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Strategies used by bacterial pathogens to suppress plant defenses

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Plant immune systems effectively prevent infections caused by the majority of microbial pathogens that are encountered by plants. However, successful pathogens have evolved specialized strategies to suppress plant defense responses and induce disease susceptibility in otherwise resistant hosts. Recent advances reveal that phytopathogenic bacteria use type III effector proteins, toxins, and other factors to inhibit host defenses. Host processes that are targeted by bacteria include programmed cell death, cell wall-based defense, hormone signaling, the expression of defense genes, and other basal defenses. The discovery of plant defenses that are vulnerable to pathogen attack has provided new insights into mechanisms that are essential for both bacterial pathogenesis and plant disease resistance.

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Abbreviations

Avr	avirulence
Bgh	*Blumeria graminis* f. sp. *hordei*
CDS	cell death suppressor
coi1	*coronatine insensitive1*
COR	coronatine
DC3000	*Pseudomonas syringae* pv. *tomato* strain DC3000
FLS2	FLAGELLIN INSENSITIVE2
HR	hypersensitive response
HST	host-selective toxin
JA	jasmonic acid
jai1	*jasmonic acid insensitive1*
NHO1	*NONHOST RESISTANCE1*
PCD	programmed cell death
Pph	*Pseudomonas syringae* pv. *phaseolicola*
PR	*pathogenesis-related* gene
R	resistance
SA	salicylic acid
TTSS	type III secretion system
Xcv	*Xanthomonas campestris* pv. *vesicatoria*

Introduction

Plants have evolved both general and highly specialized defense responses that function to prevent diseases caused by the majority of microbial pathogens they encounter. These defenses include preformed and induced antimicrobial compounds to repel pathogen attack, cell wall reinforcements to prevent pathogen entry, and programmed cell death (PCD) to limit pathogen establishment and spread (Figure 1). When disease develops, the virulent pathogen commonly infects only a particular plant species or cultivar, suggesting that pathogens have evolved highly specialized tactics to promote disease. It has long been hypothesized that successful pathogens depend, at least in part, on their ability to avoid or actively suppress plant defense responses [1,2]. That is, successful disease formation may rely on pathogen factors that function to induce susceptibility in an otherwise resistant or tolerant host. Several such pathogenicity factors have been identified, including type III effectors [3] and toxins [4] from phytopathogenic bacteria, host-selective toxins (HSTs) [5] and small molecule suppressors [6] from phytopathogenic fungi, and suppressors of post-transcriptional gene silencing from plant viruses [7].

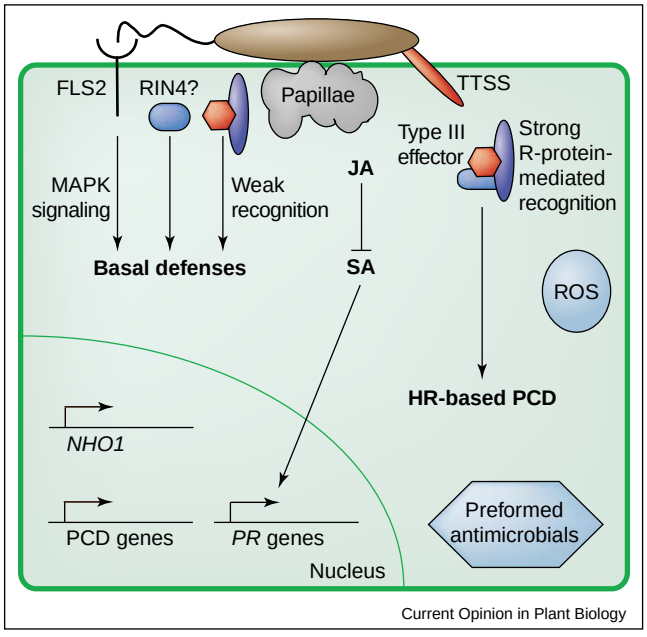
Until recently, the mechanisms used by bacteria to suppress plant immunity have mostly remained uncharacterized. New findings reveal, however, that bacteria employ diverse strategies to undermine plant defenses and target core components of plant immunity, such as hypersensitive response (HR)-based PCD, cell wall-based defenses, jasmonic acid (JA) signaling, and the expression of defense genes (Figure 1, Table 1). The identification of plant defenses that are vulnerable to pathogen attack has provided new insights into processes that are essential for both bacterial pathogenesis and plant immunity. These findings have great potential for the improvement of plant disease resistance.

