


MICROREVIEW

Pathogen effectors: What do they do at plasmodesmata?

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Abstract

Plants perceive an assortment of external cues during their life cycle, including abiotic and biotic stressors. Biotic stress from a variety of pathogens, including viruses, oomycetes, fungi, and bacteria, is considered to be a substantial factor hindering plant growth and development. To hijack the host cell's defence machinery, plant pathogens have evolved sophisticated attack strategies mediated by numerous effector proteins. Several studies have indicated that plasmodesmata (PD), symplasmic pores that facilitate cell-to-cell communication between a cell and neighbouring cells, are one of the targets of pathogen effectors. However, in contrast to plant-pathogenic viruses, reports of fungal- and bacterial-encoded effectors that localize to and exploit PD are limited. Surprisingly, a recent study of PD-associated bacterial effectors has shown that a number of bacterial effectors undergo cell-to-cell movement via PD. Here we summarize and highlight recent advances in the study of PD-associated fungal/oomycete/bacterial effectors. We also discuss how pathogen effectors interfere with host defence mechanisms in the context of PD regulation.

KEYWORDS

bacterial effectors, fungal effectors, host defence mechanism, plant immune response, plasmodesmata

1 | INTRODUCTION

In plants, cells are connected by symplasmic tunnels, plasmodesmata (PD). PD facilitate intercellular trafficking of essential molecules such as proteins, sugars, hormones, and RNAs, the movement of which is

controlled by the permeability of PD to the molecule (Zambryski, 2008). One of the regulatory components in PD permeability is callose, a polysaccharide in the form of β -1,3-glucan that is localized in the neck region of PD (Sager & Lee, 2018; Wu et al., 2018). Callose accumulation is dynamic, controlled by the competitive activity of

Abbreviations: BAK1, BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1; BAS1, biotrophy-associated secreted protein 1; BIK1, BOTRYTIS-INDUCED KINASE 1; CalS, callose synthase; CaM1, calmodulin 1; CML41, calmodulin-like protein 41; CRK2, cystein-rich kinase 2; DAMP, damage-associated molecular pattern; Elf18, elongation factor EF-Tu; ETI, effector-triggered immunity; FLS2, FLAGELLIN SENSING 2; GFP, green fluorescent protein; GSL, glucan-synthase like; LRR-RLK, leucine-rich repeat receptor-like kinase; MAMP, microbe-associated molecular pattern; MAP65, microtubule-associated protein 65; MAPK, mitogen-activated protein kinase; MP, movement protein; NLR, nucleotide-binding domain and leucine-rich repeat containing receptors; PAMP, pathogen-associated molecular pattern; PD, plasmodesmata; PDLP, PD-localizing protein; PM, plasma membrane; PMR4, powdery mildew resistance 4; PRR, pattern-recognition-receptor; PTI, pathogen-associated molecular pattern-triggered immunity; PWL2, Pathogenicity toward weeping lovegrass; RBOHD, respiratory burst oxidase protein D; REMs, remorins; ROS, reactive oxygen species.

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callose synthases (CaSs) and β -(1,3)-glucanases (Tilsner et al., 2016), and their enzyme activities are highly regulated in response to plant developmental stages as well as environmental factors (Brunkard & Zambryski, 2017; Tilsner et al., 2016). For instance, callose deposition/degradation around PD necks or/and cell walls often occur during plant defence responses (Kumar et al., 2015; Ngou et al., 2021; Nomura et al., 2006; Reagan & Burch-Smith, 2020).

