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REVIEW PAPER

Callose balancing at plasmodesmata

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Abstract

In plants, communication and molecular exchanges between different cells and tissues are dependent on the apoplastic and symplastic pathways. Symplastic molecular exchanges take place through the plasmodesmata, which connect the cytoplasm of neighboring cells in a highly controlled manner. Callose, a β-1,3-glucan polysaccharide, is a plasmodesmal marker molecule that is deposited in cell walls near the neck zone of plasmodesmata and controls their permeability. During cell differentiation and plant development, and in response to diverse stresses, the level of callose in plasmodesmata is highly regulated by two antagonistic enzymes, callose synthase or glucan synthase-like and β-1,3-glucanase. The diverse modes of regulation by callose synthase and β-1,3-glucanase have been uncovered in the past decades through biochemical, molecular, genetic, and omics methods. This review highlights recent findings regarding the function of plasmodesmal callose and the molecular players involved in callose metabolism, and provides new insight into the mechanisms maintaining plasmodesmal callose homeostasis.

Keywords: β-1,3-glucanase, callose, cell-to-cell communication, glucan synthase-like, plasmodesmata, symplastic trafficking.

Introduction

Callose, a β-1,3-glucan polysaccharide with β-1,6 branches, is involved in numerous plant processes, such as plasmodesmal and sieve pore regulation, pollen development, vascular differentiation, cell plate formation during cytokinesis, and responses to biotic and abiotic stresses (Chen and Kim, 2009; Amsbury *et al.*, 2017). Plasmodesmata (PDs), symplastic junctions between cells, provide symplasmic nanochannels crossing the cell walls and connecting the plasma membranes of neighboring cells, and function as important pathways for intercellular communication and molecular exchanges. The cell-to-cell movements of a wide range of molecules through PDs are regulated by callose-dependent and callose-independent mechanisms (Lucas *et al.*, 2009; Sager and Lee, 2014; Iswanto and Kim, 2017). The callose-independent mechanisms include alterations in PD frequency, changes in PD structure, such as from the simple to the complex branched form, involvement of the actin cytoskeleton, and regulation of PD permeability by PD gating proteins (Lucas *et al.*, 2009; Kumar *et al.*, 2015). In callose-dependent regulation, the level of callose in the PD neck zone is important; high levels of callose close PD channels, while and low levels of callose open them. In a recent review, Amsbury *et al.* (2017) provided an additional model wherein the structural and mechanical properties of callose are potentially linked to PD regulation. The level of callose is highly regulated by two antagonistic enzymes, callose synthase (CalS) or glucan synthase-like (GSL) and β-1,3-glucanase (BG) (Chen and Kim, 2009) (Fig. 1, Table 1).

Numerous insightful reviews have been published on topics such as PD components and regulation (Lucas *et al.*, 2009; Burch-Smith and Zambryski, 2012; Sager and Lee, 2014; Kumar *et al.*, 2015; Kim, 2018), PD callose (Chen and Kim,

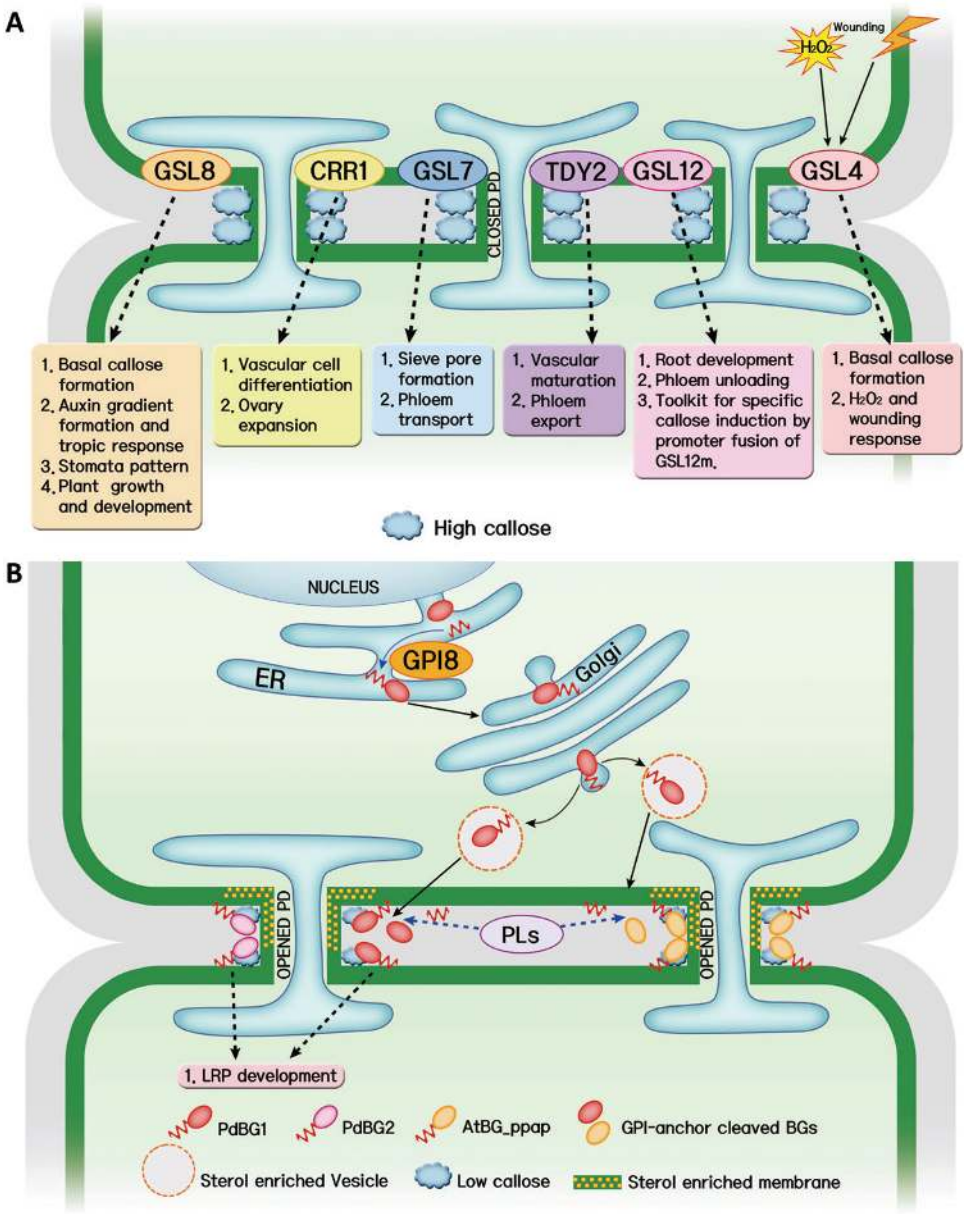

Fig. 1. Callose homeostasis at plasmodesmata (PDs) during plant development. (A) PD-associated callose synthases during diverse developmental processes. The glucan synthase-like (GSL) family and their homologs accumulate callose at the PD neck zone to close the PD channels. GSL8 and GSL4 are the two main enzymes that produce basal callose at PDs. CANNOT REACH THE ROOF 1 (CRR1) is an ortholog of Arabidopsis GSL8 in rice and is involved in vascular cell differentiation and ovary expansion. Arabidopsis GSL7 plays a role in callose synthesis at the PDs during sieve pore formation and phloem transport. Tie-dyed2 (TDY2) is a callose synthase of maize that regulates vascular maturation and phloem export. GSL12 and its gain-of-function form, *cals3m*, synthesize PD-associated callose in roots and in phloem companion cells. GSL4 synthesizes callose at PDs under normal conditions and is activated in response to reactive oxygen species and wounding. (B) Synthesis, delivery, and function of PD-localized β-1,3-glucanases (BGs). PdBG1, PdBG2, and AtBG_ppap are PD-localized BGs. GPI8 regulates GPI anchor attachment for GPI-anchored proteins in the endoplasmic reticulum (ER). Sterol-enriched vesicles and sterol-enriched membranes are required for the PD targeting of all three BGs. PdBG1 and PdBG2 are required for PD callose degradation during lateral root primordia (LRP) formation. Phospholipases (PLs) might play a role as a potential regulator of post-translational BG modification.

2009; Zavaliev *et al.*, 2011; De Storme and Geelen, 2014; Amsbury *et al.*, 2017), the PD membrane (Tilsner *et al.*, 2016), the plant vascular system (Lucas *et al.*, 2013; Heo *et al.*, 2017), intercellular movement (Gallagher *et al.*, 2014; Han *et al.*, 2014*b*; Kitagawa and Jackson, 2017), cell-to-cell and long-distance transport of phytohormones (Han and Kim, 2016; Lacombe and Achard, 2016), and PD regulation during biotic stress (Stahl and Faulkner, 2016; Cheval and Faulkner, 2018). In this article, we highlight recent findings regarding the function of PD callose and the molecular players involved in callose metabolism, and provide new insights into the mechanisms maintaining PD callose homeostasis.

Enzymes responsible for callose synthesis and degradation and plant development

Callose synthases in higher plants

There are 12, 10, 12, and ~10–13 *GSL* genes in *Arabidopsis*, rice, maize, and tomato, respectively, and most of them have a

Table 1. *Callose synthases and degrading enzymes*

Gene name	Gene ID	Function
GSL1/CalS11	AT4G04970	Formation of the callose wall to separate microspores
GSL2/CalS5	AT2G13680	Callose synthesis during male gametophyte development
GSL4/CalS8	AT3G14570	Maintenance of basal PD callose and H_2O_2-induced PD callose
GSL5/CalS12	AT4G03550	Pollen development and fertility Response to SA-, wound-, iron-, and pathogen-induced callose deposition
GSL6/CalS1	AT1G05570	Callose synthesis at the cell plate SA-dependent callose synthesis
GSL7/CalS7	AT1G06490	Callose synthesis on phloem sieve elements
GSL8/CalS10	AT2G36850	Microspore development Callose deposition at cell plates during cytokinesis Callose deposition at PDs during the phototropic response
GSL10/CalS9	AT3G07160	Microspore development and plant growth
GSL12/CalS3	AT5G13000	Callose deposition at PDs in the stele, roots and phloem
AtBG_ppap	At5g42100	Degradation of PD-associated callose
PdBG1	At3g13560	Callose degradation at LRP during lateral root development
PdBG2	At2g01630	Callose degradation at LRP during lateral root development
BG6	At4g16260	Copper-induced PD callose degradation

LRP, Lateral root primordia; PD, plasmodesma; SA, salicylic acid.

molecular weight of approximately 200 kDa (Verma and Hong, 2001; Slewinski *et al.*, 2012; Shi *et al.*, 2014; https://solgenomics.net). Biochemical and genetic evidence that GSLs produce callose was first obtained in studies of barley and tobacco pollen tubes (Turner *et al.*, 1998; Li *et al.*, 2003) and further supported by research reports in Arabidopsis (Hong *et al.*, 2001a, b; Huang *et al.*, 2009; Thiele *et al.*, 2009; Guseman *et al.*, 2010; Vatén *et al.*, 2011).

GSLs possess a large central catalytic domain, which includes a UDP-glucose catalytic site and a glycosyltransferase domain, surrounded by multiple transmembrane domains (TMDs) and a putative N-terminal regulatory region (Hong *et al.*, 2001a, b; Verma and Hong, 2001; Thiele *et al.*, 2009). Although GSLs have no signal peptide that is required for the conventional secretion pathway, it seems that they need EXO70 family-mediated exocysts for their subcellular localization. For example, EXO70H4 was essential for the appropriate GSL5/PMR4 secretion and subcellular targeting in trichome cells (Kulich *et al.*, 2018). Highly specialized protein complexes of GSLs are required to regulate callose synthase activity in a sophisticated manner (Schneider *et al.*, 2016). In addition to GSLs, GSL complexes include components such as UDP-glucose transferase 1 (UGT1), Rho-like GTPase (Rop), RabA4c, tubulin, phragmoplastin (Phr), sucrose synthase (SuSy), and annexin (ANN), which are involved in activity regulation or targeting (Shin and Brown, 1999; Hong *et al.*, 2001a, b; Verma and Hong, 2001; Aidemark *et al.*, 2009). However, the composition of the GSL complexes may be different in the various callose synthesis processes. For example, the GSL complex contains phragmoplastin in the cell plate but may not contain it at PDs (De Storme and Geelen, 2014). Identification of tissue-specific components of the GSL complexes will be the next challenge in this field, and new biochemical proteomic approaches such as proximity-dependent biotin identification (BioID), which was recently used in a rice protoplast system (Lin *et al.*, 2017), will be advantageous for the identification of those components.