Pathogens enhance their growth by modulating host PCD

The cultivar-specificity of many microbial pathogens is conditioned by gene-for-gene interactions. In a traditional view of gene-for-gene immunity, disease resistance is observed if a pathogen expresses an avirulence (Avr) protein that is specifically recognized by a host resistance (R) protein (as reviewed in [8]). Pathogen recognition often results in HR-based PCD, which limits pathogen establishment and spread by killing both the pathogen and the infected host cell. Given the central role of HR-based PCD in plant immunity, this defense has obvious potential as a target for pathogen attack.

Phytopathogenic bacteria, such as *Pseudomonas syringae*, elicit the HR by introducing effector proteins into the

Figure 1



Plant defenses targeted by pathogens to promote disease. The plant defenses shown are targeted by plant pathogens as described in Table 1 and the text. Here, we describe a few salient features of these vulnerable defenses. HR-based PCD is activated by gene-for-gene strong recognition of pathogen Avr factors and is a rapid response that functions to limit the establishment of pathogen infection. Basal defenses are elicited by the recognition of common pathogen signatures, such as flagellin or chitin, or by weak recognition of Avr factors. Basal defenses result in the activation of defense gene expression or the induction of late-onset cell death. Cell wall-based defenses include cell wall thickening and the formation of papillae near a nascent bacterial colony or fungal penetration site. JA signaling activates defenses that protect plants from insects and necrotrophic pathogens. The activation of JA signaling inhibits SA-dependent signaling and suppresses specific *PR* genes. Reactive oxygen species (ROS) are directly toxic to bacteria and also act as signaling molecules in plant immunity and PCD. Preformed antimicrobials, such as saponins, are toxic to pathogens and function to repel pathogen attack. Genes that encode acidic and basic PR proteins are activated by SA- and JA-dependent signalling pathways, respectively, and produce antimicrobial compounds. PCD genes are required to signal and execute cell death and are regulated in response to pathogen attack. The expression of the *NHO1* gene is activated by nonhost and avirulent bacterial pathogens and is required in some cases of nonhost resistance.

plant cell via the type III secretion system (TTSS). Type III effectors are also essential agents of pathogenicity: the growth and pathogenesis of *P. syringae* is abrogated in the absence of a functional TTSS. As a result of the recent genome sequencing of *P. syringae* pv. *tomato* strain DC3000 (DC3000), a pathogen of both tomato and *Arabidopsis*, more than 40 DC3000 type III effectors have been identified [9•]. Discovering the virulence functions of these effectors is now recognized as a key step in understanding bacterial pathogenesis.

Bacterial type III effectors suppress HR-based PCD

Initial studies demonstrating that bacteria suppress the HR were performed using the bean pathogen *P. syringae* pv. *phaseolicola* (*Pph*). Jakobek *et al.* [10] observed that *Pph* actively suppressed the induction of defense genes in bean plants undergoing the HR. Later, in a remarkable pair of studies, the *Pph* type III effectors VirPphA, AvrPphC and AvrPphF were found to enable *Pph* to evade HR-based defenses in specific bean cultivars [11,12]. For example, AvrPphC blocked the HR triggered by AvrPphF in the Canadian Wonder bean cultivar. Interestingly, AvrPphF, which triggered the HR in Canadian Wonder, blocked the HR caused by another unknown *Pph* avirulence protein in the Tendergreen bean cultivar. Therefore, both the virulence and avirulence activities of AvrPphC and AvrPphF are cultivar specific and both effectors function to modulate the HR response.