Plant defences to pathogens arise from both cell-autonomous and non-cell-autonomous immune systems. The cell-autonomous immune system relies on pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones & Dangl, 2006; Yan et al., 2019). PTI relies on cell-surface-receptor or pattern-recognition-receptor (PRR) of extra-cellular pathogen/microbe/damage-associated molecular patterns (PAMPs/MAMPs/DAMPs, respectively) to subsequently activate innate immune responses (Jones & Dangl, 2006; Meisrimler et al., 2020). In PTI, the activation of PRRs initiates the induction of immune responses through multiple biological and physiological processes such as reactive oxygen species (ROS) accumulation, the activation of mitogen-activated protein kinase (MAPK) signalling, and transcriptional regulation of immunity-related genes (Baxter et al., 2014; Pitzschke et al., 2009). Moreover, it has been shown that callose deposition is typically triggered by conserved PAMPs. Examples of bacterial PAMPs are the 22 amino acid sequence of the conserved N-terminal part of flagellin (flg22) and the bacterial elongation factor EF-Tu (Elf18) (Kunze et al., 2004). The flg22-induced callose response requires FLAGELLIN SENSING 2 (FLS2), BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 (BAK1), BOTRYTIS-INDUCED KINASE 1 (BIK1), (Lu et al., 2010)- and some PD-associated proteins such as calmodulin-like protein 41 (CML41) and cysteine-rich receptor-like kinase (CRK2) (Kimura et al., 2020; Xu et al., 2017). Chitin, a β -(1-4)-linked polymer of *N*-acetylglucosamine, and chitosan, a randomly distributed β -(1-4)-linked polymer of *D*-glucosamide and acetylglucosamine, are examples of potent callose-inducing PAMPs from the fungal cell wall (Gong et al., 2020). The lysin motif (LysM) domain-containing protein CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) and LYSIN MOTIF DOMAIN-CONTAINING GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN 2 (LYM2) were identified as chitin PRRs in *Arabidopsis* (Faulkner et al., 2013; Miya et al., 2007). Even though both CERK1 and LYM2 are called "chitin PRR", they are segregated in the chitin perception machinery. It seems that CERK1 predominantly mediates chitin-activated signals in the plasma membrane (PM), whereas LYM2 is required for chitin-induced callose accumulation at PD (Faulkner et al., 2013). Moreover, LYM2-mediated chitin signalling at the PD-PM also requires two additional LysM-RKs, LYK4, and LYK5. LYK4 and LYK5 behave differently in response to chitin. LYK5 associates with CERK1 in the PM, whereas LYK4 associates with LYM2 in the PD-PM. Admittedly, both LYK4 and LYK5 are required for chitin-induced callose deposition and PD closure, but their partners are different (Cheval et al., 2020).

To counteract the PTI response, several pathogens inject an assortment of effectors, molecules that manipulate host cell structure

and function, mediating disease (caused by virulence effectors) or defence responses (caused by avirulence effectors) (Kamoun, 2006; Nguyen et al., 2021). Effectors can be targeted either to the cytosol or specifically to a host compartment. In the case of bacteria (both gram-negative and -positive), protein secretion systems are classified into 12 systems that include the general secretion (Sec), twin-arginine translocation (Tat), SecA2, injectosome, sortase, type I secretion system (T1SS), T2SS, T3SS, T4SS, T5SS, T6SS, and T7SS. For instance, many gram-negative plant-pathogenic bacteria use a type III secretion system (T3SS) to subvert and colonize their hosts (Green & Meccas, 2016). Like plant-pathogenic bacteria, to suppress the PTI and manipulate the cellular activities plant-pathogenic fungi also secrete effectors (Stergiopoulos & de Wit, 2009) that can be delivered either into the plant apoplast or inside the plant cell (Djamei et al., 2011; Selin et al., 2016). For biotrophic and hemibiotrophic fungal pathogens, cytosolic effectors are delivered into the host cells using unique structures such as appressoria, haustoria, invasive hyphae (IH), and biotrophic interfacial complex (BIC) (Catanzariti et al., 2006; Giraldo et al., 2013; Kemen et al., 2005; Khang et al., 2010; Petre & Kamoun, 2014; Rafiqi et al., 2010). For example, the rice effector proteins Pathogenicity toward weeping lovegrass (PWL2) and biotrophy-associated secreted protein 1 (BAS1) preferentially accumulate in the BIC and then translocate to the rice cytoplasm through IH (Khang et al., 2010). Furthermore, the apoplastic effectors are delivered by conventional fungal endoplasmic reticulum (ER)-Golgi secretory processes; in contrast, the cytoplasmic effectors require exocyst components Exo70 and Sec5 for delivery into rice cells (Giraldo et al., 2013). Both intracellular fungal and bacterial effectors can be recognized by plant resistance proteins, typically nucleotide-binding domain and leucine-rich-repeat containing receptors (NLR) (Nguyen et al., 2021). Such recognition leads to stronger defence responses, for example the hypersensitive cell death response at the injection site (Jones & Dangl, 2006), resulting in local disease resistance (Kim et al., 2009; Su et al., 2018; Thomma et al., 2011), increased callose deposition (Ngou et al., 2021), and systemic acquired resistance (Lim et al., 2016). Because fungal/oomycete/bacterial-encoded effectors involve a variety of cellular activities within the host cell and can result in systemic resistance, it is logical that some of these effectors localize at and modify symplasmic PD channels. Indeed, it has been demonstrated that PD modification mediates cell-to-cell movement and disseminates effectors to expand their infection (Cao et al., 2018; Iswanto et al., 2021). This raises the possibility that fungal/oomycete/bacterial effectors interfere with a host innate immune response by a direct mechanism involving PD regulation.

