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Plant cells under siege: plant immune system versus pathogen effectors

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Pathogen-secreted effector proteins enable pathogens to manipulate plant immunity for successful infection. To penetrate host apoplastic space, pathogens reopen the stomata. Once the invasion into the apoplast occurs, pathogens deceive the host detection system by deploying apoplastic effectors. Pathogens also deliver an arsenal of cytosolic effectors into the host cells, which undermine host immunity such as salicylic acid (SA)-dependent immunity. Here we summarize recent findings that highlight the functions of the effectors from fungal, oomycete and bacterial pathogens in the key steps of infection at the stomata, in the apoplast, and inside the cell. We also discuss cell type-specific responses in the host during infection and the necessity of further investigation of plant–pathogen interactions at spatial and temporal resolution.

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Introduction

Plant immunity is shaped by millions of years of coevolution between plants and their pathogens resulting in enormous complexity at the molecular level. The invading pathogens thus need special weapons to conquer the established immune system of their corresponding host, as the host would defend itself like a fortified castle. These weapons are known as effectors, which modulate plant immunity and enable parasitic infection. For example, some effectors attack the first layer of the plant immune system conferred by cell surface pattern-recognition receptors (PRRs) that detect broadly conserved pathogen molecules (pathogen/microbe-associated molecular patterns, PAMP/MAMPs). This system is known as PAMP- (or pattern)-triggered immunity (PTI). To overcome the system, some effectors are targeted to the plant apoplast whilst other effectors are delivered inside the host cytoplasm. To detect such effectors, plants have evolved other types of receptors called Resistance (R) proteins, which recognize the presence of pathogen effectors directly or indirectly and launch strong counter attack, known as effector-triggered immunity (ETI) [1].

Although the functions of most effectors remain elusive, recent studies revealed a set of host targets, greatly assisting our understanding of host colonization strategies of the pathogens and the key plant defense pathways involved. Recent technologies in live-cell imaging and histochemical analysis of effector-target complexes during pathogen invasions have also greatly facilitated our understanding of plant–pathogen interactions. In addition, the latest transcriptional analyses in several pathosystems have shown that the expression of effectors is tightly regulated [2–4]. In response, plants show cell type-specific and organ-specific gene expression during pathogen infection [2,5••,6••]. These reports indicate that the investigation of plant–pathogen interactions at spatial and temporal resolution is highly informative to understand intimate relationships during infection. In this review, we provide a brief overview of the latest advances in our understanding of the functions of fungal, oomycete and bacterial effector proteins.

Pathogen catapult system: effector delivery

Certain filamentous pathogens, such as the causal agent of tomato leaf mold *Cladosporium fulvum*, enter through pre-existing openings in the plant's surface, such as wounds and stomatal pores. In contrast, other filamentous pathogens are able to enter plant tissue by direct penetration through the cuticle by forming specialized structures, such as appressoria, and/or by secreting cell wall-degrading enzymes [7]. Following successful penetration, intracellular hyphae extend or form specialized infection structures that penetrates host cells known as haustoria to secrete additional effectors as well as to uptake nutrients. These structures are enveloped by the plant plasma membrane, forming a biotrophic interface known as the extrainvasive hyphal matrix or the extrahaustorial matrix (reviewed in [8]). At this interface, apoplastic effectors play key roles to prevent PAMP recognition and PRR activation whilst cytoplasmic effectors undermine host immunity by controlling gene expression, or by interfering with cellular signal and secretion (see below). 7Although bacteria deploy the well-studied type III secretion system to deliver cytoplasmic effectors inside