The activity of callose synthase can be directly suppressed by free fatty acids such as linoleic and α-linolenic acids (Blümke *et al.*, 2014). In addition, post-translational modifications such as phosphorylation and proteolysis may affect the activity and stability of the enzyme or its trafficking. Several Arabidopsis GSL members, including GSL5, GSL10, and GSL12, are phosphorylated in response to various abiotic and biotic stresses (Schneider *et al.*, 2016). In addition, some range of proteolysis is responsible for controlling the activity of callose synthase in plant species (Girard and Maclachlan, 1987). Recently, it has been suggested that the protease activity of OsMMP1 plays a role in the activation of callose synthase (Das *et al.*, 2018). However, the exact molecular mechanisms of enzyme activation by phosphorylation and proteolysis are not yet known.

Plasmodesmal callose synthases and plant development

Callose deposition determines the size exclusion limit (SEL) of PDs and hence their permeability (as well as the movement of large macromolecules). The *gsl8/chorus* (*chor*) Arabidopsis mutant shows reduced callose deposition in the cell plate and at PDs, higher symplastic permeability, and an increased SEL in epidermal leaf cells in comparison to wild-type plants. Moreover, SPEECHLESS (SPCH), a stomatal development regulator, shows ectopic distribution in the *chor* mutant due to a large SEL that allows the abnormal spread of SPCH to neighboring cells. Hence, GLS8-mediated callose synthesis is crucial in the regulation of stomatal differentiation by restricting the allocation of SPCH (Fig. 1A) (Chen and Kim, 2009; Guseman *et al.*, 2010). GSL8-mediated PD regulation is also essential for a normal phototropic response; a transcriptional feedback loop of auxin-ARF7-GSL8 has been identified (Han *et al.*, 2014*a*). However, considering the rapid auxin gradient formation and relatively slow callose deposition by transcriptionally regulated GSL8, it seems likely that rapid accumulation of callose during the phototropic response might require modulation by faster post-translational regulation.

CANNOT REACH THE ROOF 1 (*CRR1*) in rice encodes a protein homologous to Arabidopsis GSL8. Unlike the *gsl8* mutant (Chen and Kim, 2009; Thiele *et al.*, 2009), a rice mutant of *CRR1* did not show any defect in callose deposition in the cell plate and sieve pores (Song *et al.*, 2016), but caused PD callose reduction in a small number of vascular cells. The *crr1* mutants showed delayed ovary expansion and defective vascular cell patterning. Thus, the callose produced by CRR1 plays a role in determining vascular cell differentiation and initial ovary expansion (Fig. 1A). *crr1* mutants exhibited aggregated sieve elements, similar to the clustered stomatal cells found in *gsl8* mutants, suggesting that increased PD permeability may induce cell fate determinants to diffuse from sieve element

precursor cells to their neighboring dedifferentiated parenchymal cells, resulting in clustered sieve element formation (Song *et al.*, 2016).

Arabidopsis *GSL7* knockout mutants exhibit reduced accumulation of callose at the PDs in early-stage sieve plates and radial sieve element walls during sieve pore formation, resulting in sieve elements with fewer PD pores (Fig. 1A) (Xie *et al.*, 2011). Arabidopsis *gsl7* mutant plants exhibit a reduced flowering stem length and smaller floral organs, which may be caused by carbohydrate starvation (Barratt *et al.*, 2011). Pulse-chase experiments with $^{14}CO_2$ revealed that the transport of sucrose produced in leaves is slower in *gsl7* mutants than in wild-type plants. In contrast to the report by Xie *et al.* (2011), Barratt *et al.* (2011) claimed that pore function, rather than pore formation, is hampered. They proposed that callose deposition may reduce the effective pore length by restricting wall growth or render the pore a rigid and non-compressible tube with good flow characteristics. An alternative explanation might be that thick callose cylinders present in the phloem sieve pores of wild-type plants provide an extension of pore size by its dynamic turnover (Barratt *et al.*, 2011). In maize, *Tie-dyed2* (*tdy2*), a callose synthase mutant, shows compromised early vein development along with defective phloem export (Fig. 1A) (Baker and Braun, 2008; Slewinski *et al.*, 2012). Transmission electron microscopy of *tdy2* yellow leaf regions showed incomplete vascular differentiation, suggesting that callose synthesis is required for vascular maturation. TDY2 interacts genetically with TDY1, an endoplasmic reticulum (ER)-localized transmembrane protein; the two mutants show very similar phenotypes, with excessive oil-droplet accumulation in companion cells and defective phloem export. However, how TDY1 and TDY2 interact to promote the symplastic transport of both solutes and to control vascular development remains to be determined.

In another study, the dominant mutant *cals3m/gsl12m* showed elevated callose levels in the cell wall domain surrounding the PDs, decreased PD aperture, and impaired root development and phloem unloading (Fig. 1A) (Vatén *et al.*, 2011). Green fluorescent protein (GFP)-GSL12 is localized at PDs and the plasma membrane in plasmolyzed cotyledon epidermal cells, consistent with a role in PD callose synthesis. However, the mechanism by which *cals3m* functions as a dominant mutant is still unknown. The dominant property of *cals3m* also provides a tool for spatially and temporally controlling the PD aperture between plant tissues by establishing a transgenic line with a specific promoter driving the expression of *cals3m*. For example, PD-dependent movement of the transcription factor SHORTROOT and microRNA-165 between the stele and the endodermis has been identified (Vatén *et al.*, 2011). Recently, using inducible *cals3m*, Wu *et al.* (2016) elegantly showed that symplastic signaling through PDs at the cell boundaries of the endodermis is critical for the coordinated growth and development of the root (Wu *et al.*, 2016). Endodermal-specific expression of *cals3m* resulted in an increase in the number of cell layers and a misspecification of surrounding cells, including stele and ground cells, in the root (Wu *et al.*, 2016). In addition, *cals3m* has been successfully used to study PD function in the shoot apical meristem; *cals3m* expression resulted in reduced PD permeability, limited WUSCHEL (WUS) trafficking, and abnormal stem cell initiation and maintenance, suggesting that PD function in organizing cells is essential for shoot apical meristem activity (Daum *et al.*, 2014).

In summary, modulation of PD callose by GSLs is critical for plant development via the mechanism of controlling the symplastic transport of developmental signals and diverse solutes. How the functional redundancy of GSLs is modulated and whether other uncharacterized Arabidopsis GSLs function at PDs remain to be determined.

β-1,3-glucanases degrade callose

BGs are hydrolytic enzymes that can catalyze the endo-type cleavage of 1,3-β-D-glucosidic linkages into single β-1,3-glucan units. In plants, there are diverse sets of BG isoforms, classified by primary structure, protein size, subcellular localization, isoelectric point, and catalytic activity (Leubner-Metzger and Meins, 1999). Bioinformatic analyses have identified approximately 50 BG-related genes in Arabidopsis, with some members that are inactive BGs; these genes are subdivided into 13 clusters on the basis of genealogical and expression analyses (Doxey *et al.*, 2007; Levy *et al.*, 2007). A sequence analysis defined five protein domain architectural classes of these Arabidopsis BGs; all contain an N-terminal secretion signal and a core glucosyl hydrolase family 17 domain, and some carry one or two repeats of carbohydrate binding module 43 (CBM43). Furthermore, some members contain a C-terminal hydrophobic sequence, which includes a predicted glycosylphosphatidylinositol (GPI)-anchor attachment motif for targeting the protein to the cell membrane (Borner *et al.*, 2002; Levy *et al.*, 2007; Benitez-Alfonso *et al.*, 2013).

Plasmodesmal callose degradation pathways and plant development

BGs have been reported to degrade callose in a hydrolytic manner (Bachman and McClay, 1996). In *Arabidopsis thaliana*, the following three GPI-anchored BG proteins have been found to play a role in callose degradation at the PD: *A. thaliana* β-1,3-glucanase_putative PD-associated protein (AtBG_ppap), PD-localized β-1,3-glucanase 1 (PdBG1), and PD-localized β-1,3-glucanase 2 (PdBG2) (Levy *et al.*, 2007; Benitez-Alfonso *et al.*, 2013) (Fig. 1B). PdBG3, a PdBG1/2-related protein, is also localized at the PDs, but its role in callose degradation is uncertain (Benitez-Alfonso *et al.*, 2013). PdBG1 and PdBG2 are both expressed in lateral root primordia and are co-localized with the PD callose-binding protein 1 (PDCB1) at PDs (Benitez-Alfonso *et al.*, 2013). Double *pdbg1/pdbg2* mutants show an increased accumulation of callose at the PDs during lateral root development, with reduced cell-to-cell macromolecular trafficking, while this phenomenon was reversed in PdBG1 overexpression lines. In *pdbg1* and *pdbg2* single mutants and the *pdbg1/pdbg2* double mutant, lateral root density was significantly higher than that in wild-type plants, but the PdBG1 overexpression line showed reduced lateral root density. This phenotype results from the formation of clustered primordia with expanded domains of auxin-responsive *DR5* and *GATA23* expression in the *pdbg1/pdbg2* double

mutant (Benitez-Alfonso *et al.*, 2013). Lateral root initiation begins from the formation of auxin maximal founder cells in the pericycle cell layer, correlated with an increase in callose deposition. Thus, lateral root initiation is a highly regulated process that depends on the balance between callose synthesis and degradation. Although some sets of callose synthases and BGs are predicted to work simultaneously or subsequently in the same tissue, very few integrative studies characterizing the coordinated action of both antagonistic enzymes have been performed. The GSL enzymes involved in lateral root initiation remain to be determined.

AtBG_ppap, a GPI-anchored protein, was found to be localized in the ER membrane and along the cell periphery in association with PDs. Genetic studies revealed that AtBG_ppap promotes intercellular trafficking through the degradation of callose (Fig. 1B) (Levy *et al.*, 2007; Zavaliev *et al.*, 2013). GPI modification of AtBG_ppap is sufficient and necessary for the PD targeting of the protein, as revealed by domain-swap analysis (Zavaliev *et al.*, 2016). The importance of GPI-anchored proteins in PD regulation was shown by a missense mutation in Arabidopsis GPI8, a Cys protease that transfers an assembled GPI anchor to proteins (Bundy *et al.*, 2016). The *gpi8-1* mutation leads to a reduced accumulation of GPI-anchored proteins, higher levels of callose, and reduced PD permeability. PD targeting and trafficking of GPI-anchored proteins seem to be dependent on a membrane lipid raft that contains a high proportion of sterols and sphingolipids (Fig. 1B) (Grison *et al.*, 2015; Iswanto and Kim, 2017).

In contrast to most GSLs, which are post-translationally regulated, PdBGs have not been reported to be subject to such regulation; they are mostly regulated at the transcriptional level. A potential post-translational regulation can be performed by the cleavage of the lipid component of GPI by a range of phospholipases, including GPI-specific phospholipase D (GPI-PLD), phosphatidylinositol-specific phospholipase C (PI-PLC), GPI-PLC, and phosphoesterases (Fig. 1B) (Takos *et al.*, 2000). The release of free BGs might modulate protein conformation, enzyme activity and stability, and their freedom to travel through the apoplasm. Considering the fact that callose deposits through the thick cell wall center the neck region of PDs and that GPI-anchored enzymes are attached to the plasma membrane, the mobility of PdBGs might be a critical factor for enzyme activity. PD callose regulation by phospholipases remains to be determined, and a reverse genetic approach will be useful to characterize the putative function of phospholipases in the PD callose degradation pathway. A putative candidate protein (At1g74010) belongs to the calcium-dependent phosphotriesterase family, and was identified from the PD proteomic approach used in a recent study (Kraner *et al.*, 2017).

Plasmodesmal callose homeostasis in response to stress

In addition to the essential role of callose homeostasis during plant development, dynamic callose turnover by callose synthases and degradation enzymes allows plants to rapidly modulate symplastic signaling in response to varying environmental signals. There has been some important progress in understanding callose-mediated PD regulation in response to various biotic and abiotic stresses.