In parallel to the cell-autonomous immune system, the non-cell-autonomous immune system responds to the pathogens via signals delivered from infected cells to uninfected cells through apoplastic and symplasmic routes (Lee & Lu, 2011; Yan et al., 2019). It has been proposed that deposition of callose at PD determines the effectiveness of some pathogen effectors and the movement of signalling molecules from one cell to neighbouring cells (Cheval & Faulkner, 2018; Li et al., 2021; Liu et al., 2021). Interestingly, a recent study

showed that several effectors from *Pseudomonas syringae* move from one cell to another cell through PD (Li et al., 2021). Notwithstanding the recent research focus, how these effectors modify PD in relation to host defence mechanisms is poorly understood.

In this review, we summarize the current status of knowledge, focusing on fungal/oomycete/bacterial effector-modulated PD flux regulation related to plant defence responses. We also discuss possible mechanisms of PD defence against fungal/oomycete/bacterial effectors after being targeted by effectors.

2 | PATHOGEN EFFECTORS INTERFERE WITH PTI-INDUCED CALLOSE ACCUMULATION

In the early PTI responses following fungal or bacterial infection, multiple plant cellular activities enhance the deposit of callose, release ROS, and activate a variety of gene-related defence responses (Cheval et al., 2020; Ellinger & Voigt, 2014; Kimura et al., 2020; Vu et al., 2020; Xu et al., 2017). However, numerous secreted effectors with broadly varied localizations and functions can suppress these signalling pathways in host cells (Ellinger & Voigt, 2014; Fu et al., 2007). As shown in Table 1 and Figure 1, many fungal/oomycete/bacterial effectors hamper callose accumulation, the one characteristic they have in common, although it remains unclear whether callose accumulation at PD is affected (Blumke et al., 2014; Castaneda-Ojeda et al., 2017; Cheng et al., 2017; Deb et al., 2018; Di et al., 2017; Fabro et al., 2011; Fu et al., 2007; Guo et al., 2016; Jin et al., 2003; Kim et al., 2005; Lan et al., 2019; Lei et al., 2020; Li et al., 2016, 2018; Medina et al., 2018; Nomura et al., 2006, 2011; Qi et al., 2016; Rodriguez-Herva et al., 2012; Sakulkoo et al., 2018; Sohn et al., 2007; Tomczynska et al., 2020; Xu et al., 2019; Zhang et al., 2007).

For instance, the *Pseudomonas syringae* HopE1 effector localizes to the cytoplasm and nucleus in cells and suppresses the basal immune response through physical interaction with calmodulin 1 (CaM1) and microtubule-associated protein 65 (MAP65) (Guo et al., 2016). *Plasmopara viticola*, the grapevine downy mildew oomycete, secretes the PvRXLR131 effector, which is targeted to a leucine-rich repeat receptor-like kinase (LRR-RLK) protein, the BRASSINOSTEROID INSENSITIVE1 (BR1) KINASE INHIBITOR1 (Lan et al., 2019). Even though both HopE1 and PvRXLR131 effectors are known to suppress innate immunity, the molecular mechanism of callose attenuation remains unknown. Similarly, the signalling pathways of other effectors leading to diminished callose accumulation remain unidentified. The *P. syringae* HopAI1 effector directly targets MPK3 and MPK6 to suppress RBOHD-activated PMR4/CalS12/GSL5 function (Zhang et al., 2007). Interestingly, RxRL3, an effector protein secreted by the oomycete *Phytophthora brassicae*, was found to localize at PD and to suppress PD callose accumulation via direct interaction with CalS1/GLS1, CalS2/GLS3, and CalS3/GLS12, resulting in enhanced intercellular movement of green fluorescent protein (GFP) (Tomczynska et al., 2020). In addition to RxRL3, *Melampsora*

larici-populina, the widespread and catastrophic rust pathogen of poplar, deploys effectors including Mlp37347, an effector that reportedly localizes at PD (Germain et al., 2018). It was also reported that PD callose accumulation was highly reduced in *Arabidopsis thaliana* transgenic lines expressing Mlp37347-GFP (Rahman et al., 2021). The wide variety of effector targets and known functions indicates that the regulation of callose accumulation/degradation is affected by many factors.