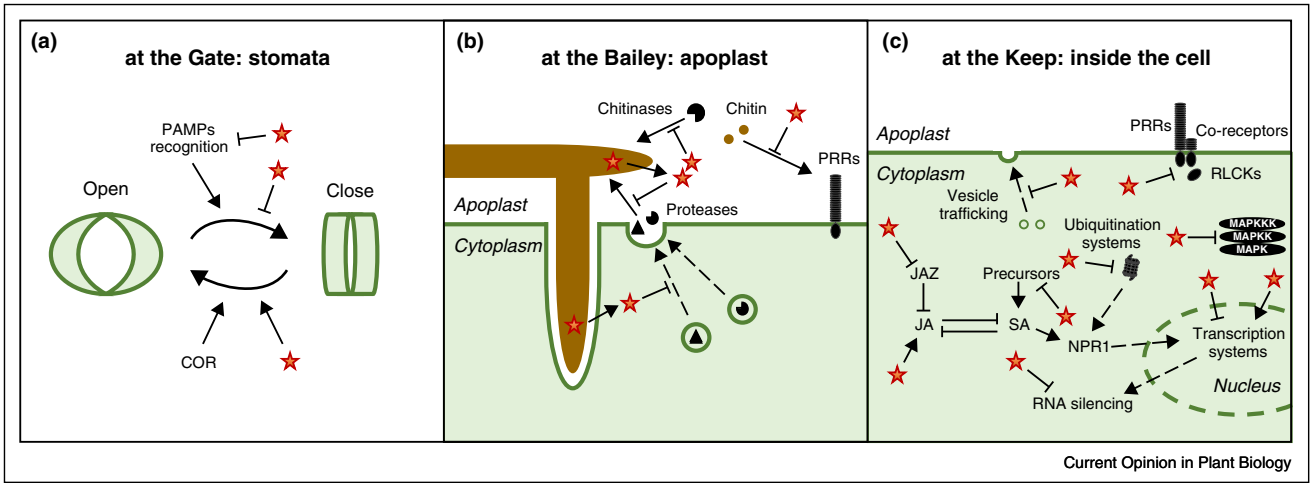
plant cells [9], the mechanisms of effector translocation in filamentous pathogens are still under debate [8]. In some oomycetes, such as *Phytophthora* spp. and downy mildews, cytoplasmic effectors have modular structures with conserved amino acid motifs associated with host translocation, such as the RxLR-motif and Crinkler effectors [10]. In contrast, bioinformatic analysis has yet failed to identify any putative translocation motif in fungal effectors [11].

Once invaded, some translocated cytoplasmic effectors, such as PWL2 and BAS1 from the rice blast fungus *Magnaporthe oryzae*, preferentially accumulate in the biotrophic interfacial complex (BIC), a plant-derived membrane-rich compartment forming at the primary hyphal tips of invasive hyphae [12,13•]. Interestingly, the effectors that accumulate in BIC require a specific secretory toolkit different from the conventional ER-Golgi based secretion [13•]. Some translocated effectors from *M. oryzae* and the smut fungus *Ustilago maydis* are shown to move from the pathogen-invaded cell to the neighboring uninvaded cells, presumably to prepare these cells for their colonization or to prevent systemic defense signals [12,14]. However, the molecular mechanism of how these filamentous pathogens deliver their effectors to particular locations remains elusive.

Battle at the Gate: stomata

While many plants fend off most microbes by the physical wall such as the cuticle layer, some successful pathogens are able to find and open a gate to enter without damaging the wall. Stomata, for example, are the main gate for many pathogens to enter host apoplastic space. In the case of some filamentous pathogens such as the oomycete downy mildew pathogen *Hyaloperonospora arabidopsidis*, conidiophores enwrapping asexual reproductive conidiospores emerge through the stomata [15]. Similarly, bacterial pathogens also often invade through the stomata. The control of stomatal opening by pathogens is therefore a key step for successful penetration and release (Figure 1a and Table 1) [16]. By contrast, plants promote stomatal closure upon the perception of PAMPs such as fungal cell wall component chitin as well as bacterial flagellin and lipopolysaccharide [17,18]. The stomatal closure is normally mediated by a plant hormone abscisic acid (ABA), but the recent work by Montillet *et al.* [19] reveals that the stomatal closure triggered by a flagellin-derived peptide flg22 does not depend on ABA. Instead, the stomatal closure requires another plant hormone salicylic acid (SA) [17]. To counter the SA-mediated closure of the stomata, some bacterial pathogens, such as *Pseudomonas syringae*, secrete coronatine (COR) to manipulate hormone regulated signaling. COR mimics a derivative of the plant hormone jasmonic acid (JA) known as JA-isoleucine. JA plays an antagonistic role to SA, thereby COR secretion facilitates the opening of the stomata [17]. Some *P. syringae* pathovars also inject effectors, such as HopX1 and HopZ1a, which induce JA responses in the host to repress SA-mediated responses (see also the following section Battle at the Keep: inside the cell) [5••,20•,21]. The HopX1-producing *P. syringae* strain no longer produces COR [20•], suggesting that the COR function is compensated by HopX1. Similarly, HopM1 from *P. syringae*, also inhibits the stomatal closure by targeting the 14-3-3 protein GRF8/AtMIN10 [18,22]. Another *P. syringae* effector HopF2 can also suppress the stomatal immunity [23]. Interestingly, although HopF2 has a mono-ADP-ribosyltransferase activity that disables PTI by targeting BAK1 and MAPK kinase 5 (MKK5) [24,25], this activity is not required for stomatal reopening by HopF2 [23]. How HopF2 suppresses the stomatal immunity is therefore still unclear.