Studies involving the DC3000 type III effector AvrPtoB have provided new insights into how effectors suppress HR-based PCD [13••]. AvrPtoB is recognized by the tomato R protein Pto, and HR-based PCD results when both proteins are expressed in tomato leaves [14]. Surprisingly, when AvrPtoB was co-expressed with Pto in the wild tobacco species *Nicotiana benthamiana*, PCD was not observed, suggesting that AvrPtoB may suppress Pto-mediated PCD in *N. benthamiana*. Indeed, AvrPtoB suppressed PCD elicited by the R proteins Pto and Cf-9, as well as by the pro-apoptotic mouse protein Bax in *N. benthamiana*. AvrPtoB also functioned in yeast, suppressing PCD caused by oxidative stress. These findings demonstrated that AvrPtoB acts downstream of R-protein-mediated recognition, and functions generally as a eukaryotic cell death suppressor (CDS). Unexpectedly, mutations that disrupted the CDS activity of AvrPtoB uncovered a hidden avirulence domain within AvrPtoB, which triggered a Pto-independent HR in tomato and *N. benthamiana*. This finding indicates that AvrPtoB is recognized by a second R protein, termed Rsb, but that intact AvrPtoB normally suppresses PCD signaled by Rsb-mediated recognition. In tomato plants that lacked Pto, intact AvrPtoB suppressed Rsb-mediated immunity and induced tomato susceptibility to DC3000 infection, demonstrating that defense suppression by AvrPtoB can function to define cultivar–pathogen specificity [13••].

CDS activity has since been demonstrated for several other DC3000 effectors. HopPtoD2 suppresses *pathogenesis-related* gene (*PR*) expression, inhibits HR-based PCD and is required for full DC3000 virulence in tomato and *Arabidopsis* [15•,16•]. HopPtoD2 exhibits tyrosine phosphatase activity that is required for the suppression of plant defense, suggesting that HopPtoD2 targets phosphoproteins or signal transduction cascades. Recently, a screen of several DC3000 effectors for CDS activity revealed that AvrPtoB, HopPtoE, AvrPphE$_{Pto}$, AvrPpiB1$_{Pto}$,

Table 1

Proposed mechanisms of defense suppression by various plant pathogens.

Pathogen species	Pathogen agent	Targeted defense	Observed experimental phenotype and biochemical mechanism if known	Reference(s)
P. syringae pv. *tomato* DC3000	AvrPtoB	HR-based PCD	Suppresses PCD in plants and yeast. Induces susceptibility in otherwise resistant tomato.	[13••]
	HopPtoD2	HR-based PCD, *PR* gene expression	Suppresses PCD and other defenses. Required for full pathogenesis in tomato and *Arabidopsis*. Tyrosine phosphatase activity required to suppress defenses.	[15•,16•]
	AvrPtoB, HopPtoE, $AvrPphE_{Pto}$, $AvrPpiB1_{Pto}$, and HopPtoF	HR-based PCD, *PR* gene expression	Suppress PCD in plants and yeast. Deletion of each effector causes enhanced HR in tobacco.	[17•]
	HopPtoN	HR-based PCD	Suppresses PCD associated with both plant disease and immunity. Function dependent on cysteine protease activity.	(a)
	AvrPto	Cell wall-based defense	Inhibits papillae formation in *Arabidopsis* and alters expression of cell wall-associated genes.	[36••]
	TTSS-dependent	*NHO1* expression	*NHO*1 provides resistance to non-host pathogens. Virulent DC3000 downregulates *NHO1* in a *COI1*-dependent manner.	[49•]
	Coronatine toxin and type III effectors	Defenses regulated by JA signaling	Activate *COI1* and *JAI1* pathways in *Arabidopsis* and tomato to suppress SA-dependent defenses.	[40,41•,45]
	AvrRpt2	*PR* gene expression, basal defenses	Suppresses *PR* gene expression in *Arabidopsis*. Enhances susceptibility to DC3000 infection. Possibly targets RIN4 for suppression of basal defenses.	[53–55]
	TTSS-dependent	PCD gene expression	Virulent DC3000 induces expression of the ACD5 ceramide kinase, a negative regulator of cell death.	[21]
P. syringae *pv. phaseolicola*	VirpPhA, AvrPphC, and AvrPphF	HR-based defense	Block HR-based resistance in a cultivar-specific manner in bean.	[11,12]
X. campestris pv. *vesicatoria*	TTSS-dependent	Cell wall-based defense	Virulent *Xcv* suppresses papillae formation in pepper.	[35]
Rhizobium sp. NGR234	NopL	*PR* gene expression	Suppresses *PR* gene expression in tobacco and *L. japonica*.	[56]
B. graminis f.sp. *hordei*	Unknown	HR-based PCD and cell wall-based defense	MLO and BI-1, both negative regulators of cell death, are involved in successful *Bgh* penetration and infection.	[23,24••]
S. lycopersici	Tomatinase	Preformed antimicrobials and HR-based PCD	Pathogenesis requires tomatinase, which degrades preformed saponin defenses in *N. benthamiana*. Saponin degradation products subsequently suppress HR-based PCD.	[27••]
P. infestans	Soluble glucans	ROS and HR-based defenses	Inhibits ROS burst and the HR.	[25]
M. pinodes	Glycopeptide suppressin	*PR* gene expression and HR-based defense	Targets plasma-membrane-associated ATPase activity to suppress defense.	[26]