3 | PATHOGEN EFFECTORS TARGET PD AND PD-ASSOCIATED PROTEINS

Many effectors have been shown to suppress PAMP-induced callose accumulation, but the main mechanism of this suppression is poorly understood. The surprising latest discovery by Li and co-workers suggests an explanation: PD-dependent movement of bacterial effectors from cell to cell (Li et al., 2021). In these experiments, 16 effectors from *P. syringae* pv. *tomato* DC3000 (HopK1, HopY1, HopF2, HopU1, HopH1, HopC1, HopN1, HopAA1-1, HopAF1, HopP1, HopAB2, HopE1, HopAO1, HopA1, HopX1, and HopB1) moved in the plant cells (Figure 2), but the movement was restricted when excessive callose accumulated at PD (Li et al., 2021). It is plausible to assume that other mobile effectors can move within the host cells and are assisted by the attenuation of callose accumulation at PD. Interestingly, HopA1 from *P. syringae* pv. *syringae* strain 61 (HopA1_{Pss61}) was shown to be located at PD (Kang et al., 2021). However, PD localization and cell-to-cell movement analyses of HopA1_{DC3000} and HopA1_{Pss61}, respectively, have not been determined.

Given that the translocation of effectors through PD is necessary for the success of plant colonization by several pathogens (Cao et al., 2018; Liu et al., 2021), effectors have employed multiple strategies to attack PD-callose regulation. To counter PD closure and callose accumulation, effectors can target the PD (Cao et al., 2018; Rahman et al., 2021; Tomczynska et al., 2020), thereby enlarging the PD gate. On *Fusarium oxysporum* infection, Avr2 and Six5 effectors physically interact at PD to expand the pore size (Cao et al., 2018). Experimental evidence suggests that the Six5 effector is required for Avr2 effector movement between cells. In the absence of the Six5 effector, PD permeability to Avr2 is restricted. However, when Avr2 and Six5 interact, PD are opened, resulting in the intercellular movement of Avr2 through PD. Indeed, the Six5 function is thought to resemble the viral movement protein (MP) action that facilitates viral spread between plant cells via PD (Waigmann & Zambryski, 1995). Unlike MPs or other viral-encoded proteins that directly interact with PD-associated proteins (Garnelo Gómez et al., 2021; Levy, 2015; Lewis & Lazarowitz, 2010; Rosas-Diaz et al., 2018; Uchiyama et al., 2014), the downstream signalling pathway of Six5-Avr2 interaction in regulating PD function is not known.

The oomycete pathogen *P. brassicae* RxLR3 effector, containing a conserved RxLR amino acid motif (Arg-any amino acid-Leu-Arg), is an archetypal effector that targets PD regulation (Tomczynska et al.,

TABLE 1 List of pathogen effectors that suppress pathogen-associated molecular pattern (PAMP)-induced callose deposition

Pathogen	Effector	Molecular target	Subcellular localization	References
<i>Fusarium graminearum</i>	FGL1	nd	nd	Blumke et al. (2014)
<i>Fusarium oxysporum</i>	Avr2	nd	Cytoplasm	Di et al. (2017)
<i>Hyaloperonospora arabidopsidis</i>	HaRxLs	nd	Nucleus	Fabro et al. (2011)
	HaRxL23	nd	nd	Deb et al. (2018)
<i>Hyaloperonospora parasitica</i>	ATR13	nd	nd	Sohn et al. (2007)
<i>Melampsora larici-populina</i>	Mlp37347	Glutamate decarboxylase 1 (GAD1)	Plasmodesmata	Rahman et al. (2021), Germain et al. (2018)
<i>Phakopsora pachyrhizi</i>	PpEC23	Soybean transcription factor GmSPL121	Cytoplasm	Qi et al. (2016)
<i>Phytophthora brassicae</i>	RxLR3	CaI51, CaI52, and CaI53	Plasmodesmata	Tomczynska et al. (2020)
<i>Phytophthora sojae</i>	PsCRN63	nd	Nucleus	Li et al. (2016)
<i>Plasmopara viticola</i>	PvRXLR131	PM-located BRI1 kinase inhibitor 1 (BK11)	nd	Lan et al. (2019)
<i>Pseudomonas savastanoi</i> pv. <i>savastanoi</i> NCPPB 3335	HopAO1, HopAO2	nd	nd	Castaneda-Ojeda et al. (2017)
<i>Pseudomonas syringae</i> pv. <i>maculicola</i>	AvrRpm1	RIN4	Plasma membrane	Nimchuk et al. (2000), Kim et al. (2005)
<i>P. syringae</i> pv. <i>tomato</i>	HopU1	Glycine-rich RNA-binding protein GRP7	Cytoplasm and nucleus	Fu et al. (2007)
	HopAI1	MPK3 and MPK6	nd	Zhang et al. (2007)
	HopN1	PsbQ, a member of the oxygen evolving complex of photosystem II	Chloroplast	Rodriguez-Herva et al. (2012)
	AvrPtoB	FLS2, BAK1, and Snf1-related kinase 2.8 (SnRK2.8)	Cytoplasm	Gohre et al. (2008), Lei et al. (2020)
	AvrE	nd	nd	Deb et al. (2018)
	HopM1	MIN7	Trans-Golgi network/early endosome	Nomura et al. (2006, 2011)
	HopE1	CaM and microtubule-associated protein 65 (MAP65)	Cytoplasm and nucleus	Guo et al. (2016)
<i>Puccinia striiformis</i> f. sp. <i>tritici</i>	AvrRpt2	RIN4	Cytoplasm and nucleus	Jin et al. (2003), Kim et al. (2005)
	Pst_12806	Wheat TalSP protein	Chloroplast	Xu et al. (2019)
	PSTha5a23	nd	Cytoplasm	Cheng et al. (2017)
<i>Xanthomonas axonopodis</i> pv. <i>manihotis</i> CIO151	XopR, AvrBs2, XopAO1	nd	nd	Medina et al. (2018)
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	pthXo3 _{JXOV}	OsSWEET14	nd	Li et al. (2018)