Modulation of plasmodesmal callose in response to abiotic stresses

Representative studies on PD callose regulation in response to abiotic stresses include those on chilling stress (Rinne *et al.*, 2005; Bilska and Sowiński, 2010; Fromm *et al.*, 2013), wounding (Xie *et al.*, 2011; Cui and Lee, 2016), heat (Iglesias and Meins, 2000; Rinne *et al.*, 2005), and heavy metals (Sivaguru *et al.*, 2000; Ueki and Citovsky, 2002, 2005) (Fig. 2A). Maize leaf tips stimulated by chilling produced action potentials through the phloem and showed severely reduced intercellular movement from the mesophyll to bundle sheath cells and reduced phloem transport of photoassimilates (Fromm *et al.*, 2013). This reduction was correlated with increased callose content in the chilled leaves and was most likely caused by the occlusion of PDs and phloem sieve pores. The mechanism by which action potentials can induce callose accumulation is not clear, but the influx of calcium accompanied with an action potential might trigger the activity of callose synthases.

Oxidative stress or other stresses that result in the accumulation of reactive oxygen species (ROS) or nitric oxide (NO) can induce callose deposition at PDs (Benitez-Alfonso *et al.*, 2009; Cui and Lee, 2016; Xiao *et al.*, 2018). Studies of *gfp arrested trafficking 1* (*gat1*), which encodes an m-type thioredoxin, indicated that ROS are key regulators of callose homeostasis in Arabidopsis. The *gat1* mutation resulted in increased PD-localized callose, similar to the response of wild-type plants treated with chemical oxidants (Benitez-Alfonso *et al.*, 2009). More recently, CalS8/GSL4 has been recognized as the key enzyme synthesizing PD callose in response to ROS, since the *cals8-1* mutant arrests the induction of callose in response to H_2O_2 treatment and wounding (Fig. 2A) (Cui and Lee, 2016). As *GSL4* gene expression was not changed by H_2O_2 treatment, ROS-dependent GSL4 activation should be under translational or post-translational regulation. The manner in which GSL4 activity is controlled remains to be elucidated. One potential mechanism is that signaling players such as receptor-like proteins (RLPs) or receptor-like kinases (RLKs) possessing a ROS sensor ectodomain may link the signaling between ROS and GSL4 activation. PDLP members and cysteine-rich RLKs (CRKs) containing two DUF26 domains or NOVEL CYSTEINE RICH RLK (NCRK) carrying WXCXCX13–18CX3CXC repeats may be strong candidates for ROS sensors.

Tocopherols (vitamin E) are chloroplast lipophilic antioxidants involved in the response to oxidative stress. In dicot and monocot plants, tocopherol deficiency induces callose deposition in vascular tissues and compromises transport of photoassimilates from source leaves (Botha *et al.*, 2000; Hofius *et al.*, 2004). Mutants with knocked-out or knocked-down expression of tocopherol cyclase, such as *sucrose-export-defective* (*sxd1*) in maize and StSXD1:RNAi in potato, exhibited a drastic reduction in tocopherols and vascular-specific callose deposition in source leaves, and displayed a defective sucrose

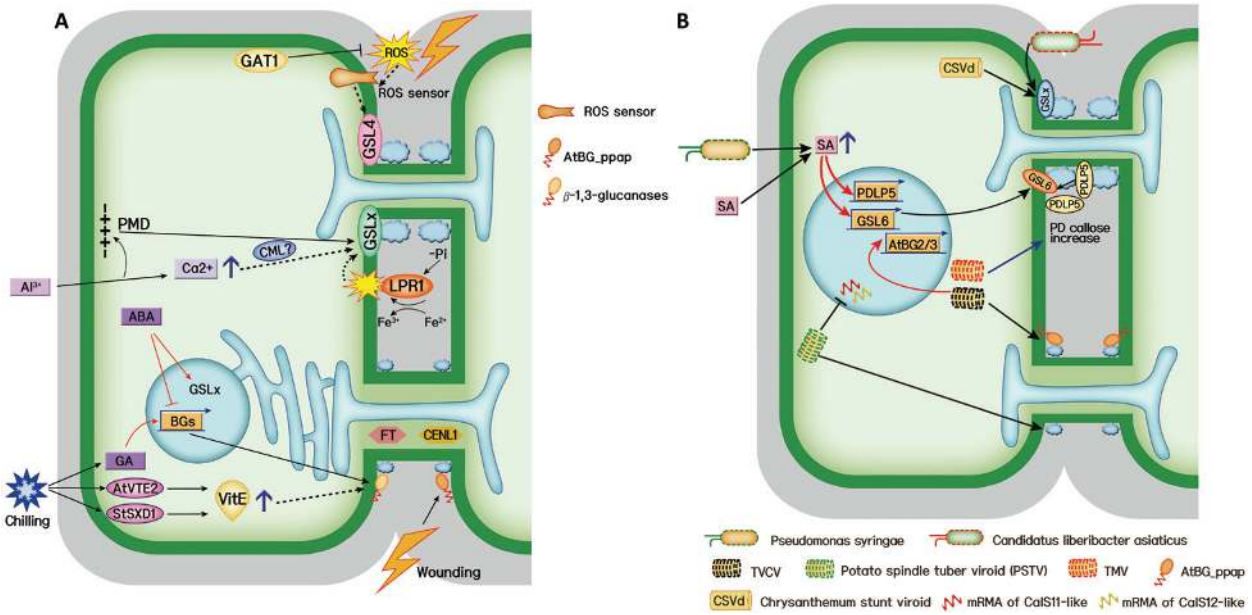

Fig. 2. Plasmodesmata (PDs)-associated callose balancing in response to abiotic and biotic stresses. (A) Callose balancing in response to abiotic stresses. In *Arabidopsis thaliana*, callose deposition at PDs upon wounding or treatment with reactive oxygen species (ROS) is dependent on GSL4. This signaling transduction happens through hypothetical ROS sensors. AtGAT1, an m-type thioredoxin, plays a role in blocking ROS-induced callose deposition. Under phosphate starvation conditions, LOW PHOSPHATE ROOT1 (LPR1) converts Fe^{2+} to Fe^{3+}, generating ROS. In turn, high levels of accumulated ROS promote callose synthesis and deposition at the PD zone of root cells. Al^{3+}-induced plasma membrane depolarization (PMD) and cytoplasmic calcium accumulation contribute to PD callose deposition and PD transport inhibition. In response to chilling, potato StSXD1 and Arabidopsis AtVTE2 function in tocopherol (vitamin E) synthesis, potentially causing a reduction in PD callose through BG-controlled callose degradation. Abscisic acid (ABA) suppresses BGs and induces callose synthase, resulting in callose deposition. In *Populus*, chilling induces the expression of several GA synthesis genes and subsequently up-regulates the transcription of several BGs. Functional BGs remove the PD callose and facilitate the plasmodesmal trafficking of FLOWERING LOCUS T (FT) and CENTRORADIALIS-LIKE1 (CENL1) to their target cells for release of dormancy. Arabidopsis AtBG_ppap promotes PD callose degradation in response to wounding. Dotted arrows indicate potential activation. (B) Callose balancing in response to biotic stresses. In response to *Pseudomonas syringae* infection or exogenous treatment with salicylic acid (SA), transcription of PDLP5 and GSL6 is induced through SA signaling; in addition, PDLP5-regulated callose deposition is dependent on GSL6. PDLP5 might form a complex with GSL6 or indirectly activate GSL6. '*Candidatus* Liberibacter asiaticus'-infected citrus trees accumulate callose and show interrupted export of photoassimilates. Chrysanthemum stunt viroid (CSVd) infects the sensitive cultivars of *Argyranthemum,* resulting in high levels of PD callose in the shoot apical meristem. AtBG2 and AtBG3 were transcriptionally induced by turnip vein clearing virus (TVCV) and cucumber mosaic virus (CMV) infection; however, these two proteins are not involved in regulating PD callose degradation. TVCV-induced callose degradation is dependent on PD-localized AtBG_ppap. Potato spindle tuber viroid (PSTV) variants drive small RNAs that can suppress the tomato callose synthase genes *CalS11-like* and *CalS12-like* and might increase PD permeability.

export phenotype (Russin *et al.*, 1996; Botha *et al.*, 2000; Hofius *et al.*, 2004). In Arabidopsis, a similar phenomenon was observed during phloem loading under low-temperature conditions (Maeda *et al.*, 2006). Arabidopsis *vitamin E2* (*vte2*) mutants, which lack α-tocopherol (the major tocopherol in leaves), exhibited aberrant cell wall callose accumulation in the phloem parenchyma 'transfer' cells and impaired photoassimilate export (Fig. 2A) (Maeda *et al.*, 2006, 2014). GSL5 is responsible for the overaccumulation of callose in *vte2* under low-temperature conditions. However, *gsl5* mutants cannot suppress the photoassimilate export phenotype, suggesting that other GSLs function in the regulation of photoassimilate export (Maeda *et al.*, 2014). Thus, tocopherol prevents abnormal callose deposition in phloem parenchyma cell walls and is required to maintain the transport of photosynthesis products under cold conditions.

Several reports have demonstrated that heavy metal ions such as Al^{3+} and Cd^{2+} are also involved in regulating PD callose accumulation in plants (Bhuja *et al.*, 2004; Piršelová *et al.*, 2012; O'Lexy *et al.*, 2018). The increase in calcium in the cytoplasm triggered by aluminum treatment induces callose accumulation (Bhuja *et al.*, 2004). In addition, depolarization of the plasma membrane is required for aluminum-induced callose deposition in tobacco cells (Fig. 2A) (Sivaguru *et al.*, 2005). A very recent study from O'Lexy *et al.* (2018) showed that applying an excess iron treatment to Arabidopsis seedlings resulted in the accumulation of callose in the phloem, which restricted the symplasmic movement of free GFP and carboxyfluorescein (O'Lexy *et al.*, 2018). In contrast, copper-treated roots showed a significant decrease in callose levels. Using genetic mutant screening, the authors identified GSL5/CalS12 enzymes as being responsible for the iron response and AtBG_PPAP and BG6 for the copper response. Interestingly, copper levels in the meristem were dramatically higher in both *atbg_ppap* and *bg6* mutants relative to wild-type roots treated with excess copper (O'Lexy *et al.*, 2018). This finding suggested that PD signaling/movement underlies the ability of the plant to properly maintain partitioning of copper when grown under conditions of excess copper.

Nutrient deficiencies such as phosphate deficiency can be sensed by plants. Interestingly, phosphate deficiency induces callose accumulation only in the presence of a sufficient iron

supply (Müller *et al.*, 2015). Under low Pi/Fe ratio conditions, LOW PHOSPHATE ROOT1 (LPR1) functions as an apoplast ferroxidase, converting Fe^{2+} to Fe^{3+}, and generates ROS, which induce callose synthesis and deposition at the PD zone of root cells in Arabidopsis (Fig. 2A) (Müller *et al.*, 2015). The enhanced PD trafficking in the *lpr1/lpr2* mutant in the absence of phosphate was visualized by a wide range of movement of GFP and SHR-GFP expressed from pSUC2::GFP and pSHR::SHR:GFP, respectively. The authors indicated that 12 GSL genes have no transcriptional changes in response to phosphate status. This result suggested potential post-transcriptional regulations of callose synthases under conditions of limited phosphate. In addition, available knockout lines of GSLs including GSL4 (responsible for ROS response during wounding or exogenous H_2O_2 treatment) could not suppress the callose deposition phenotype, suggesting potential redundancy among GSL members. Otherwise, GSL8 and GSL10, which were not tested due to their lethality in the seedling or gametophyte stages, might be the responsible enzymes (Müller *et al.*, 2015).

BG also plays an important role in regulating PD callose during stress. AtBG_PPAP is a PD-localized BG, and *atbg_ppap* mutants showed an increase in callose levels at the PDs in comparison with wild-type plants in response to wounding (Levy *et al.*, 2007). However, the expression level of *AtBG_PPAP* was not affected by wounding (Zavaliev *et al.*, 2011).