(a) A Collmer, pers. com.

and HopPtoF suppress HopPsyA-initiated PCD in tobacco and *Arabidopsis*, as well as Bax-initiated PCD in tobacco [17•]. Deletion mutants for each of these effectors elicited an enhanced HR in tobacco. In a separate study, the effector HopPtoN suppressed cell death associated with both immunity and disease (A Collmer, pers. com.). Cysteine protease activity has been demonstrated for HopPtoN, indicating that this effector may suppress PCD by proteolytic cleavage of a host factor. Overall, the discovery of at least seven effectors that have CDS activity reveals that the inhibition of plant PCD plays a key role in DC3000 pathogenesis.

It is perhaps surprising that so many type III effectors have CDS activity. Some effectors, such as AvrPtoB, suppress PCD that is associated with gene-for-gene resistance and act as qualitative pathogenicity factors. Alternatively, it is possible that some CDS effectors act as quantitative virulence factors. It has been proposed that PCD might be triggered when a threshold of death-inducing signals is detected by the plant [18,19]. Therefore, multiple CDS effectors may be required to sufficiently downregulate cell death signals derived from gene-for-gene recognition or the activation of basal defenses (see below), and to delay the plant from reaching a PCD-initiating threshold.

The discovery of CDS effectors lends a new perspective to our understanding of gene-for-gene interactions. A traditional gene-for-gene model generally assumes that R-protein-mediated recognition of effectors is dominant over effector virulence activity. However, the CDS activity of some effectors can dominantly suppress HR-based PCD signaled by recognition. In such cases, plants might 'recognize' a CDS effector but the HR is not observed. When studying plant–microbe interactions, therefore, observing disease does not necessarily indicate the absence of an Avr–R protein pair. Deletion of a single CDS effector from the pathogen might reveal a normally hidden resistance phenotype [11,12,13••]. The identification of hidden gene-for-gene specificities will increase the repertoire of functional *R* genes and perhaps enable plant breeders to develop more durable resistance.

CDS effectors are a diverse group of proteins that have differing proposed biochemical activities, indicating that CDS effectors probably target different positive or negative regulators of plant PCD (recently reviewed in [20•]). Interestingly, virulent *P. syringae* upregulates the expression of a ceramide kinase that negatively regulates PCD [21], demonstrating that modulating the expression of PCD regulators may be a mechanism used by *P. syringae* to promote a PCD-suppressive environment. CDS effectors represent a new class of tools for identifying plant processes that are essential for HR-based PCD. Once identified, the targets of CDS effectors will be excellent candidates to allow the manipulation of host PCD in ways that enhance plant disease resistance.