nd, not determined.

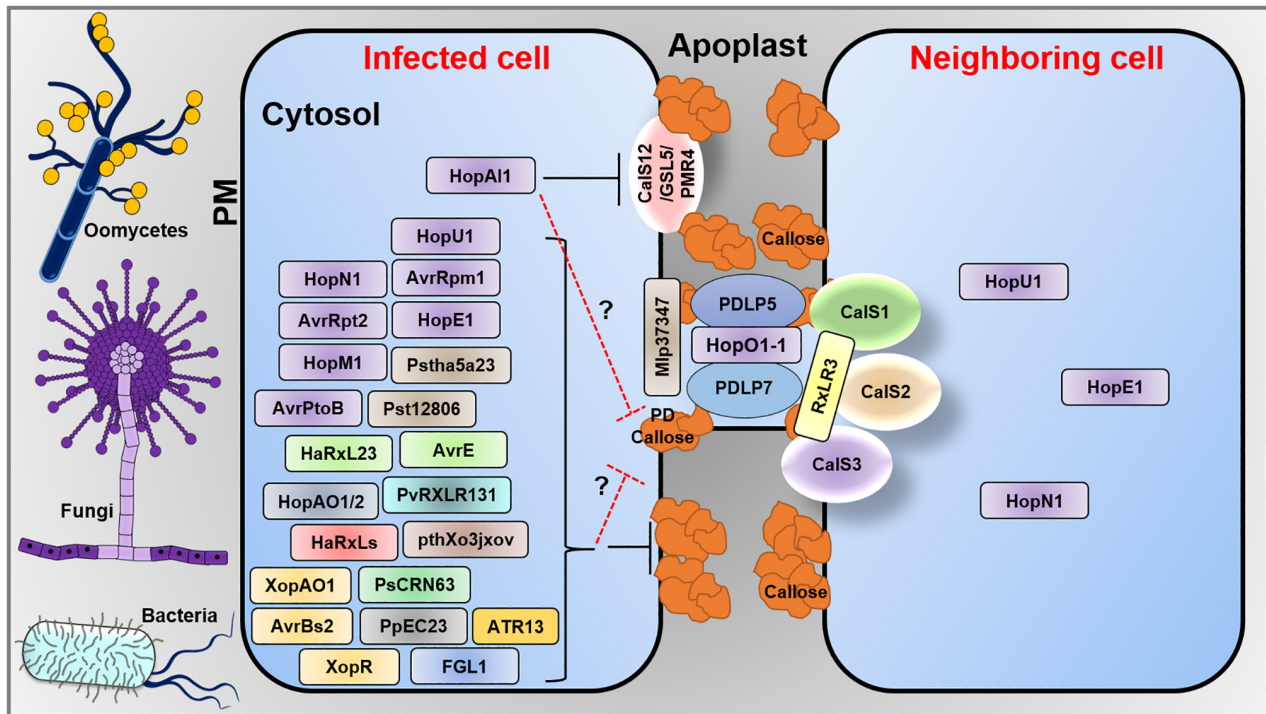


FIGURE 1 Effectors suppress pathogen-associated molecular pattern (PAMP)-induced callose deposition. Several fungal/oomycete/bacterial pathogens, including *Pseudomonas syringae*, *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335, *Phytophthora sojae*, *Phytophthora brassicae*, *Hyaloperonospora arabidopsidis*, *Hyaloperonospora parasitica*, *Plasmopara viticola*, *Xanthomonas manihotis*, *Xanthomonas oryzae* pv. *oryzae*, *Fusarium graminearum*, *Melampsora larici-populina*, *Puccinia striiformis* f. sp. *tritici*, and *Phakopsora pachyrhizi* deploy effectors to suppress PAMP-triggered immunity (PTI) and lead to reduced callose accumulation in the host cells. HopA11 