Modulation of plasmodesmal callose in response to biotic stresses

In a similar fashion, diverse biotic stresses also lead to callose deposition at PDs. Cell-to-cell spread of viruses and the blast fungus was restricted after PD callose deposition (Beffa *et al.*, 1996), suggesting that PD callose deposition may be an early defense mechanism of plants in response to viral attack. Many viruses have been reported to induce PD callose accumulation, including the tobacco mosaic virus (TMV) (Wu and Dimitman, 1970; Moore and Stone, 1972; Leisner and Turgeon, 1993; Beffa *et al.*, 1996), maize dwarf mosaic virus (Choi, 1999), potato virus X (PVX) (Allison and Shalla, 1974), tomato bushy stunt virus (Pennazio *et al.*, 1978), and broad bean wilt virus (Xie *et al.*, 2016). Ironically, some viral movement proteins (MPs) can induce host BGs, which accumulate in ER-derived vesicles (Epel, 2009). It is still controversial whether the increase in BG expression is part of the plant's defense or a viral strategy to open PDs to facilitate spread into neighboring cells (Vögeli-Lange *et al.*, 1988; Epel, 2009). Zavaliev *et al.* (2013) found that two AtBGs, *AtBG2* and *AtBG3*, were transcriptionally induced by turnip vein clearing virus (TVCV) and cucumber mosaic virus (CMV) infection. AtBG2 is secreted into the apoplast in the stress response mediated by salicylic acid (SA) or co-localized with TMV MP in ER bodies at the leading edge of TMV infection. AtBG2 seems to not be involved in regulating viral spread through PDs, as neither the *atbg2* mutant nor AtBG2 overexpression in *Nicotiana benthamiana* affected TVCV infection, PD permeability, or callose deposition. Interestingly, another isoenzyme, AtBG_PPAP, was shown to localize at the PDs, and the knockout mutant of AtBG_PPAP showed increased PD callose deposition and decreased spread of TVCV, even though the transcript level of AtBG_PPAP is not changed by TVCV and CMV (Fig. 2B) (Zavaliev *et al.*, 2013).

Recently, it was reported that chrysanthemum stunt viroid (CSVd) showed a differential ability to invade the shoot apical meristem in different *Chrysanthemum* cultivars. A major difference between sensitive and insensitive cultivars was the level of PD callose in the shoot apical meristem; a lower level of callose was found in the sensitive cultivars (Zhang *et al.*, 2015). Interestingly, certain potato spindle tuber viroid variants have a virulence-modulating region that drives a small RNA that suppresses the tomato callose synthase genes *CalS11-like* and *CalS12-like* (Fig. 2B) (Adkar-Purushothama *et al.*, 2015). The target sequence was also perfectly conserved in potato *CalS11-like* and *CalS12-like* mRNAs, suggesting evolutionary conservation in Solanaceae.

In response to *Pseudomonas syringae,* the SEL of PDs also reduces due to callose deposition at the PDs. Treatment with a bacterial pathogen induced the expression of *PLASMODESMAL-LOCALIZED PROTEIN 5* (*PDLP5*) (Lee *et al.*, 2011). Further, PDLP5 affected the plant's vulnerability to bacterial pathogen invasion; PLDP5 overexpression lines were resistant to the virulent *P. syringae,* while *pdlp5-1* mutants were sensitive (Lee *et al.*, 2011). Additionally, the dye loading DANS assay showed that bacterial infection leads to reduced PD permeability through PDLP5-mediated callose deposition.

Upstream regulators of plasmodesmal callose balancing

Some PD-localized proteins are not directly involved in callose synthesis or degradation, but they can regulate the PD callose balance indirectly. Here, we mainly discuss the function of hormonal regulation, RLKs/RLPs, callose-binding proteins, and remorin-like proteins in PD regulation, and potential crosstalk between callose-dependent and actin-mediated PD regulation.

Hormonal regulation

Several phytohormones, such as indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellin (GA), and SA, have been reported to be involved in PD callose regulation through the transcriptional modulation of callose synthase or callose-degrading enzymes. Formation of an auxin gradient by polar auxin transport is an essential step in a range of auxin responses, including phototropism and gravitropism (Bennett *et al.*, 1996; Noh *et al.*, 2003; Sorefan *et al.*, 2009). An auxin gradient across the Arabidopsis hypocotyl was correlated with the asymmetric deposition of PD callose during the phototropic response (Han *et al.*, 2014*a*). However, in *GSL8*-knockdown hypocotyls, asymmetric callose deposition and the auxin gradient are not formed, and thus no phototropic response occurs (Han *et al.*, 2014*a*). The perturbation in the auxin gradient seems to be caused by increased movement of auxin through open PDs in *GSL8* RNAi lines. Interestingly, auxin can induce *GSL8* expression through AUXIN RESPONSE FACTOR 7

(ARF7), a key auxin response transcription factor. Collectively, this work provides evidence that a positive feedback auxin–callose-mediated regulation of PDs is essential to successfully form and maintain an auxin gradient during the phototropic response (Fig. 3A–C) (Han *et al.*, 2014*a*).

ABA induces callose deposition at PDs during viral challenge; this serves as a mechanism that restricts viral cell-to-cell movement (Fraser and Whenham, 1989; Iriti and Faoro, 2008; Alazem *et al.*, 2017). Application of exogenous ABA suppressed BG and induced callose synthase activity in rice (Fig. 2A) (Liu *et al.*, 2017). ABA-mediated transcriptional down-regulation of BG was shown to be a key cause of increased callose deposition (Oide *et al.*, 2013). Modulation of symplastic trafficking by ABA also acts on the photoperiodically controlled dormancy mechanism in hybrid aspen. Short photoperiods increase the ABA level and induce the expression of the ABA receptor in *Populus* buds (Ruttink *et al.*, 2007; Karlberg *et al.*, 2010), and *abi1-1* dominant mutants have a disrupted dormancy phenotype. During a short-photoperiod treatment, Tylewicz *et al.* (2018) found that CALLOSE SYNTHASE 1, GERMIN-LIKE 10, and REMORIN-LIKE 1 and 2 were up-regulated, and that GH17-39, a glucanase, was down-regulated in wild-type hybrid aspen, but that this expression pattern was altered in *abi1-1* mutant plants. Consistent with these data, in contrast to the wild type, *abi1-1* plants showed failure to close PDs by callosic dormancy sphincters after 5 weeks of the short-photoperiod treatment (Fig. 3D–M). The defective dormancy phenotype in *abi1-1* can be rescued by the induction of callose deposition using PDLP1 overexpression without suppressing the defective ABA response, suggesting that PD closure is essential to dormancy and occurs downstream of ABA-mediated control of dormancy. From transcriptomic analysis, PICKLE (PKL), an antagonist of polycomb repression complex 2, was shown to work in the suppression of the callosic dormancy sphincter (Fig. 4A) (Tylewicz *et al.*, 2018). Reopening of closed PDs in dormant buds occurs only after prolonged exposure to chilling. Chilling or GA treatment induces the expression of several GA synthesis genes, subsequently resulting in BG production. Ten putative *Populus* 1,3-β-glucanase genes encoding orthologs of *A. thaliana* BG_ppap were found and were observed to be differentially regulated by photoperiod, chilling (5 °C), and GA (Figs 2A and 4A). The BG-mediated PD opening may allow movement of FLOWERING LOCUS T (FT) and CENTRORADIALIS-LIKE1 (CENL1) to their target

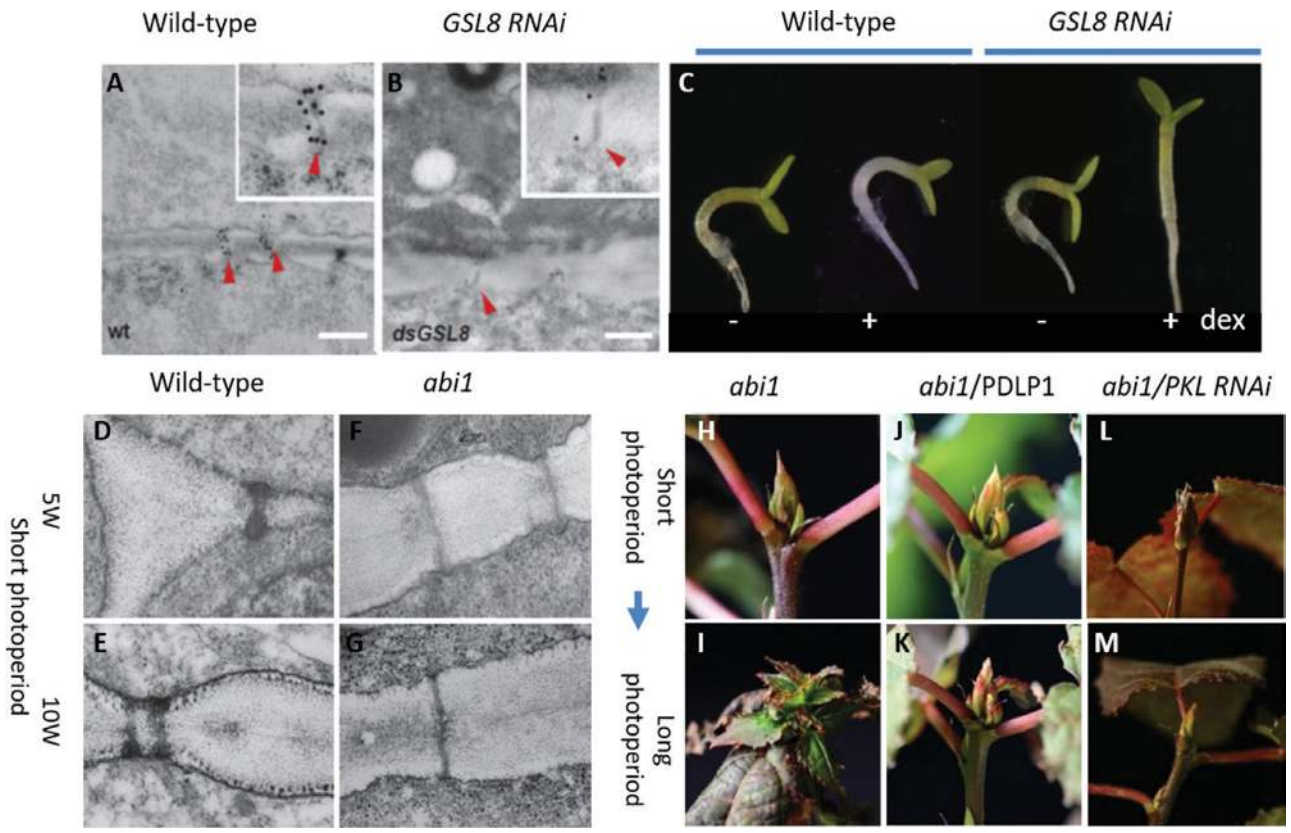

Fig. 3. PD callose controls tropism and shoot dormancy. (A, B) Callose staining in a GSL8 RNAi line using immunogold transmission electron microscopy (TEM). Immunogold labeling of callose (black dots) in PDs (red arrowheads) of wild-type (A) and dsGSL8+dexamethasone (dex) (B) hypocotyls. Insets: higher magnification images. (C) Phototropic response in the GSL8 RNAi line and Col-0 wild type under –/+dex conditions. dsGSL8+dex seedlings exhibit defective phototropism. (D–G) Callose staining in an *abi1* mutant using TEM. TEM micrographs of the apices of actively growing wild-type plants (D, E) and *abi1-1* lines (F, G) showing callose-enriched electron-dense dormancy sphincters. Sphincters are observed during short photoperiods in the apices of wild-type plants but not *abi1* mutants. (H–M). Control of dormancy break. PDLP1 expression and PKL down-regulation restore bud dormancy in *abi1* plants. Buds after 11 weeks of a short photoperiod (upper panels) and buds following the shift to a long photoperiod (lower panels) are shown. From Han *et al.* 2014*a*. Auxin-callose-mediated plasmodesmal gating is essential for tropic auxin gradient formation and signaling. Developmental Cell 28, 132–146, and Tylewicz *et al.* 2018. Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. Science 360, 212–215, with permission.