Targeting of PCD by other plant pathogens

Pathogenic fungi and oomycetes also target cell death as part of pathogenesis (reviewed in [6,22]), and these strategies provide an interesting contrast to mechanisms used by bacterial pathogens. Observations of diseases caused by biotrophic fungi such as *Blumeria graminis* f. sp. *hordei*(*Bgh*) provided early evidence that fungi suppress cell death. Infection by these fungi can cause a 'green-island' effect: leaf tissue near the fungal infection is kept alive while the surrounding tissue undergoes senescence. Suppression of cell death in barley, governed by plant genes such as *MLO* and *BI-1*, has been implicated in the ability of the powdery mildew pathogen *Bgh* to penetrate its host and sustain infectious growth [23,24••]. A large body of literature exists that describes soluble molecules and enzymes that are produced by pathogenic fungi that suppress HR-based plant defenses (reviewed in [6]). In one well-studied example, the oomycete *Phytophthora infestans* produces soluble glucans in its spore germination fluids that suppress the oxidative burst and the HR in potato [25]. Also, the pea pathogen *Mycosphaerella pinodes* produces a low-molecular-weight glycopeptide in its spore germination fluid that suppresses plant defenses and conditions the disease susceptibility of pea to *M. pinodes* and the avirulent pathogen *Alternaria alternata* [6,26]. The presence of the suppressor in the spore germination fluid in both cases suggests that HR suppression is especially crucial early in infection. Another interesting case is HR suppression by the tomato leaf spot fungus *Septoria lycopersici* [27••]. The *S. lycopersici* tomatinase enzyme acts as a pathogenicity factor by degrading preformed antimicrobial saponins and by suppressing defenses in *N. benthamiana*. Surprisingly, the saponin degradation products were identified as the compounds that suppress HR-based defenses. It will not be surprising if bacterial factors are identified that suppress the HR by mechanisms analogous to the fungal strategies discussed above.

In contrast to biotrophic fungi and bacteria, several strains of necrotrophic fungi promote host cell death as part of their pathogenesis and use HSTs to parasitize a specific host cultivar [5]. Plants are generally resistant to these fungi if they do not produce the HST; therefore, the cultivar specificity of these pathogens is conditioned by the killing of host cells. In this manner, HST-mediated cell death can be considered a complete suppression of plant defenses by the killing of the host cell. The need of necrotrophic fungi to target cell death actively is further supported by the observation that the expression of the baculovirus cell death suppressor p35 in tomato enhances disease resistance to necrotrophic fungi [28]. Bacterial factors and toxins that induce cell death, such as syringomycin, may play a similar role

in undermining basal defenses during a necrotrophic stage of bacterial growth.

What is the role of PCD in plant immunity and disease susceptibility?

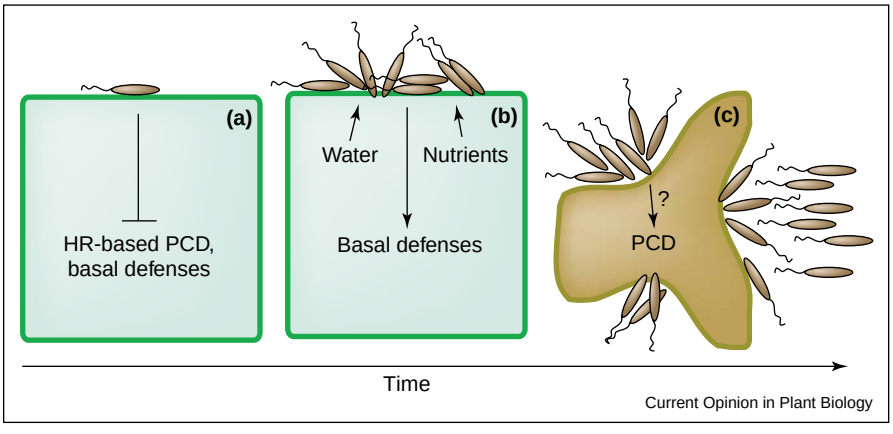
Depending on their lifestyle, pathogens can either suppress or promote host PCD to induce disease susceptibility. Given these opposing forces, the plant faces a trade-off when mounting resistance to pathogen attack. The plant must not create an overly PCD-suppressive environment because a biotrophic pathogen may take advantage of cell death that is too easily suppressed; conversely, a 'hair-trigger' easily activated cell death system, which may protect against biotrophic pathogens, might be exploited by a necrotroph. This trade-off is well illustrated by studies of the *MLO* gene in barley, in which a mutant *mlo* gene enhances cell death and provides resistance to the biotroph *Bgh*, but increases susceptibility to the heminecrotroph *Magnaporthe grisea* [29].