Figure 1



Pathogen effectors interfere with plant immunity in various plant subcellular compartments in key steps of the interaction (**(a)** stomata; **(b)** apoplast; **(c)** inside the cell). Pathogen effectors (red stars) targets host components (black shapes) related to plant immunity (see text for details).

Table 1

Examples for pathogen effectors that interfere with crucial host targets in different levels of infection

Effector name	Organism	Target	Mode of action	Refs
Stomatal immunity				
HopF2	*Pseudomonas syringae*	Unknown	Suppression of stomatal immunity independent of its ADP-ribosyltransferase activity	[23]
HopM1	*Pseudomonas syringae*	GRF8/AtMIN10	Degradation of GRF8/MIN10	[18,22]
Fungal chitin				
Avr4	*Cladosporium fulvum*	Fungal chitin	Protection of chitin against hydrolysis by chitinases	[32,33]
Ecp6	*Cladosporium fulvum*	Fungal chitin	Evasion of chitin recognition by binding to chitin Interference of chitin receptor dimerization (!?)	[30,31•]
Slp1	*Magnaporthe oryzae*	Fungal chitin	Evasion of chitin recognition by binding to chitin	[68]
Mg3LysM	*Mycosphaerella graminicola*	Fungal chitin	Evasion of chitin recognition by binding to chitin Protection of chitin against hydrolysis by chitinases	[69]
Host proteases				
Avr2	*Cladosporium fulvum*	RCR3 and PIP1	Inhibition of the protease activity of RCR3 and PIP1	[40]
AVRblb2	*Phytophthora infestans*	C14	Inhibition of the secretion of C14	[41]
EPI1	*Phytophthora infestans*	P69B	Inhibition of the protease activity of P69B	[70]
EPI10	*Phytophthora infestans*	P69B	Inhibition of the protease activity of P69B	[71]
EPIC1	*Phytophthora infestans*	RCR3 and C14	Inhibition of the protease activity of RCR3 and C14	[37,38,39•]
EPIC2B	*Phytophthora infestans*	RCR3, PIP1 and C14	Inhibition of the protease activity of RCR3, PIP1 and C14	[36–38]
PmEPIC1	*Phytophthora mirabilis*	MRP2	Inhibition of the protease activity of MRP2	[39•]
Pit2	*Ustilago maydis*	CP2, CP1A and XCP2	Inhibition of the protease activity of CP2, CP1A and XCP2	[35]
SA signaling				
HaRxL44	*Hyaloperonospora arabidopsidis*	MED19a	Degradation of MED19a	[5••]
HaRxL62	*Hyaloperonospora arabidopsidis*	Unknown	Suppression of SA-triggered immunity	[6••]
HaRxL96	*Hyaloperonospora arabidopsidis*	Unknown	Suppression of *PR1* after inoculation with an avirulent *Hpa* strain	[57]
PsAvh163	*Phytophthora sojae*	Unknown	Suppression of *PR1* after inoculation with an avirulent *Hpa* strain	[57]
PsIsc1	*Phytophthora sojae*	Host SA biosynthesis	Hydrolysis of isochorismate	[50•]
AvrE	*Pseudomonas syringae*	Unknown	Suppression of SA-dependent basal immunity	[55]
HopI1	*Pseudomonas syringae*	Hsp70	Suppression of chloroplast-synthesized SA accumulation	[51,52]
HopM1	*Pseudomonas syringae*	MIN7	Degradation of MIN7	[22,55,56]
HopX1	*Pseudomonas syringae*	JAZs	Degradation of JAZs	[20•]
HopZ1a	*Pseudomonas syringae*	JAZs	Degradation of JAZs	[21]
Cmu1	*Ustilago maydis*	Host SA biosynthesis	Conversion of chorismate to prephenate	[14]
VdIsc1	*Verticillium dahliae*	Host SA biosynthesis	Hydrolysis of isochorismate	[50•]
XopJ	*Xanthomonas campestris*	RPT6	Suppression of proteasome activity	[53,54]