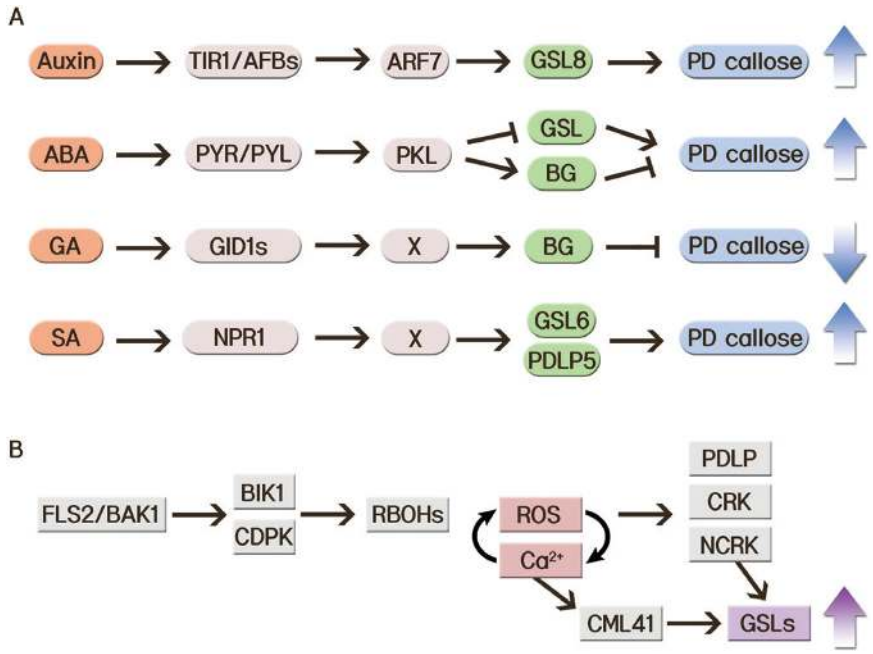

Fig. 4. Upstream regulators of PD callose balancing. (A) Hormone signaling in PD callose regulation. Auxin signaling through TIR1/AFBs auxin co-receptors and the ARF7 transcription factor regulates the expression of GSL8. ABA regulates the expression of GSL and BG in a PKL-dependent manner. GA is sensed by receptor GIDs and activates the expression of BGs. GSL6 and PDLP5 are transcriptionally regulated by SA signaling and contribute to PD callose accumulation. X, Unknown factors. (B) FLS2-triggered innate immune signaling involved in PD callose regulation. The three putative ROS sensors PDLP, CRK, and NCRK are proposed. Upward arrows represent increased callose, downward arrows represent decreased callose.

cells for release of dormancy (Rinne *et al.*, 2011). Similarly, reactivation of symplastic communication is associated with para-dormant axillary bud break of *Populus* that contains a dwarfed shoot. Para-dormant axillary buds can grow out after stem decapitation, which triggers the expression of GA-related genes, and this may lead to the induction of BGs that hydrolyze callose at the sieve plates and PDs (Rinne *et al.*, 2016).

SA and jasmonate (JA) are master hormones involved in plant defense. During the flg22-triggered innate immune response, SA signaling pathways induce PD closure through PD callose deposition, but CORONATINE INSENSITIVE 1 (COI1)-dependent JA signaling pathways suppress callose accumulation (Wang *et al.*, 2013; Cui and Lee, 2016). Interestingly, an intact JA biosynthetic pathway was found to be required for proper callose deposition during the basal defense of tomato plants against necrotrophic pathogens (Scalschi *et al.*, 2015). This finding suggests that callose-based immune responses during biotic and necrotic infections are controlled by independent callose homeostasis pathways. PD callose deposition is enhanced through the action of GSL6/CalS1 and PDLP5, which is induced by SA. PDLP5-mediated callose deposition and the regulation of PD permeability in response to pathogen infection are dependent on the EDS1/ICS/NPR1-associated SA signaling pathway. PDLP5 activates the accumulation of SA that is required for PDLP5-mediated PD closure (Fig. 4A). PDLP5 was shown to modulate both basal and induced PD permeability (Lee *et al.*, 2011; Wang *et al.*, 2013). However, SA mutants showed normal basal PD permeability but a compromised induced PD closure response (Cui and Lee, 2016). In addition to this finding, the high level of accumulated PD callose in PDLP5 overexpression lines was shown to be fully suppressed by a *cals1* mutation, suggesting the involvement of GSL6/CalS1 as well as PDLP5 in the regulation of the callose level at PDs during SA-induced callose production. It remains to be determined whether PDLP5 and GSL6/CalS1 form a complex in response to environmental stimuli or up-regulated SA biosynthesis (Fig. 2B) (Cui and Lee, 2016). It would be interesting to determine how GSL6/CalS1 is involved in the mechanism that produces PD-associated callose in response to *P. syringae* infection or SA treatment.

Receptor-like kinases/receptor-like proteins

Many RLKs and RLPs, such as CRINKLY4 (CR4), FLAGELLIN SENSING 2 (FLS2), and PDLPs, have been reported to be localized at PDs (Thomas *et al.*, 2008; Faulkner *et al.*, 2013; Stahl *et al.*, 2013). Maize CR4, a receptor kinase implicated in the determination of aleurone cell fate, promotes the lateral movement of signaling molecules between aleurone cells (Tian *et al.*, 2007). Arabidopsis CR4 is also co-localized with callose stained by aniline blue at the PDs of root meristem cells, but it is not clear whether this functions upstream of callose metabolism (Stahl *et al.*, 2013). Pattern recognition receptors (PRR) such as the RLK FLS2 recognize the pathogen-associated molecular pattern (PAMP) flagellin. Activated FLS2 interacts with the LRR-RK BRASSINOSTEROID RECEPTOR 1-ASSOCIATED KINASE 1 (BAK1, also known as SERK3) (Chinchilla *et al.*, 2007; Heese *et al.*, 2007; Roux *et al.*, 2011; Sun *et al.*, 2013). This complex phosphorylates the receptor-like cytoplasmic kinase BOTRYTIS INDUCED KINASE 1 (BIK1) (Veronese *et al.*, 2006; Lu *et al.*, 2010; Zhang and Zhou, 2010). Flagellin-triggered responses include an increase in cytosolic calcium ion concentration, an oxidative burst (a rapid increase in ROS), the activation of mitogen-activated protein

kinases (MAPKs) and calcium-dependent protein kinases (CDPKs), callose deposition, and stomatal closure (Fig. 4B) (Segonzac and Zipfel, 2011; Zipfel and Robatzek, 2010).

The generation of apoplastic ROS after the recognition of PAMPs such as flg22 might function upstream of callose deposition. Apoplastic ROS can be produced by the plasma membrane NADPH oxidases (RBOHs) when they are activated by BIK1 and CDPK (Kadota *et al.*, 2014). Recently, the phosphoinositide-specific phospholipase C2 (PI-PLC2) was shown to function downstream of FLS2 and upstream of RBOHD by direct binding. PI-PLC2 RNAi lines showed a reduced ROS level and reduced ROS-dependent responses such as callose deposition and stomatal closure (D'Ambrosio *et al.*, 2017). However, the mechanism by which flg22 PAMP-induced ROS controls callose deposition remains to be identified. One possibility is that ROS may act through Ca^{2+} that can be sensed by CML proteins. A PD-localized Ca^{2+} binding protein, CALMODULIN-LIKE 41 (CML41), whose expression is transcriptionally up-regulated by flg22, was required for PD callose deposition (Fig. 4B) (Xu *et al.*, 2017). The action of CML41 was specific to the flg22-FLS2 signaling pathway, but not fungal chitin, although chitin-LYSIN MOTIF DOMAIN-CONTAINING GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN 2 (LYM2) signaling can mediate a reduction in PD permeability through an unknown pathway (Faulkner *et al.*, 2013). Since callose synthases were reported to be activated by calcium ions, and we detected through yeast two-hybrid screening that a CML member can bind to GSL8 (S.W. Wu *et al.,* unpublished results), it is highly plausible that CML41 can directly activate some GSL members (Fig. 4B). It will be interesting to test this hypothesis in the near future. As previous studies revealed that flg22 induced callose deposition in a PMR4/GSL5-dependent pathway, GSL5 might be a targeted regulation enzyme of CML41 in response to flg22 treatment (Luna *et al.*, 2011; Leslie *et al.*, 2016).

PDLP1 was identified from a proteomic analysis of cell wall extracts from suspension-cultured Arabidopsis cells (Bayer *et al.*, 2006). The PDLP family is composed of eight members, all of which show PD targeting. PDLPs carry two extracellular DUF26 domains, one TMD domain in the middle, and one cytoplasmic C-terminal short tail. The DUF26 domain contains a C-X(8)-C-X(2)-C motif that forms three intramolecular disulfide bridges (Miyakawa *et al.*, 2009). The DUF26 domain was found in the large superfamily of cysteine-rich RLKs (CRKs) and cysteine-rich secreted proteins (CRSPs); the role of this domain is not clear, although it may function to sense the apoplastic ROS status.

PDLPs are targeted to the PDs through a COPII-dependent secretory pathway; the signal peptide and TMD domain are both necessary and sufficient for PD localization. The double knockout mutants *pdlp1/pdlp2* and *pdlp1/pdlp3* both showed increased PD permeability, while in PDLP1 overexpression lines the intercellular traffic of free GFP through PDs was dramatically blocked (Thomas *et al.*, 2008). Another report indicated that PDLP1 is highly expressed in *Hyaloperonospora arabidopsidis* (a downy mildew pathogen)-infected cells and that PDLP1 overexpression enhances callose deposition in the encasement of *H. arabidopsidis* haustoria and restricts infection by this pathogen (Caillaud *et al.*, 2014). By contrast, the *pdlp1/pdlp2/pdlp3* triple mutant has reduced callose deposition and showed more sensitivity to *H. arabidopsidis* inoculation. Overexpression of the TMD-C-terminal (TMCT) from PDLP1 also results in elevated callose deposition, suggesting that TMCT domains are involved in triggering callose deposition (Caillaud *et al.*, 2014).

Callose-binding proteins

PDCBs are PD-localized proteins with specific *in vitro* callose-binding activity. PDCBs comprise an N-terminal signal peptide, an X8 domain (CBM43), an unstructured region in the middle, and a GPI anchor motif at the C-terminus (Simpson *et al.*, 2009). There are 11 PDCB-like proteins in Arabidopsis. PDCB2 and PDCB3 are the most conserved homologs of PDCB1, and all three proteins are localized in the neck zone of PDs. *pdcb2* and *pdcb3* single mutants and the *pdcb2/pdcb3* double mutant did not exhibit any clearly defective PD phenotype, suggesting that they are functionally redundant with PDCB1. In addition, PDCB1 overexpression lines showed enhanced accumulation of PD callose and arrested intercellular trafficking through PDs (Simpson *et al.*, 2009). Interestingly, researchers failed to generate PDCB2 overexpression lines; this finding hints at the vital role of PDCB2 in plant development. PDCB1 acts as a positive regulator of PD callose deposition. To explore the true function of the PDCB family would require a *pdcb1* knockout line or a *pdcb1/pdcb2/pdcb3* triple mutant in order to analyze the *in vivo* function of PDCBs. The CRISPR-associated protein-9 nuclease system provides new and helpful technology to address such issues, and would be effective at revealing the mechanism by which PDCBs regulate PD-associated callose accumulation.

Regarding callose homeostasis, accumulated callose should activate the callose degradation pathway. A plausible speculation is that callose can be perceived by the CBM43 of PdBGs or RLPs/RLKs that possess lectin domain(s) that can bind to callose and trigger negative feedback signaling to maintain callose homeostasis. There are 75 lectin RLKs in Arabidopsis and 173 in rice (Vaid *et al.*, 2013). In mammals, dectin-1, which has a C-type lectin domain, was characterized as a callose PAMP receptor protein and functions in defense signaling in response to fungal infection. One unique member of the RLKs with a C-type lectin domain with significant homology to dectin-1 is found in both Arabidopsis and rice. It will be interesting to explore whether these C-type lectin domain RLKs have certain roles in callose homeostasis.

Remorin-like proteins

Recently, the rice gene *GRAIN SETTING DEFECT1* (*GSD1*), which encodes a remorin protein specific to vascular plants, was shown to function in controlling grain setting (Gui *et al.*, 2014). GSD1 was localized in the plasma membrane and PDs of phloem companion cells. Its overexpression or dominant mutation resulted in increased callose deposition and abnormalities in the phloem transport of photoassimilates, resulting in a higher sugar concentration in the leaves and a

lower sugar concentration in the phloem exudates. GSD1 requires *S*-acylation for plasma membrane attachment and interacts with ACTIN1 in association with PDCB1, a potential PD callose regulator (Gui *et al.*, 2015). A high level of remorins affects the ability of PVX to increase PD permeability and reduces its movement in transgenic tomatoes (Raffaele *et al.*, 2009; Perraki *et al.*, 2014). Remorin proteins have been suggested to be typical lipid raft proteins. Functional lipid rafts are essential for proper PD function. PD lipid rafts display enriched levels of sterols. Blocking sterol synthesis impaired the PD localization of the GPI-anchored proteins PdBG2 (a β-1,3-glucanase) and PDCB1, resulting in altered callose homeostasis (Grison *et al.*, 2015). In cotton, suppressed expression of the gene *GhSCP2D*, which encodes a putative sterol carrier protein, reduced the sterol content during fiber development and was associated with callose deposition at the PDs due to reduced expression of PD-targeting β-1,3-glucanases (*GhPdBG3-2A/D*), leading to reduced fiber PD permeability (Zhang *et al.*, 2017). These data support the notion that sterol, a component of lipid rafts, is required for maintaining symplastic permeability by PdBG-mediated callose degradation. It will be interesting in future studies to investigate how the amount of sterol in lipid rafts directs the PD localization of GPI-anchored proteins and the delivery mechanism of other non-GPI-anchored proteins to the PDs.