To further complicate the role of PCD in disease susceptibility, a pathogen might switch from biotrophic growth to necrotrophic growth during pathogenesis. In an early biotrophic stage, the suppression of cell death might enable the pathogen to avoid host detection and to establish infection. However, a late necrotrophic stage may be required to undermine basal defenses (discussed below), to enhance pathogen multiplication and access to nutrients, or to aid pathogen dissemination and dispersal (Figure 2). Experimental data support the idea that an individual pathogen might both suppress and induce PCD for successful pathogenesis [30,31]. For example, CDS activity of AvrPtoB is required for DC3000 to cause disease on a Pto-lacking tomato line [13••], but expression of the cell death suppressor p35 in tomato enhances resistance to DC3000 [28]. In addition, the uncoupling of disease-associated cell death from bacterial growth in plant leaves supports the active induction of cell death as part of the complete pathogen lifecycle [18,30,32]. Pathogens may target common cell death pathways to induce or suppress host PCD. For example, individual proteins from both the pathogen and plant, such as HopPtoN from DC3000 (A Collmer, pers. com.) and mitogen-activated protein kinase (MAPK) kinase kinase from *N. benthamiana* (O del Pozo, GB Martin, unpublished), modulate cell death that is associated with both disease and immunity, establishing a mechanistic link between these two cell death phenomena. Therefore, the late-onset, disease-associated 'necrosis' that can be seen as bacterial specks or collapsed tissue may actually be a form of PCD. These new data provide further support for the longstanding hypothesis that modulating the timing of PCD is a key determinant of disease outcome.

Pathogens enhance their growth by suppressing host basal defenses

Most plants are nonhosts for the vast majority of plant pathogens present in nature. This nonhost resistance is the result of many factors including preformed and passive defenses, such as physical barriers to infection and constitutively expressed antimicrobial compounds (reviewed in [33]). Nonhost defenses also include

Figure 2



A model for the pathogen-mediated modulation of plant PCD during the course of disease. Pathogens such as DC3000, *Xcv* or *P. infestans* may switch from biotrophic to necrotrophic growth during the course of pathogenesis. Experimental evidence suggests that the timing of host PCD is a crucial determinant of disease outcome. Early HR-based PCD in the host leads to resistance, whereas late-activated basal defenses and PCD are observed during disease. This model illustrates how a pathogen may modulate PCD to suppress both HR-based and basal defenses during pathogenesis. **(a)** Early in pathogenesis, the successful pathogen may need to suppress HR-based PCD and basal defenses to delay host detection and establish infection. **(b)** Once infection is established, the pathogen may manipulate the host to release the water and nutrients necessary for multiplication. With time and increasing bacterial multiplication, however, the slowly induced basal defenses may become sufficiently activated to limit pathogen growth effectively. **(c)** To overcome activated basal defenses, to gain access to nutrients or to aid dissemination, the pathogen may induce host PCD during late infection. This cell death is seen as specks, spots or disease-associated 'necrosis'. The distinction between PCD and necrosis observed late in infection, however, remains unclear.

induced defenses, which are initiated by the recognition of common signatures of bacterial and fungal challenge, such as flagellin perception by FLS2 [34] or by 'weak recognition' of Avr proteins (Figure 1). This recognition activates defense signaling pathways, which lead to the expression of induced antimicrobial compounds, localized reinforcement of the cell wall, and nonhost-based PCD. Together, these defenses represent a basal defense that limits disease. In general, basal defenses are activated more slowly than HR-based defenses. Many new findings present a molecular basis for the bacterial suppression of basal defenses, which functions to promote bacterial growth.