FLS2, the PRR for flg22, which is expressed in guard cells as well as epidermal cells is required for flg22-induced stomatal closure [17]. Although *P. syringae* carries multiple PAMPs and *Arabidopsis* stomata can respond to other PAMPs than flg22 [17], stomata in *fls2* mutant plants do not respond to the COR-deficient strain of the bacteria [26], suggesting that plants recognize the pathogen's stomata entry mainly by FLS2. Since flagellin is essential for bacteria to penetrate the apoplastic space through stomata, it is reasonable to see FLS2 plays a key role in stomata immunity. Indeed, the amino acid residue in flagellin required for recognition by FLS2 is essential for bacterial motility and virulence [27]. By contrast, bacterial pathogens produce several effectors targeting FLS2 and components in the PRR complex (reviewed in [28]). However, it remains to be determined whether the effectors that participate in stomatal opening are directly delivered into guard cells to carry out the function. Further investigation of stomatal cell-specific responses in host plants and the spatial functional analysis of pathogen effectors (e.g. by using guard cell-specific promoters) are therefore necessary.

Battle at the Bailey: apoplast

Once the invasion into the apoplast occurs, pathogens often aim to deceive the host detection system by deploying apoplastic effectors (Figure 1b and Table 1). For example, diverse fungal pathogens secrete LysM domain-containing

effectors that either sequester chitin fragments to prevent recognition or prevent the release of these fragments from fungal cell walls (reviewed in [29]). Ecp6 of *C. fulvum* is a LysM domain-containing effector that suppresses chitin-triggered immunity in two different ways; the sequestration of chitin fragments [30] and a potential mechanism that prevents the chitin receptor dimerization required for the activation of immune signaling [31•]. Another example is the *C. fulvum* effector Avr4, which prevents release of free chitin molecules by protecting the fungal cell wall from plant chitinases [32,33].

Proteases are integral components of the plant immune response in the apoplast [34]; therefore successful pathogens have evolved means to suppress the defense responses by inhibiting host proteases. For instance, Pit2 of *U. maydis* inhibits a set of apoplastic maize cysteine proteases by the conserved 14-amino-acid motif that serves as a protease inhibitor domain [35]. Similarly, two secreted cystatin-like proteins from the oomycete late blight pathogen *Phytophthora infestans* and the other effector Avr2 from *C. fulvum* inhibit papain-like cysteine proteases RCR3, PIP1, and C14 [36–40]. Interestingly, the *P. infestans* cytoplasmic effector Avrblb2 prevents the secretion of C14 into the apoplast [41], suggesting that blocking host apoplastic proteases are important for virulence. Genomic studies showed that diversification of such pathogen protease inhibitors plays an important role in host specificity upon host jump [39•].