Potential crosstalk between callose-dependent and actin-mediated PD regulation

In addition to callose-dependent PD regulation, the actin cytoskeleton-mediated pathway seems to be important in modulating PDs (Aaziz *et al.*, 2001; Lucas *et al.*, 2009; Chen *et al.*, 2010; Kumar *et al.*, 2015; Pitzalis and Heinlein, 2017). Actin and some actin-associated proteins were detected at PDs (Radford and White, 1998; Faulkner *et al.*, 2009), but the existence of actin filaments in PDs is not clear. The function of actin filaments at PDs is indirectly supported by pharmacological studies; destabilization of actin filaments increases PD permeability, while increased stabilization of actin filaments is associated with reduced PD permeability (Ding *et al.*, 1996; Su *et al.*, 2010). Similarly, it has been suggested that CMV MP increases PD channels by severing actin filaments (Ding *et al.*, 1996; Su *et al.*, 2010), possibly by controlling F-actin networks functioning as a molecular filter to control the permeability of PDs (Chen *et al.*, 2010).

So far, a direct relationship between PD callose dynamics and actin filament dynamics has not been determined. However, there are a few clues indicating a potential link between them. A study using tobacco pollen tubes showed that actin filaments and endomembrane dynamics are critical for the distribution of callose synthase, suggesting that callose synthases are assembled in the ER and transported through Golgi bodies and/or vesicles moving along actin filaments (Cai *et al.*, 2011). Whether PD-localized GSL complexes use similar pathways for their targeting remains to be determined. Next, the PD-localized remorin protein GSD1 interacts with ACTIN1 (Gui *et al.*, 2015). Although the biological significance of this interaction has not yet been determined, it is very plausible that actin may function to organize PD localization of the remorin proteins that affect the recruitment of callose-associated proteins. Interestingly, a recent report showed that a mutation in ACTIN DEPOLYMERIZING FACTOR 3 reduced the level of total callose during the later stages of aphid herbivory (Mondal *et al.*, 2017). Our own studies have also found that ADF3 modulates the level of PD callose (R. Kumar *et al.*, unpublished results). Further exploration of some endogenous actin-binding proteins will reveal crosstalk between PD callose and actin filament dynamics.

Concluding remarks and future perspectives

Callose is a polysaccharide that plays a critical role in regulating the PD SEL and the symplastic pathway. Several callose synthases and β-1,3-glucanases have been identified as being involved in maintaining callose homeostasis at the PD zone; these findings have dramatically expanded our knowledge of callose-dependent PD regulation. In addition to developmental cues, several environmental cues, such as light, ROS, viruses, pathogens, and cold, could act as upstream signals and affect callose turnover. In recent decades, various signaling components have been found that coordinate PD callose accumulation and PD permeability in response to external cues, but the mechanisms by which they act to control PD callose at the molecular level remain to be explored.

Future research might uncover details of how transcription factors regulate the expression pattern of glucan synthases and β-1,3-glucanase in response to specific signals. In addition, post-translational regulation seems likely to be responsible for the rapid and effective callose deposition and degradation processes at the PD zone. An understanding of how the interaction partners regulate the enzymatic activity of GSLs and BGs will provide more information to link specific signals with callose homeostasis.

The identification of tissue-specific components of the GSL complexes, ROS sensors involved in PD callose regulation, signal-transducing factors of secondary messengers such as Ca^{2+} and ROS, and post-translational modulators such as kinases and PI-PLC will be the next challenge in this field. Finally, it will be interesting to investigate what characteristic of lipid rafts is required for PD localization of GPI-anchored proteins, and what relationship exists between PD callose and actin filament dynamics.

One long-term and meaningful field of research in agriculture is how to efficiently increase yield and elevate the assimilation of photosynthetic products into the harvested organs. The temporal and spatial control of callose deposition and degradation during phloem transport offers an opportunity to enhance the transport of photoassimilates from the source tissue to sink tissues, where the sucrose is converted to starch as a major portion of the harvest yield. Exploring PD regulation mechanisms might be helpful in improving agricultural productivity. In addition, the development of stress-tolerant crops can enhance crop productivity. One practical possibility is that by using CRISPR/Cas9 genome editing technology, callose

synthase can be modified to produce viroid resistance alleles. Understanding the molecular mechanisms underlying callose-mediated PD regulation during biotic and abiotic challenges will provide ways to apply PD engineering for the improvement of agronomical crop traits.

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References

Aaziz R, Dinant S, Epel BL. 2001. Plasmodesmata and plant cytoskeleton. Trends in Plant Science **6,** 326–330.

Adkar-Purushothama CR, Brosseau C, Giguère T, Sano T, Moffett P, Perreault JP. 2015. Small RNA derived from the virulence modulating region of the potato spindle tuber viroid silences callose synthase genes of tomato plants. The Plant Cell **27,** 2178–2194.

Aidemark M, Andersson CJ, Rasmusson AG, Widell S. 2009. Regulation of callose synthase activity *in situ* in alamethicin-permeabilized *Arabidopsis* and tobacco suspension cells. BMC Plant Biology **9,** 27.

Alazem M, He MH, Moffett P, Lin NS. 2017. Abscisic acid induces resistance against *bamboo mosaic virus* through *Argonaute2* and *3*. Plant Physiology **174,** 339–355.

Allison A, Shalla T. 1974. The ultrastructure of local lesions induced by potato virus X: a sequence of cytological events in the course of infection. Phytopathology **64,** 784–793.

Amsbury S, Kirk P, Benitez-Alfonso Y. 2017. Emerging models on the regulation of intercellular transport by plasmodesmata-associated callose. Journal of Experimental Botany **69,** 105–115.

Bachman ES, McClay DR. 1996. Molecular cloning of the first metazoan β-1, 3 glucanase from eggs of the sea urchin *Strongylocentrotus purpuratus*. Proceedings of the National Academy of Sciences, USA **93,** 6808–6813.

Baker RF, Braun DM. 2008. *Tie-dyed2* functions with *tie-dyed1* to promote carbohydrate export from maize leaves. Plant Physiology **146,** 1085–1097.

Barratt DH, Kölling K, Graf A, Pike M, Calder G, Findlay K, Zeeman SC, Smith AM. 2011. Callose synthase GSL7 is necessary for normal phloem transport and inflorescence growth in Arabidopsis. Plant Physiology **155,** 328–341.

Bayer EM, Bottrill AR, Walshaw J, Vigouroux M, Naldrett MJ, Thomas CL, Maule AJ. 2006. *Arabidopsis* cell wall proteome defined using multidimensional protein identification technology. Proteomics **6,** 301–311.

Beffa RS, Hofer RM, Thomas M, Meins F Jr. 1996. Decreased susceptibility to viral disease of β-1,3-glucanase-deficient plants generated by antisense transformation. The Plant Cell **8,** 1001–1011.

Benitez-Alfonso Y, Cilia M, San Roman A, Thomas C, Maule A, Hearn S, Jackson D. 2009. Control of *Arabidopsis* meristem development by thioredoxin-dependent regulation of intercellular transport. Proceedings of the National Academy of Sciences, USA **106,** 3615–3620.

Benitez-Alfonso Y, Faulkner C, Pendle A, Miyashima S, Helariutta Y, Maule A. 2013. Symplastic intercellular connectivity regulates lateral root patterning. Developmental Cell **26,** 136–147.

Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B, Feldmann KA. 1996. *Arabidopsis AUX1* gene: a permease-like regulator of root gravitropism. Science **273,** 948–950.

Bhuja P, McLachlan K, Stephens J, Taylor G. 2004. Accumulation of 1,3-β-D-glucans, in response to aluminum and cytosolic calcium in *Triticum aestivum*. Plant & Cell Physiology **45,** 543–549.

Bilska A, Sowiński P. 2010. Closure of plasmodesmata in maize (*Zea mays*) at low temperature: a new mechanism for inhibition of photosynthesis. Annals of Botany **106,** 675–686.

Blümke A, Falter C, Herrfurth C, Sode B, Bode R, Schäfer W, Feussner I, Voigt CA. 2014. Secreted fungal effector lipase releases free fatty acids to inhibit innate immunity-related callose formation during wheat head infection. Plant Physiology **165,** 346–358.

Borner GH, Sherrier DJ, Stevens TJ, Arkin IT, Dupree P. 2002. Prediction of glycosylphosphatidylinositol-anchored proteins in Arabidopsis. A genomic analysis. Plant Physiology **129,** 486–499.

Botha C, Cross R, Van Bel A, Peter C. 2000. Phloem loading in the sucrose-export-defective (*SXD-1*) mutant maize is limited by callose deposition at plasmodesmata in bundle sheath–vascular parenchyma interface. Protoplasma **214,** 65–72.

Bundy MG, Kosentka PZ, Willet AH, Zhang L, Miller EJ, Shpak ED. 2016. A mutation in the catalytic subunit of the glycosylphosphatidylinositol transamidase disrupts growth, fertility and stomata formation. Plant Physiology **171,** 974–985.

Burch-Smith TM, Zambryski PC. 2012. Plasmodesmata paradigm shift: regulation from without versus within. Annual Review of Plant Biology **63,** 239–260.

Cai G, Faleri C, Del Casino C, Emons AM, Cresti M. 2011. Distribution of callose synthase, cellulose synthase, and sucrose synthase in tobacco pollen tube is controlled in dissimilar ways by actin filaments and microtubules. Plant Physiology **155,** 1169–1190.

Caillaud MC, Wirthmueller L, Sklenar J, Findlay K, Piquerez SJ, Jones AM, Robatzek S, Jones JD, Faulkner C. 2014. The plasmodesmal protein PDLP1 localises to haustoria-associated membranes during downy mildew infection and regulates callose deposition. PLoS Pathogens **10,** e1004496.

Chen XY, Kim JY. 2009. Callose synthesis in higher plants. Plant Signaling & Behavior **4,** 489–492.

Chen Z, Borek D, Padrick SB, Gomez TS, Metlagel Z, Ismail AM, Umetani J, Billadeau DD, Otwinowski Z, Rosen MK. 2010. Structure and control of the actin regulatory WAVE complex. Nature **468,** 533–538.

Cheval C, Faulkner C. 2018. Plasmodesmal regulation during plant-pathogen interactions. New Phytologist **217,** 62–67.

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, Felix G, Boller T. 2007. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature **448,** 497–500.

Choi CW. 1999. Modified plasmodesmata in Sorghum (*Sorghum bicolor* L Moench) leaf tissues infected by maize dwarf mosaic virus. Journal of Plant Biology **42,** 63.

Cui W, Lee JY. 2016. *Arabidopsis* callose synthases CalS1/8 regulate plasmodesmal permeability during stress. Nature Plants **2,** 16034.

D'Ambrosio JM, Couto D, Fabro G, Scuffi D, Lamattina L, Munnik T, Andersson MX, Álvarez ME, Zipfel C, Laxalt AM. 2017. Phospholipase C2 affects MAMP-triggered immunity by modulating ROS production. Plant Physiology **175,** 970–981.

Das PK, Biswas R, Anjum N, Das AK, Maiti MK. 2018. Rice matrix metalloproteinase OsMMP1 plays pleiotropic roles in plant development and symplastic-apoplastic transport by modulating cellulose and callose depositions. Scientific Reports **8,** 2783.

Daum G, Medzihradszky A, Suzaki T, Lohmann JU. 2014. A mechanistic framework for noncell autonomous stem cell induction in *Arabidopsis*. Proceedings of the National Academy of Sciences, USA **111,** 14619–14624.

De Storme N, Geelen D. 2014. Callose homeostasis at plasmodesmata: molecular regulators and developmental relevance. Frontiers in Plant Science **5,** 138.