suppresses MPK3/6-induced PMR4/GSL5/CaS12 activity. Even though some effectors exhibit cell-to-cell movement via plasmodesmata (PD), the molecular mechanisms of effector-modulated PD function have not been unequivocally determined. Unlike other effectors, HopO1-1 and RxLR3 control symplasmic continuity through direct interaction with PD-associated proteins such as PDL5/7 and CaS1/2/3, respectively

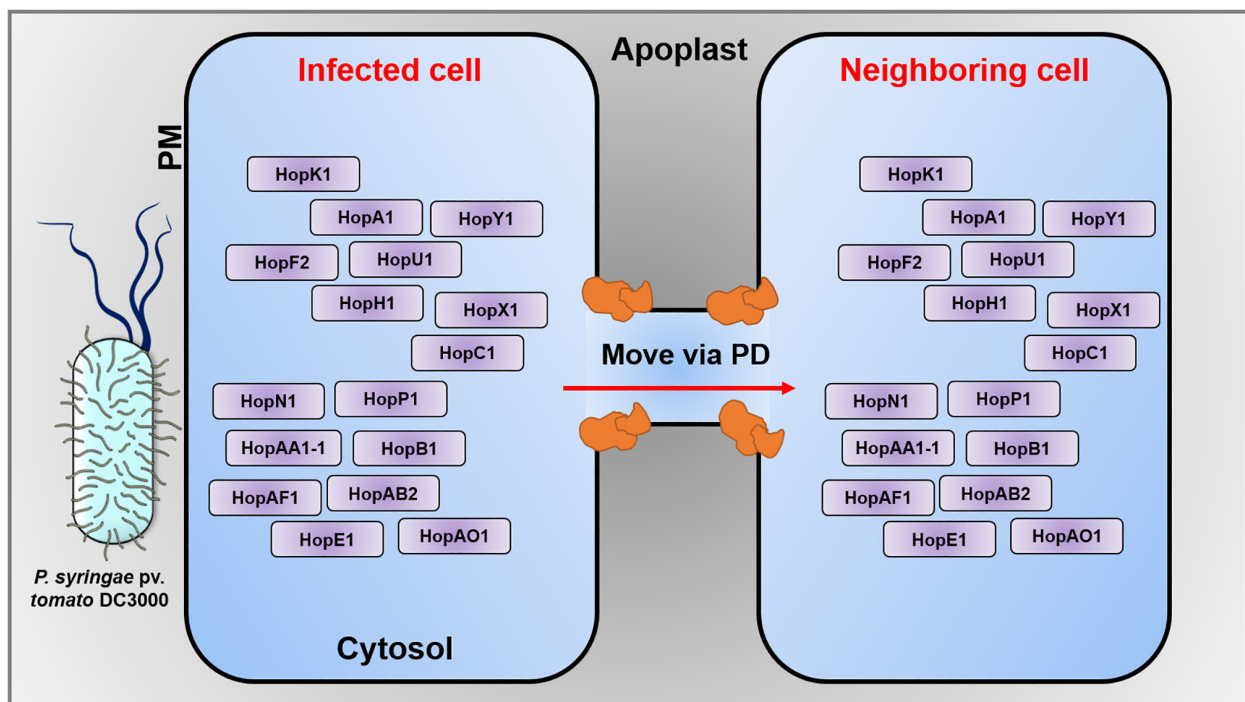


FIGURE 2 *Pseudomonas syringae* effector proteins undergo cell-to-cell movement via plasmodesmata (PD). Sixteen effectors (HopK1, HopY1, HopF2, HopU1, HopH1, HopC1, HopN1, HopAA1-1, HopAF1, HopP1, HopAB2, HopE1, HopAO1, HopA1, HopX1, and HopB1) move from infected cells to neighbouring cells through PD