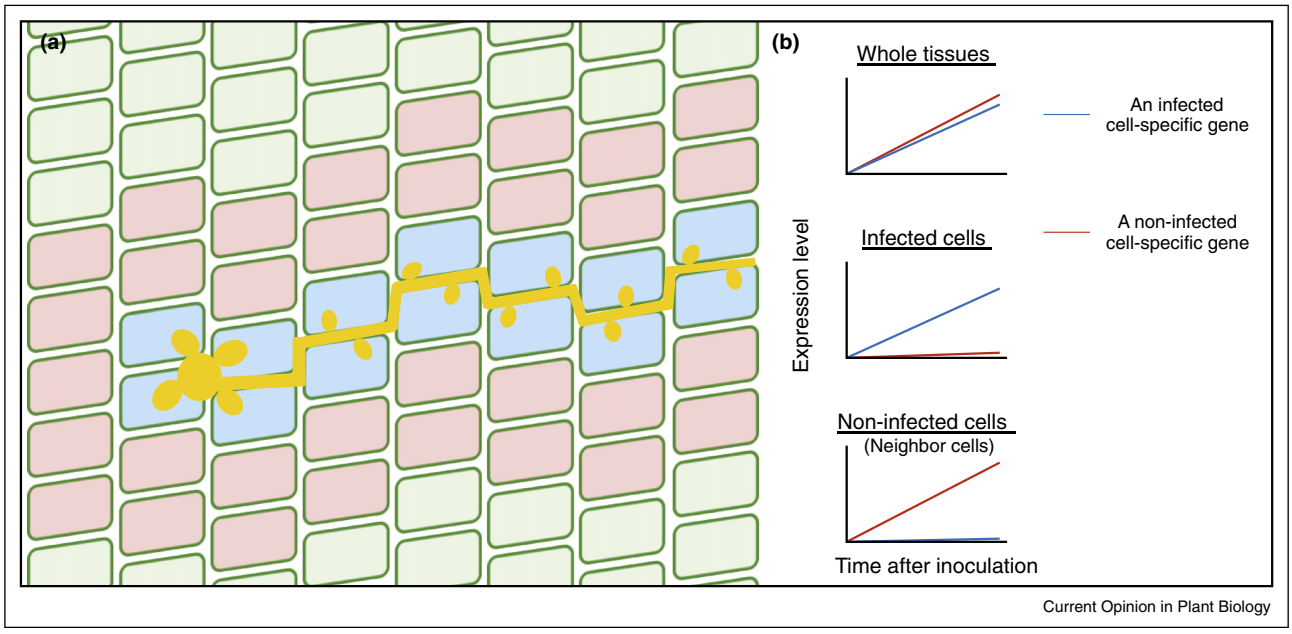
Battle at the Keep: inside the cell

To capture the Keep, pathogens deliver an arsenal of cytosolic effectors into the host cells. Cytosolic effectors suppress plant defense responses and modulate plant physiology to accommodate pathogenic invaders and provide them with nutrients in many ways; i.e. by inactivating or degrading key components in the PRR complexes as well as receptor-like cytoplasmic kinases, or by inactivating downstream components like MAPK cascades, ubiquitination systems, hormone signaling, transcription systems, vesicle trafficking systems, secondary metabolisms, or gene silencing (Figure 1c and Table 1). Here, we focus on cytosolic effectors targeting host hormone signaling, especially SA signaling. For a more elaborate description of other effectors targeting the other host immune systems, we refer to several recent reviews [8,28,42–45].

SA is a phytohormone essential for many immune responses against biotrophic pathogens [46]. SA-dependent immunity responses are mostly antagonistic to those of JA, which is often required for immunity against necrotrophic pathogens and herbivores [47–49]. As SA also acts as a crucial component in systemic immunity [46], pathogens need to attenuate SA signals to promote their fitness. To do so, several pathogen effectors target the SA biosynthesis. For, example, *U. maydis* Cmu1, *Phytophthora sojae* PsIsc1, and *Verticillium dahlia* VdIsc1 prevent formation of SA precursors [14,50•]. Similarly, *P. syringae* HopI1 suppresses chloroplast-synthesized SA accumulation [51,52]. Other effectors target key regulatory components required for the SA signaling pathway. For instance, the bacterial pathogen *Xanthomonas campestris* effector XopJ inhibits the proteasome activity by degrading RPT6, a subunit of the proteasome complex, to impair the proteasomal turnover of NONEXPRESSOR OF PR GENES1 (NPR1), the key regulator of the SA signaling pathway [53,54]. The *P. syringae* effector HopM1 promotes proteasome-dependent degradation of the vesicle trafficking-related AtMIN7, which is also required for SA-triggered immunity [22,55,56]. In addition, some effectors also alter the antagonistic relationship between SA and JA. One example is the *H. arabidopsidis* effector HaRxL44, which triggers degradation of the mediator subunit MED19a to alter the balance between the SA-mediated and JA-mediated signaling pathways by perturbing transcription [5••]. On the other hand, the *P. syringae* effectors HopX1 and HopZ1a promote the degradation of JASMONATE ZIM DOMAIN (JAZ) repressors to activate the JA pathway, resulting in suppression of SA signaling [20•,21]. Additionally, *P. syringae* AvrE, *Ralstonia solanacearum* PopS, *P. sojae* PsAvh163, *H. arabidopsidis* HaRxL62 and HaRxL96 interfere with the recognition of SA and/or downstream signaling, but not SA biosynthesis [6••,55,57,58]. However, targets of these effectors are still unknown, and identification of their host targets might reveal unknown components in the SA signaling pathway.