Ding B, Kwon MO, Warnberg L. 1996. Evidence that actin filaments are involved in controlling the permeability of plasmodesmata in tobacco mesophyll. The Plant Journal **10,** 157–164.

Doxey AC, Yaish MW, Moffatt BA, Griffith M, McConkey BJ. 2007. Functional divergence in the *Arabidopsis* β-1,3-glucanase gene family inferred by phylogenetic reconstruction of expression states. Molecular Biology and Evolution **24,** 1045–1055.

Epel BL. 2009. Plant viruses spread by diffusion on ER-associated movement-protein-rafts through plasmodesmata gated by viral induced host β-1,3-glucanases. Seminars in Cell & Developmental Biology **20,** 1074–1081.

Faulkner C, Petutschnig E, Benitez-Alfonso Y, Beck M, Robatzek S, Lipka V, Maule AJ. 2013. LYM2-dependent chitin perception limits

molecular flux via plasmodesmata. Proceedings of the National Academy of Sciences, USA **110,** 9166–9170.

Faulkner CR, Blackman LM, Collings DA, Cordwell SJ, Overall RL. 2009. Anti-tropomyosin antibodies co-localise with actin microfilaments and label plasmodesmata. European Journal of Cell Biology **88,** 357–369.

Fraser R, Whenham R. 1989. Abscisic acid metabolism in tomato plants infected with tobacco mosaic virus: relationships with growth, symptoms and the *Tm*-1 gene for TMV resistance. Physiological and Molecular Plant Pathology **34,** 215–226.

Fromm J, Hajirezaei MR, Becker VK, Lautner S. 2013. Electrical signaling along the phloem and its physiological responses in the maize leaf. Frontiers in Plant Science **4,** 239.

Gallagher KL, Sozzani R, Lee CM. 2014. Intercellular protein movement: deciphering the language of development. Annual Review of Cell and Developmental Biology **30,** 207–233.

Girard V, Maclachlan G. 1987. Modulation of pea membrane β-glucan synthase activity by calcium, polycation, endogenous protease, and protease inhibitor. Plant Physiology **85,** 131–136.

Grison MS, Brocard L, Fouillen L, *et al.* 2015. Specific membrane lipid composition is important for plasmodesmata function in Arabidopsis. The Plant Cell **27,** 1228–1250.

Gui J, Liu C, Shen J, Li L. 2014. *Grain setting defect1*, encoding a remorin protein, affects the grain setting in rice through regulating plasmodesmatal conductance. Plant Physiology **166,** 1463–1478.

Gui J, Zheng S, Shen J, Li L. 2015. Grain setting defect1 (GSD1) function in rice depends on *S*-acylation and interacts with actin 1 (OsACT1) at its C-terminal. Frontiers in Plant Science **6,** 804.

Guseman JM, Lee JS, Bogenschutz NL, Peterson KM, Virata RE, Xie B, Kanaoka MM, Hong Z, Torii KU. 2010. Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in *Arabidopsis CHORUS* (*GLUCAN SYNTHASE-LIKE 8*). Development **137,** 1731–1741.

Han X, Hyun TK, Zhang M, Kumar R, Koh EJ, Kang BH, Lucas WJ, Kim JY. 2014a. Auxin-callose-mediated plasmodesmal gating is essential for tropic auxin gradient formation and signaling. Developmental Cell **28,** 132–146.

Han X, Kim JY. 2016. Integrating hormone- and micromolecule-mediated signaling with plasmodesmal communication. Molecular Plant **9,** 46–56.

Han X, Kumar D, Chen H, Wu S, Kim JY. 2014b. Transcription factor-mediated cell-to-cell signalling in plants. Journal of Experimental Botany **65,** 1737–1749.

Heese A, Hann DR, Gimenez-Ibanez S, Jones AM, He K, Li J, Schroeder JI, Peck SC, Rathjen JP. 2007. The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proceedings of the National Academy of Sciences, USA **104,** 12217–12222.

Heo JO, Blob B, Helariutta Y. 2017. Differentiation of conductive cells: a matter of life and death. Current Opinion in Plant Biology **35,** 23–29.

Hofius D, Hajirezaei MR, Geiger M, Tschiersch H, Melzer M, Sonnewald U. 2004. RNAi-mediated tocopherol deficiency impairs photoassimilate export in transgenic potato plants. Plant Physiology **135,** 1256–1268.

Hong Z, Delauney AJ, Verma DP. 2001a. A cell plate-specific callose synthase and its interaction with phragmoplastin. The Plant Cell **13,** 755–768.

Hong Z, Zhang Z, Olson JM, Verma DP. 2001b. A novel UDP-glucose transferase is part of the callose synthase complex and interacts with phragmoplastin at the forming cell plate. The Plant Cell **13,** 769–779.

Huang L, Chen XY, Rim Y, Han X, Cho WK, Kim SW, Kim JY. 2009. *Arabidopsis* glucan synthase-like 10 functions in male gametogenesis. Journal of Plant Physiology **166,** 344–352.

Iglesias VA, Meins F Jr. 2000. Movement of plant viruses is delayed in a β-1,3-glucanase-deficient mutant showing a reduced plasmodesmatal size exclusion limit and enhanced callose deposition. The Plant Journal **21,** 157–166.

Iriti M, Faoro F. 2008. Abscisic acid is involved in chitosan-induced resistance to tobacco necrosis virus (TNV). Plant Physiology and Biochemistry **46,** 1106–1111.

Iswanto ABB, Kim J-Y. 2017. Lipid raft, regulator of plasmodesmal callose homeostasis. Plants **6,** 15.

Kadota Y, Sklenar J, Derbyshire P, *et al.* 2014. Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Molecular Cell **54,** 43–55.

Karlberg A, Englund M, Petterle A, Molnar G, Sjödin A, Bako L, Bhalerao RP. 2010. Analysis of global changes in gene expression during activity-dormancy cycle in hybrid aspen apex. Plant Biotechnology **27,** 1–16.

Kim J-Y. 2018. Symplasmic intercellular communication through plasmodesmata. Plants **7,** 23.

Kitagawa M, Jackson D. 2017. Plasmodesmata-mediated cell-to-cell communication in the shoot apical meristem: how stem cells talk. Plants **6,** 12.

Kraner ME, Link K, Melzer M, Ekici AB, Uebe S, Tarazona P, Feussner I, Hofmann J, Sonnewald U. 2017. Choline transporter-like1 (CHER1) is crucial for plasmodesmata maturation in *Arabidopsis thaliana*. The Plant Journal **89,** 394–406.

Kulich I, Vojtíková Z, Sabol P, Ortmannová J, Neděla V, Tihlaříková E, Žárský V. 2018. Exocyst subunit EXO70H4 has a specific role in callose synthase secretion and silica accumulation. Plant Physiology **176,** 2040–2051.

Kumar R, Kumar D, Hyun TK, Kim J-Y. 2015. Players at plasmodesmal nano-channels. Journal of Plant Biology **58,** 75–86.

Lacombe B, Achard P. 2016. Long-distance transport of phytohormones through the plant vascular system. Current Opinion in Plant Biology **34,** 1–8.

Lee JY, Wang X, Cui W, *et al.* 2011. A plasmodesmata-localized protein mediates crosstalk between cell-to-cell communication and innate immunity in *Arabidopsis*. The Plant Cell **23,** 3353–3373.

Leisner SM, Turgeon R. 1993. Movement of virus and photoassimilate in the phloem: a comparative analysis. Bioessays **15,** 741–748.

Leslie ME, Rogers SW, Heese A. 2016. Increased callose deposition in plants lacking *DYNAMIN-RELATED PROTEIN 2B* is dependent upon *POWDERY MILDEW RESISTANT 4*. Plant Signaling & Behavior **11,** e1244594.

Leubner-Metzger G, Meins F Jr. 1999. Functions and regulation of plant β-(PR-2). In: Datta SK, Muthukrishnan S, eds. Pathogenesis-related proteins in plants. Boca Raton: CRC Press, 49–76.

Levy A, Erlanger M, Rosenthal M, Epel BL. 2007. A plasmodesmata-associated β-1,3-glucanase in Arabidopsis. The Plant Journal **49,** 669–682.

Li J, Burton RA, Harvey AJ, Hrmova M, Wardak AZ, Stone BA, Fincher GB. 2003. Biochemical evidence linking a putative callose synthase gene with (1→3)-β-D-glucan biosynthesis in barley. Plant Molecular Biology **53,** 213–225.

Lin Q, Zhou Z, Luo W, Fang M, Li M, Li H. 2017. Screening of proximal and interacting proteins in rice protoplasts by proximity-dependent biotinylation. Frontiers in Plant Science **8,** 749.

Liu J, Du H, Ding X, Zhou Y, Xie P, Wu J. 2017. Mechanisms of callose deposition in rice regulated by exogenous abscisic acid and its involvement in rice resistance to *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). Pest Management Science **73,** 2559–2568.

Lu D, Wu S, Gao X, Zhang Y, Shan L, He P. 2010. A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. Proceedings of the National Academy of Sciences, USA **107,** 496–501.

Lucas WJ, Groover A, Lichtenberger R, *et al.* 2013. The plant vascular system: evolution, development and functions. Journal of Integrative Plant Biology **55,** 294–388.

Lucas WJ, Ham BK, Kim JY. 2009. Plasmodesmata – bridging the gap between neighboring plant cells. Trends in Cell Biology **19,** 495–503.

Luna E, Pastor V, Robert J, Flors V, Mauch-Mani B, Ton J. 2011. Callose deposition: a multifaceted plant defense response. Molecular Plant-Microbe Interactions **24,** 183–193.

Maeda H, Song W, Sage TL, DellaPenna D. 2006. Tocopherols play a crucial role in low-temperature adaptation and phloem loading in *Arabidopsis*. The Plant Cell **18,** 2710–2732.

Maeda H, Song W, Sage T, Dellapenna D. 2014. Role of callose synthases in transfer cell wall development in tocopherol deficient *Arabidopsis* mutants. Frontiers in Plant Science **5,** 46.

Miyakawa T, Miyazono K, Sawano Y, Hatano K, Tanokura M. 2009. Crystal structure of ginkbilobin-2 with homology to the extracellular domain of plant cysteine-rich receptor-like kinases. Proteins **77,** 247–251.

Mondal HA, Louis J, Archer L, Patel M, Nalam VJ, Sarowar S, Sivapalan V, Root DD, Shah J. 2018. Arabidopsis *ACTIN-DEPOLYMERIZING FACTOR3* is required for controlling aphid feeding from the phloem. Plant Physiology **176,** 879–890.

Moore AE, Stone BA. 1972. Effect of infection with TMV and other viruses on the level of a β-1,3-glucan hydrolase in leaves of *Nicotiana glutinosa*. Virology **50,** 791–798.

Müller J, Toev T, Heisters M, Teller J, Moore KL, Hause G, Dinesh DC, Bürstenbinder K, Abel S. 2015. Iron-dependent callose deposition adjusts root meristem maintenance to phosphate availability. Developmental Cell **33,** 216–230.

Noh B, Bandyopadhyay A, Peer WA, Spalding EP, Murphy AS. 2003. Enhanced gravi- and phototropism in plant *mdr* mutants mislocalizing the auxin efflux protein PIN1. Nature **423,** 999–1002.

O'Lexy R, Kasai K, Clark N, Fujiwara T, Sozzani R, Gallagher KL. 2018. Exposure to heavy metal stress triggers changes in plasmodesmatal permeability via deposition and breakdown of callose. Journal of Experimental Botany **69,** 3715–3728.

Oide S, Bejai S, Staal J, Guan N, Kaliff M, Dixelius C. 2013. A novel role of PR2 in abscisic acid (ABA) mediated, pathogen-induced callose deposition in *Arabidopsis thaliana*. New Phytologist **200,** 1187–1199.

Pennazio S, D'Agostino G, Appiano A, Redolfi P. 1978. Ultrastructure and histochemistry of the resistant tissue surrounding lesions of tomato bushy stunt virus in *Gomphrena globosa* leaves. Physiological Plant Pathology **13,** 165–171.

Perraki A, Binaghi M, Mecchia MA, Gronnier J, German-Retana S, Mongrand S, Bayer E, Zelada AM, Germain V. 2014. StRemorin1.3 hampers *Potato virus X* TGBp1 ability to increase plasmodesmata permeability, but does not interfere with its silencing suppressor activity. FEBS Letters **588,** 1699–1705.