Bacterial type III effectors suppress plant cell wall-based defense

Plants have active cell wall-based defenses that limit the ability of bacterial and fungal pathogens to establish infectious growth. These cell wall alterations, observed microscopically as papillae, consist of callose, cross-linked phenolics and hydroxyproline-rich glycoprotein deposits and are thought to form a strong reinforcement of the cell wall that limits infection. Recent studies using phytopathogenic bacteria have revealed that type III effectors suppress papillae formation. In an early study, mutant strains of *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) that lacked a functional TTSS elicited papillae formation in pepper plants, whereas wildtype *Xcv* did not cause the formation of papillae [35], indicating that effectors may downregulate papillae formation. Recently, the DC3000 type III effector AvrPto was demonstrated to limit callose deposition and papillae formation when overexpressed in transgenic *Arabidopsis* [36••]. In this study, *hrcC* TTSS mutants of DC3000 grew poorly in *Arabidopsis* and strongly induced the deposition of callose. In AvrPto-expressing *Arabidopsis*, however, growth of the *hrcC* mutant was restored and papillae formation was reduced.

Interestingly, a connection between cell wall-based defense and cell death may exist. For example, the barley *MLO* gene suppresses both cell death and the formation of papillae [37]. Furthermore, a recent analysis of single and double mutants of DC3000 revealed that the AvrPto and AvrPtoB proteins are partially redundant in tomato pathogenesis, suggesting that they have shared functional activities (N Lin, GB Martin, unpublished). Indeed, AvrPto has been reported to suppress HR-based cell death [38], and AvrPtoB enables *P. syringae* to overcome cell wall-based defenses (J Mansfield, pers. comm.). However, the mechanistic connection between cell wall-based defense and cell death remains to be determined.

Bacterial toxins and effectors target JA defense signaling to suppress defense

Salicylic acid- (SA), JA- and ethylene-dependent signaling pathways coordinate plant defense responses to pathogen attack (recently reviewed in [39]). SA-dependent signaling activates defenses, such as the expression of antimicrobial acidic PR proteins, that provide resistance against diverse microbial pathogens. The JA and ethylene signaling pathways function in defense against wounding by insects and necrotrophic pathogens, such as *Alternaria brassicicola* and *Botrytis cinerea*. Complex patterns of cross-talk exist between these pathways. For example, JA signaling inhibits SA-dependent responses in both *Arabidopsis* [40] and tomato [41•].

The link between JA signaling and pathogenesis was made by studying the responses of *Arabidopsis* and tomato to the *P. syringae* toxin coronatine (COR). COR is a virulence factor that significantly increases lesion formation and bacterial growth in infected plants (reviewed in [4]). In early studies, the virulence activity of COR was associated with the suppression of defense gene expression in *Arabidopsis* [42]. Plants that are treated with COR demonstrate similar responses to those treated with methyl-jasmonate (MeJA), suggesting that COR may mimic MeJA to activate JA signaling. Indeed, COR-insensitive mutants of *Arabidopsis* (*coronatine insensitive1* [*coi1*] and tomato (*jasmonic acid insensitive1* [*jai1*]) have phenotypes that are consistent with JA-signaling defects [40,41•,43], further suggesting that COR targets JA signaling. Recently, *JAI1* has been shown to be the tomato homolog of *COI1* [44]. Both *coi1* and *jai1* mutants have increased resistance to *P. syringae*, and this resistance is associated with the more rapid SA-dependent expression of *PR* genes [40,41•]. Furthermore, using microarray gene expression profiling in tomato, COR was shown to upregulate several *JAI1*-dependent genes [41•]. Together, these findings provide strong evidence that COR activates JA-signaling responses that, through unknown mechanisms, result in the suppression of SA-dependent defenses.