2020). Physical interaction of RxLR3 with callose synthases CalS1, CalS2, and CalS3 hinders callose accumulation at PD (Figure 1) and subsequently promotes symplasmic trafficking of free GFP (Tomczynska et al., 2020). The authors also provided evidence that the RxLR3 effector interferes with callose accumulation in papillae forming at the penetration site and cell periphery in plant leaves expressing RxLR3 and response to penetration by germinating cysts of *P. brassicae*. However, the subgroup of CalS limiting callose deposition at the penetration site was not determined. The authors suggested that RxLR3 promotes PD cell-to-cell trafficking to counteract systemic acquired resistance, a plant defence that protects uninfected plant tissues and employs PD-mediated trafficking of signalling molecules (Lim et al., 2016). Possibly RxLR3 targets an entire subgroup of CalS to interrupt callose synthesis by multiple enzymes. Alternatively, the synthases may function redundantly, and the pathogen has acquired the ability to counteract all of them although only one is needed. It will be interesting to see the results of future experiments interrogating the relationship between RxLR3 delivery into the host cell, PD dynamic regulation, and systemic acquired resistance signalling.

In addition to the effector-targeted PD-associated proteins, an effector protein delivered by *P. syringae*, HopO1-1, localizes at PD and modulates PD permeability (Aung et al., 2020). In particular, *Arabidopsis* stably expressing HopO1-1 increases the distance of PD-dependent molecular flux, although it does not apparently affect the size exclusion limit of PD. HopO1-1 was shown to have the mono-ADP-ribosyltransferase activity required for the proper subcellular localization of HopO1-1 to modulate host target proteins. Moreover, HopO1-1 was also found to selectively associate with and reduce the levels of the *Arabidopsis* PD regulators, PDLP5 and PDLP7 (Figure 1). Surprisingly, this destabilization is not caused by ribosylation activity but apparently involves a proteasome-dependent degradation pathway that particularly affects PDLP7. The short intracellular C-tail (7–19 amino acid residues) of the transmembrane PDLP7 is required for the specific interaction between PDLP7 and HopO1-1. Removing two putative ribosylation sites in the C-tail domain prevents degradation of PDLP7 by HopO1-1 (Aung et al., 2020). Bacterial multiplication increased in mutant plants lacking PDLP7 or PDLP5 (Li et al., 2021) as was previously shown for mutant *pdlp5* plants (Liu et al., 2020). In those experiments, PDLP5 was shown to be an important regulator of callose accumulation in response to bacterial infection, and callose accumulation was inversely related to bacterial multiplication and invasion (Liu et al., 2020). It is not as clear how PDLP7 increases the molecular flux and the colonization by bacteria, although apparently PDLP5 and PDLP7 work together. Because PDLP7 is particularly targeted for degradation, its absence may leave PDLP5 as the dominant PDLP and thus limit callose accumulation. Alternatively, other PD components such as ER-plasma membrane (PM) contact sites, ER-derived desmotubules, cytoplasmic sleeves, and spoke-like tethers (Park et al., 2019; Yan & Liu, 2019) may regulate PD permeability. Further research to determine whether and how pathogen effectors target other PD components and alter PD architectures would be interesting.

4 | DO FUNGAL/OOMYCETE/BACTERIAL EFFECTORS TARGET PD-LIPID RAFTS?

Because PD are PM-lined pores, the PM may be important in defining the functionality of the PD. The PD-PM has unique compartments, lipid rafts/lipid nanodomains that are enriched in sterols and sphingolipids (Grison et al., 2015; Iswanto & Kim, 2017; Iswanto et al., 2020). Plant Remorins (REMs) are one of the best-characterized membrane lipid nanodomain-associated proteins in the plant (Raffaele et al., 2009), but the participation of REMs has been identified in only a few plant-microbe interactions and hormonal signalling. There are conflicting reports of the REM1.3 effect in viral spread (Raffaele et al., 2009), and one in which it promotes susceptibility to the oomycete *Phytophthora infestans* (Bozkurt et al., 2014), indicating that plant REM1.3 has a variety of mechanisms for responding to viral and fungal attacks. Effector HopZ1a from *P. syringae* was shown to interact with *Nicotiana benthamiana* REM NbREM4 at the PM, and overexpression of NbREM4 increases the expression of defence genes. Also, NbREM4 is phosphorylated in vitro by a cytoplasmic kinase known to act in early defence responses, avrPphB-susceptible1 (PBS1) (Albers et al., 2019). Furthermore, treatment with flg22, an elicitor of PTI, changes the localization of NbREM4 to punctate structures that appear to be in the PM. It will be interesting to see if these structures, which also appear to be at the borders of cells, are in the PD-PM and if they are elicited by other effectors in other plant species. However, it is still unknown how the pathogen targets the protein that modifies the lipid nanodomains.

