permeability. These findings are consistent with unexpected new findings about the roles of callose in modulating the properties of the cell wall. Addition of callose to cellulose hydrogels *in vitro* increased the elasticity of cellulose [76], a change would not be expected to constrict PD. Future studies examining callose–cellulose interactions and their interactions with other cell wall components as well as their effects on plant cells should bring novel insight into PD function and how callose regulates PD. Also noteworthy are recent studies demonstrating that chloroplasts use the MEcPP CRS pathway to influence light detection by the phytochrome photoreceptors [77,78], (Figure 2), and light regulates expression of genes encoding the MEP biosynthesis pathway [79]. The role of the MEcPP CRS pathway in mediating PD flux in response to light remains to be examined.

ONPS in response to biotic and abiotic stress

PD have important roles in mediating plant responses to biotic and abiotic stresses. Plants encounter multiple biotic stresses and have acquired adaptive tools of defense to respond to them. Chloroplasts are critical players in plant responses to biotic challenges, and include production of both defense molecules and chemicals that attract

Figure 3



ONPS and PD in stress responses. (1) Infection by pathogens including bacteria leads to the production of defense hormones which ultimately causes a defense response. For example, SA production is induced in the chloroplast and then SA enters the apoplast for SAR. (2) SA also triggers accumulation of callose at PD via PDLP5 and reduces intercellular trafficking. (3) Chloroplast ROS are also produced during defense to reduce intercellular trafficking. (4) AzA and G-3-P are induced in the chloroplast by pathogen infection and they move systemically via the symplast to mediate SAR together with SA. PD proteins PDLP1 and PDLP5 together with the AZI1-DIR1 heterodimer coordinate the symplastic trafficking of AzA and G-3-P. (5) Some PD-mediated defense responses, for example, those mediated by LYM2 in response to fungi and chitin, are local and the role of ONPS in those remains unknown. (6) Heat stress inhibits PD-mediated intercellular trafficking, reducing carbohydrate export from leaves and decreasing photosynthesis rates. (7) Cold stress causes changes to PD ultrastructure and reduces intercellular trafficking but the role of ONPS remains to be determined.

beneficial microbes and animals as reviewed [80–82]. Salicylic acid (SA), jasmonic acid (JA), and ABA are phytohormones that are partially synthesized in the chloroplast and act as defense hormones. Further, CRS components are important for defense responses to pathogens. An Arabidopsis *ceh1* mutant that accumulates MEcPP (Box 1) constitutively produces SA and constitutively expresses JA-responsive genes in an MEcPP-dependent manner [83]. In contrast, in *sal1* mutants (Box 1), SA-mediated and JA-mediated defense responses were defective, leading to increased susceptibility to pathogen infection [84].

Besides launching local defense programs in the cells encountering an invading microbe, JA and SA are also important for systemic defense responses. The role of SA in systemic acquired resistance (SAR) has been controversial, but recent results provide very strong evidence supporting its importance to SAR [85••]. In addition to SA, chloroplasts also produce the signaling molecules azelaic acid (AzA) and glycerol-3-phosphate (G3P) in response to pathogenic attack to mediate SAR [86]. AzA functions upstream of G3P and AzA-G3P branch functions in parallel to SA-derived signaling during SAR [87]. AzA and G3P move cell-cell via PD through the action of the lipid transfer proteins, AZI1 and DIR1, while SA moves via the apoplast in the cuticle under the control of transpiration [85••,86] (Figure 3). Interestingly, SA stimulates callose deposition at PD through the action of PD-LOCATED PROTEINs PDLP1 and PDLP5 to negatively regulate PD trafficking during pathogen infection [2], (Figure 3). PDLP1 and PDLP5 are essential for SAR as well as the stability of AZI1, which is required for AzA-induced and G3P-induced SAR [86]. Loss of either PDLP1 or PDLP5 increases chloroplast localization of AZI1, similar to pathogen infection

[88], suggesting that plastidial localization or partitioning of AZI1 between the plastid and cytoplasm may be important for SAR. However, in a seeming paradox, treatment of Arabidopsis leaves with exogenous SA-induced PD formation. In those leaves, the rate of complex PD formation is increased, although the final number of PD formed was the same as in control leaves [89]. These studies highlight the interplay between chloroplasts and PD in mediating defense responses to biotic stresses, and further experiments could clarify the role of CRS and ONPS in these responses. While PD are important for systemic signaling in defense, studies with the PD-localized LYM2 chitin receptor reveal that PD can also act locally to respond to pathogens [90] (Figure 3).

Compared to knowledge of ONPs in biotic stress, much less is known about ONPS in abiotic stress. In rice varieties tolerant or sensitive to heat stress, 40°C for 12 days, kernel weight was reduced due to the inhibition of the assimilate distribution [91]. The authors reported that heat stress caused callose deposition at PD at the mesophyll-phloem interfaces in both the leaf and the sheath with more callose accumulating in the heat sensitive variety (Figure 3). PD structure and function also respond to low temperatures. Chilling maize plants at 14°C inhibited photosynthesis and restricted photoassimilate export to the phloem [92]. In a chilling-sensitive line, changes in PD ultrastructure were observed particularly at the bundle sheath and Kranz mesophyll, and the bundle sheath and vascular parenchyma interfaces. These studies implicated calreticulin as responsible for the short-term restriction of PD trafficking and implied that callose was only later involved in modifying PD trafficking [92]. Low temperatures also induced expression of PDANGs in maize, including *GRMZM2G081949* encoding a putative Remorin gene [93]. While studies on PD structure and function during stress are uncommon, it should be noted that the hormone ABA mediates plant responses to drought and numerous other abiotic stresses [94]. ABA also regulates PD function during the breaking of bud dormancy [95]. Thus, it is possible that ABA regulates PD in response to abiotic stresses, although this remains to be experimentally verified.

Interestingly, ER stress has also been found to involve PD-mediated systemic signaling. In Arabidopsis plants the transcription factor bZIP60 was found to move systemically from the root to the shoot during the unfolded protein response (UPR) induced by tunicamycin [96]. The cell-to-cell trafficking of bZIP60 was inhibited by callose accumulation at PD, suggesting that the movement of this transcription factor is via the symplast. In the future it will be interesting determine whether the bZIP60 transcription factor regulates PDANGs to control PD and the extent of ER stress-associated systemic signaling. Regardless, these results demonstrate that organelles besides chloroplasts and mitochondria mediate their effects via PD.

Conclusions

In recent years we have gained significant insight into the mechanisms and importance of organelle retrograde signaling for plant development, physiology and responses to stress [97•]. These findings along with studies into the evolutionary history of plants have made new insights into how ONPS may act to regulate intercellular trafficking. Beyond what we have covered in this review, PD have essential roles in development, allowing cell-to-cell movement of hormones, small signaling RNAs and transcription factors [3,4]. Further investigations into how PD are regulated in these developmental processes as well as a better understanding of how PD are involved in stress responses, especially in non-photosynthetic tissues, will illuminate ONPS. The identification of signature PDANGs that are markers for organelle retrograde signaling and the identification of the retrograde signals themselves would go a long way toward fully characterizing ONPS and the interplay of PD with all other cellular processes that involve intercellular movement and communication.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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