Another way for successful pathogens to suppress host immunity is to control host enzymes that downregulate the SA signaling pathway. For instance, *H. arabidopsidis* induces host *DOWNY MILDEW RESISTANT 6* (*DMR6*) upon infection in the haustoriated cells into which cytosolic effectors are delivered [59]. In these haustoriated cells, *DMR6* expression is highly induced but expression of SA-responsive *PATHOGENESIS-RELATED GENE 1* (*PR1*) is greatly suppressed. However, in the non-haustoriated adjacent cells, *DMR6* is not expressed, while *PR1* expression is highly induced (Figure 2a) [5••,6••]. Thus, yet unknown *H. arabidopsidis* effector(s) probably functions in a cell autonomous manner to induce host *DMR6* to block the SA signaling. *DMR6* encodes a putative 2-oxoglutarate (2OG) oxygenase [59]. *DMR6-LIKE OXYGENASE 1* (*DLO1*), a homolog of *DMR6*, redundantly suppresses plant immunity with *DMR6*, as the *dmr6/dlo1* double mutant is completely resistant to *H. arabidopsidis* and exhibits a severe dwarf phenotype associated with high levels of SA [60•]. Consistently, overexpression of *DLO1* in the *dmr6* mutant restores the susceptibility to *H. arabidopsidis*. *DLO1* is also induced during senescence and the enzyme encoded by *DLO1* can hydroxylate SA to form 2,3-dihydroxybenzoic acid by its 2OG oxygenase activity, which reduces SA levels to the control [61].

Figure 2



Schematic view of cell type-specific responses in the *H. arabidopsidis–Arabidopsis* interaction. **(a)** *H. arabidopsidis* extends hyphae forming haustoria inside host cells (yellow shapes). Blue-shaded and red-shaded cells indicate the haustoriated (infected) and non-haustoriated adjacent (non-infected) cells, respectively. **(b)** A predicted expression pattern of cell type-specific genes after inoculation with *H. arabidopsidis* in samples from the whole tissues, infected cells and non-infected cells. Blue and red indicate infected cell-specific genes such as *DMR6* and non-infected cell-specific genes such as *PR1*, respectively.

DMR6 is also likely to catabolize SA by a similar hydroxylation reaction to DLO1 [60•].

Notably, although *DMR6* and *PR1* show distinct cell type-specific expression in the interaction with *H. arabidopsidis*, transcriptome analysis using whole tissues apparently shows no significant difference in expression patterns of these genes during infection (Figure 2b) [6••]. Analysis using whole tissues in interactions with pathogens, especially highly localized-infecting filamentous pathogens including *H. arabidopsidis*, therefore, is likely to fail to capture the cell type-specific expression pattern in the host (Figure 2). More advance technologies, such as laser microdissection [62], fluorescence-activated cell sorting [63], affinity tagging of ribosomal proteins [64] and INTACT (isolation of nuclei tagged in specific cell types) [65], will be used to further dissect cell type-specific pathogen and host interactions.

Conclusions

Pathogens establish successful colonization by effective and timely delivery of effectors. In the past decade, studies in effector biology have revealed pathogen-targeted host pathways. The emerging picture is that diverse effectors have evolved to target common host components of the plant immune system [66,67•]. However, the spatial and temporal interactions between pathogen effectors and host targets need to be further investigated. By building a unified view of the sophisticated plant immune system and pathogen strategies employed to overcome immunity, we may be able to develop novel methods to combat plant diseases in the future.

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