5 | POSSIBLE MECHANISMS OF PD DEFENCE AGAINST FUNGAL/OOMYCETE/BACTERIAL EFFECTORS

To date, the mechanism for rapid callose deposition at the PD to inhibit the movement of fungi/bacterial effectors is generally poorly understood (Li et al., 2021). Pathogen-induced callose may be regulated via a hormone signalling pathway such as salicylic acid or via the ROS signalling pathway (Cheval & Faulkner, 2018; Wang et al., 2013). PD-associated proteins specific for PD response in the ETI defence context have recently been identified and were shown to be involved in cell-to-cell trafficking. In this section, we will outline PD defence tactics to counteract effectors. The CalS family and PDLPs family play a central role in bacterial defence mechanisms in diverse plant species. At the PD-PM, the increased deposition of callose, associated reduction of molecular flux, and reduced bacterial colonization are regulated by PDLP5 and PDLP7, as described in an earlier section of this review (Aung et al., 2020). Using a different strategy, Li and coworkers demonstrated that PDLP5- and PDLP7-induced PD callose accumulation restricts the PD-dependent movement of HopAF1 (Li et al., 2021). It has been shown that GSL8/CalS10 and PDLP5 physically interact (Saatian et al., 2018), and overall it is now clear that the CalSs family and PDLPs family play conserved and central roles in bacterial-triggered immune responses across species.

However, it is still challenging to identify the exact mechanism of the CalSs/PDLPs complex when countering bacterial effectors because they are functionally redundant.

In the case of fungal infection, the attenuation of PD permeability is another strategy to limit fungal effector trafficking. For example, Avr2 and Six5, described in an earlier section as important for fungal infection by *F. oxysporum* and disease susceptibility, interact with each other in the cytoplasm. However, at a lower expression level, Avr2 and Six5 are translocated to PD and induce PD permeability (Cao et al., 2018). Previously, it was also reported that Avr2 expression suppressed FLS2-mediated immune responses, ROS production, and callose accumulation in *Arabidopsis* (Di et al., 2017), albeit the molecular target of Avr2 and its contribution to callose degradation are still elusive. These studies indicate that callose accumulation may be involved in the Six5-dependent cell-to-cell movement of Avr2 and did not exclude the possibility that PD-associated proteins interact with or recruit Six5 to the PD, thereby reducing the interaction of Avr2 and Six5 at the PD and limiting PD channel opening.

A low level of PD-callose may be an advantage in fungal resistance when coupled with the hypersensitive response. It is possible that the subcellular localization of I-2, which is commonly required for fungal effector resistance in tomato, is not expressed in cortical cells (Ma et al., 2013). Avr2 is assisted by Six5 to translocate from an infected cell to a neighbouring cell that could, if it contains I-2, trigger the immune response. While recent studies mostly focus on the PD-associated promotion of callose deposition as a resistance mechanism, it is possible that plasmodesmal proteins involved in callose degradation ultimately contribute to the innate immune response.

Collectively, on bacterial and fungal attack, the CalS family and PDL family act as key factors to regulate callose accumulation and symplasmic continuity together with the basal immune response. Moreover, it is expected that future studies will find spatiotemporal regulation in the PD-dependent time-course of pathogen infection, especially when researchers explore the involvement of effector trafficking through PD in relation to the hypersensitive response.

6 | CONCLUSIONS AND PERSPECTIVE

Opened or closed PD, the dynamic of symplasmic channels during plant immune responses results from multiple signalling pathways to adjust both PTI and ETI in host plants. The current study of fungal/bacterial-PD interactions during innate immunity has several limitations. Uncovering the molecular linkage between fungal/bacterial effectors and PD regulation is the main challenge. The recent identification of numerous effectors that move cell-to-cell and directly interact with PD-associated proteins permits potential exploration of the molecular mechanisms and characterization of other pathogenic effectors that regulate PD function. Moreover, the identification of pathogen effectors, which localize at and exploit PD, suggests that control of the symplasmic continuity might be a pivotal factor in defence that limits the dissemination of effectors. Further study is required to fully understand the function of PD in plant innate immune responses.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed.

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