Recently, type III effectors have also been associated with the modulation of JA signaling to suppress defense responses [41•,45]. Microarray experiments revealed similarities between *JAI1*-dependent and TTSS-dependent gene-expression profiles; in susceptible plants, both COR and the TTSS suppressed the expression of *PR* genes and activated the expression of JA-responsive genes. In a separate study, an *Arabidopsis* gene, *RAP2.6*, was identified that was upregulated by DC3000 infection in a *COI1*-dependent manner [45]. Both a functional TTSS and the COR toxin were required for *RAP2.6* induction, suggesting that type III effectors and COR act together in stimulating JA signaling. In DC3000, type III effectors and COR are both regulated by the HrpL alternative sigma factor [46,47], further supporting an interplay between these two factors.

Another general defense that is targeted by *P. syringae* in a *COI1*-dependent manner is *NONHOST RESISTANCE1*

(*NHO1*) expression. The *nho1 Arabidopsis* mutant supports increased growth of nonhost *Pseudomonas* spp., including *Pph* [48]. *Pph* induces the expression of *NHO1*, but virulent DC3000 actively suppresses *NHO1* expression in a *COI1*-dependent manner [49•]. These observations indicate that active suppression of *NHO1* and other nonhost defenses may be required for host compatibility. Interestingly, incompatible DC3000 induces *NHO1* gene expression, suggesting that *NHO1* is also a component of gene-for-gene immunity. This raises the possibility that *Arabidopsis* nonhost resistance to *Pph* might actually be a form of HR-based gene-for-gene immunity. Indeed, many bacteria require a functional TTSS to elicit a nonhost HR, further hinting that the recognition of type III effectors may govern some cases of nonhost resistance. It is possible, therefore, that virulent pathogens may suppress nonhost resistance and *NHO1* expression by introducing type III effectors that interfere with recognition or that suppress HR-based PCD signaled by unidentified R proteins.

Do type III effectors target 'guarded' proteins to inhibit plant defense?

Several recent studies have provided support for the 'guard hypothesis' and have demonstrated that plant resistance proteins monitor the status of host proteins that are targeted by bacterial type III effectors. For example, in *Arabidopsis*, the RPS2 R protein detects the AvrRpt2-dependent disappearance of *Arabidopsis* RIN4 [50•,51•], and RPS5 detects the AvrPphB-dependent cleavage of the *Arabidopsis* PBS1 kinase [52]. It has been hypothesized that the 'guarded' proteins are targets of *P. syringae* virulence factors, and that proteins such as RIN4 and PBS1 may be positive regulators of general defense responses. Although there is no direct evidence to suggest that RIN4 and PBS1 are targets for defense suppression, studies involving the effector AvrRpt2 offer some promising hints. Transgenic AvrRpt2-expressing *Arabidopsis* plants exhibit an enhanced susceptibility to DC3000 infection [53] that is independent of a functional DC3000 TTSS [54]. Furthermore, AvrRpt2 that is delivered by the pathogen suppresses the expression of *PR* genes in a SA-independent manner [54]. Together, these observations indicate that AvrRpt2 suppresses basal defense responses independently of SA and gene-for-gene defenses. Interestingly, mutant AvrRpt2 proteins that have reduced virulence activity also cause reduced disappearance of RIN4 [55]. This correlative evidence hints that RIN4 may be the target of the observed AvrRpt2-mediated defense suppression, although the discovery of a positive role for RIN4 in basal defense is necessary to support this hypothesis.

Conclusions and future perspectives

The importance of suppressing plant defense during bacterial pathogenesis has been highlighted by many recent studies. Pathogens target diverse components of plant defense and employ highly diverse mechanisms to subvert these defenses. In some cases, the capacity of the pathogen to suppress defense determines its ability to parasitize a host and therefore its host specificity. In other cases, defense suppression may be necessary for full disease formation. It is interesting that defense suppression also appears to play an important role in symbiotic plant–microbe interactions. For example, *Rhizobium* sp. NGR234 has a TTSS, and the NopL effector suppresses *PR* gene expression when expressed in tobacco or *Lotus japonicus* [56]. Discoveries regarding the basis of host–microbe compatibility, in both pathogenesis and symbiosis, will probably provide further insights into the suppression of host immunity by microbes. By understanding how pathogens undermine plant defenses, new strategies may be envisaged to shore-up these vulnerable defenses and generate plants that have durable resistance.

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- •• of outstanding interest

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