Piršelová B, Mistríková V, Libantová J, Moravčíková J, Matušíková I. 2012. Study on metal-triggered callose deposition in roots of maize and soybean. Biologia **67,** 698–705.

Pitzalis N, Heinlein M. 2017. The roles of membranes and associated cytoskeleton in plant virus replication and cell-to-cell movement. Journal of Experimental Botany **69,** 117–132.

Radford JE, White RG. 1998. Localization of a myosin-like protein to plasmodesmata. The Plant Journal **14,** 743–750.

Raffaele S, Bayer E, Lafarge D, *et al.* 2009. Remorin, a solanaceae protein resident in membrane rafts and plasmodesmata, impairs potato virus X movement. The Plant Cell **21,** 1541–1555.

Rinne PL, van den Boogaard R, Mensink MG, Kopperud C, Kormelink R, Goldbach R, van der Schoot C. 2005. Tobacco plants respond to the constitutive expression of the tospovirus movement protein NS(M) with a heat-reversible sealing of plasmodesmata that impairs development. The Plant Journal **43,** 688–707.

Rinne PL, Paul LK, Vahala J, Kangasjärvi J, van der Schoot C. 2016. Axillary buds are dwarfed shoots that tightly regulate GA pathway and GA-inducible 1,3-β-glucanase genes during branching in hybrid aspen. Journal of Experimental Botany **67,** 5975–5991.

Rinne PL, Welling A, Vahala J, Ripel L, Ruonala R, Kangasjärvi J, van der Schoot C. 2011. Chilling of dormant buds hyperinduces *FLOWERING LOCUS T* and recruits GA-inducible 1,3-β-glucanases to reopen signal conduits and release dormancy in *Populus*. The Plant Cell **23,** 130–146.

Roux M, Schwessinger B, Albrecht C, Chinchilla D, Jones A, Holton N, Malinovsky FG, Tör M, de Vries S, Zipfel C. 2011. The *Arabidopsis* leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. The Plant Cell **23,** 2440–2455.

Russin WA, Evert RF, Vanderveer PJ, Sharkey TD, Briggs SP. 1996. Modification of a specific class of plasmodesmata and loss of sucrose export ability in the sucrose export defective1 maize mutant. The Plant Cell **8,** 645–658.

Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao RP, Boerjan W, Rohde A. 2007. A molecular timetable for apical bud formation and dormancy induction in poplar. The Plant Cell **19,** 2370–2390.

Sager R, Lee JY. 2014. Plasmodesmata in integrated cell signalling: insights from development and environmental signals and stresses. Journal of Experimental Botany **65,** 6337–6358.

Scalschi L, Sanmartín M, Camañes G, Troncho P, Sánchez-Serrano JJ, García-Agustín P, Vicedo B. 2015. Silencing of OPR3 in tomato reveals the role of OPDA in callose deposition during the activation of defense responses against *Botrytis cinerea*. The Plant Journal **81,** 304–315.

Schneider R, Hanak T, Persson S, Voigt CA. 2016. Cellulose and callose synthesis and organization in focus, what's new? Current Opinion in Plant Biology **34,** 9–16.

Segonzac C, Zipfel C. 2011. Activation of plant pattern-recognition receptors by bacteria. Current Opinion in Microbiology **14,** 54–61.

Shi X, Sun X, Zhang Z, Feng D, Zhang Q, Han L, Wu J, Lu T. 2015. GLUCAN SYNTHASE-LIKE 5 (GSL5) plays an essential role in male fertility by regulating callose metabolism during microsporogenesis in rice. Plant Cell Physiology **56,** 497–509.

Shin H, Brown RM Jr. 1999. GTPase activity and biochemical characterization of a recombinant cotton fiber annexin. Plant Physiology **119,** 925–934.

Simpson C, Thomas C, Findlay K, Bayer E, Maule AJ. 2009. An *Arabidopsis* GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking. The Plant Cell **21,** 581–594.

Sivaguru M, Fujiwara T, Samaj J, Baluska F, Yang Z, Osawa H, Maeda T, Mori T, Volkmann D, Matsumoto H. 2000. Aluminum-induced 1→3-β-D-glucan inhibits cell-to-cell trafficking of molecules through plasmodesmata. A new mechanism of aluminum toxicity in plants. Plant Physiology **124,** 991–1006.

Sivaguru M, Yamamoto Y, Rengel Z, Ahn SJ, Matsumoto H. 2005. Early events responsible for aluminum toxicity symptoms in suspension-cultured tobacco cells. New Phytologist **165,** 99–109.

Slewinski TL, Baker RF, Stubert A, Braun DM. 2012. *Tie-dyed2* encodes a callose synthase that functions in vein development and affects symplastic trafficking within the phloem of maize leaves. Plant Physiology **160,** 1540–1550.

Song L, Wang R, Zhang L, Wang Y, Yao S. 2016. *CRR1* encoding callose synthase functions in ovary expansion by affecting vascular cell patterning in rice. The Plant Journal **88,** 620–632.

Sorefan K, Girin T, Liljegren SJ, Ljung K, Robles P, Galván-Ampudia CS, Offringa R, Friml J, Yanofsky MF, Østergaard L. 2009. A regulated auxin minimum is required for seed dispersal in *Arabidopsis*. Nature **459,** 583–586.

Stahl Y, Faulkner C. 2016. Receptor complex mediated regulation of symplastic traffic. Trends in Plant Science **21,** 450–459.

Stahl Y, Grabowski S, Bleckmann A, *et al.* 2013. Moderation of *Arabidopsis* root stemness by CLAVATA1 and ARABIDOPSIS CRINKLY4 receptor kinase complexes. Current Biology **23,** 362–371.

Su S, Liu Z, Chen C, Zhang Y, Wang X, Zhu L, Miao L, Wang XC, Yuan M. 2010. Cucumber mosaic virus movement protein severs actin filaments to increase the plasmodesmal size exclusion limit in tobacco. The Plant Cell **22,** 1373–1387.

Sun Y, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou JM, Chai J. 2013. Structural basis for flg22-induced activation of the *Arabidopsis* FLS2-BAK1 immune complex. Science **342,** 624–628.

Takos AM, Dry IB, Soole KL. 2000. Glycosyl-phosphatidylinositol-anchor addition signals are processed in *Nicotiana tabacum*. The Plant Journal **21,** 43–52.

Thiele K, Wanner G, Kindzierski V, Jürgens G, Mayer U, Pachl F, Assaad FF. 2009. The timely deposition of callose is essential for cytokinesis in Arabidopsis. The Plant Journal **58,** 13–26.

Thomas CL, Bayer EM, Ritzenthaler C, Fernandez-Calvino L, Maule AJ. 2008. Specific targeting of a plasmodesmal protein affecting cell-to-cell communication. PLoS Biology **6,** e7.

Tian Q, Olsen L, Sun B, *et al.* 2007. Subcellular localization and functional domain studies of DEFECTIVE KERNEL1 in maize and *Arabidopsis* suggest a model for aleurone cell fate specification involving CRINKLY4 and SUPERNUMERARY ALEURONE LAYER1. The Plant Cell **19,** 3127–3145.

Tilsner J, Nicolas W, Rosado A, Bayer EM. 2016. Staying tight: plasmodesmal membrane contact sites and the control of cell-to-cell connectivity in plants. Annual Review of Plant Biology **67,** 337–364.

Turner A, Bacic A, Harris PJ, Read SM. 1998. Membrane fractionation and enrichment of callose synthase from pollen tubes of *Nicotiana alata* Link et Otto. Planta **205,** 380–388.

Tylewicz S, Petterle A, Marttila S, *et al.* 2018. Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. Science **360,** 212–215.

Ueki S, Citovsky V. 2002. The systemic movement of a tobamovirus is inhibited by a cadmium-ion-induced glycine-rich protein. Nature Cell Biology **4,** 478–486.

Ueki S, Citovsky V. 2005. Identification of an interactor of cadmium ion-induced glycine-rich protein involved in regulation of callose levels in plant vasculature. Proceedings of the National Academy of Sciences, USA **102,** 12089–12094.

Vögeli-Lange R, Hansen-Gehri A, Boller T, Meins Jr F. 1988. Induction of the defense-related glucanohydrolases, β-1, 3-glucanase and chitinase, by tobacco mosaic virus infection of tobacco leaves. Plant Science **54,** 171–176.

Vaid N, Macovei A, Tuteja N. 2013. Knights in action: lectin receptor-like kinases in plant development and stress responses. Molecular Plant **6,** 1405–1418.

Vatén A, Dettmer J, Wu S, *et al.* 2011. Callose biosynthesis regulates symplastic trafficking during root development. Developmental Cell **21,** 1144–1155.

Verma DP, Hong Z. 2001. Plant callose synthase complexes. Plant Molecular Biology **47,** 693–701.

Veronese P, Nakagami H, Bluhm B, Abuqamar S, Chen X, Salmeron J, Dietrich RA, Hirt H, Mengiste T. 2006. The membrane-anchored *BOTRYTIS-INDUCED KINASE1* plays distinct roles in *Arabidopsis* resistance to necrotrophic and biotrophic pathogens. The Plant Cell **18,** 257–273.

Wang X, Sager R, Cui W, Zhang C, Lu H, Lee JY. 2013. Salicylic acid regulates plasmodesmata closure during innate immune responses in *Arabidopsis*. The Plant Cell **25,** 2315–2329.

Wu JH, Dimitman JE. 1970. Leaf structure and callose formation as determinants of TMV movement in bean leaves as revealed by UV irradiation studies. Virology **40,** 820–827.

Wu S, O'Lexy R, Xu M, Sang Y, Chen X, Yu Q, Gallagher KL. 2016. Symplastic signaling instructs cell division, cell expansion, and cell polarity in the ground tissue of *Arabidopsis thaliana* roots. Proceedings of the National Academy of Sciences, USA **113,** 11621–11626.

Xiao D, Duan X, Zhang M, Sun T, Sun X, Li F, Liu N, Zhang J, Hou C, Wang D. 2018. Changes in nitric oxide levels and their relationship with callose deposition during the interaction between soybean and *Soybean mosaic virus*. Plant Biology **20,** 318–326.

Xie B, Wang X, Zhu M, Zhang Z, Hong Z. 2011. *CalS7* encodes a callose synthase responsible for callose deposition in the phloem. The Plant Journal **65,** 1–14.

Xie L, Shang W, Liu C, Zhang Q, Sunter G, Hong J, Zhou X. 2016. Mutual association of *Broad bean wilt virus 2* VP37-derived tubules and plasmodesmata obtained from cytological observation. Scientific Reports **6,** 21552.

Xu B, Cheval C, Laohavisit A, Hocking B, Chiasson D, Olsson TSG, Shirasu K, Faulkner C, Gilliham M. 2017. A calmodulin-like protein regulates plasmodesmal closure during bacterial immune responses. New Phytologist **215,** 77–84.

Zavaliev R, Dong X, Epel BL. 2016. Glycosylphosphatidylinositol (GPI) modification serves as a primary plasmodesmal sorting signal. Plant Physiology **172,** 1061–1073.

Zavaliev R, Levy A, Gera A, Epel BL. 2013. Subcellular dynamics and role of *Arabidopsis* β-1,3-glucanases in cell-to-cell movement of tobamoviruses. Molecular Plant-Microbe Interactions **26,** 1016–1030.

Zavaliev R, Ueki S, Epel BL, Citovsky V. 2011. Biology of callose (β-1,3-glucan) turnover at plasmodesmata. Protoplasma **248,** 117–130.

Zhang J, Zhou JM. 2010. Plant immunity triggered by microbial molecular signatures. Molecular Plant **3,** 783–793.

Zhang Z, Lee Y, Spetz C, Clarke JL, Wang Q, Blystad DR. 2015. Invasion of shoot apical meristems by *Chrysanthemum stunt viroid* differs among *Argyranthemum* cultivars. Frontiers in Plant Science **6,** 53.

Zhang Z, Ruan YL, Zhou N, Wang F, Guan X, Fang L, Shang X, Guo W, Zhu S, Zhang T. 2017. Suppressing a putative sterol carrier gene reduces plasmodesmal permeability and activates sucrose transporter genes during cotton fiber elongation. The Plant Cell **29,** 2027–2046.

Zipfel C, Robatzek S. 2010. Pathogen-associated molecular pattern-triggered immunity: veni, vidi…? Plant Physiology **154,** 551–554.