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Ill Communication: Host Metabolites as Virulence-Regulating Signals for Plant-Pathogenic Bacteria

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Abstract

Plant bacterial pathogens rely on host-derived signals to coordinate the deployment of virulence factors required for infection. In this review, I describe how diverse plant-pathogenic bacteria detect and respond to plant-derived metabolic signals for the purpose of virulence gene regulation. I highlight examples of how pathogens perceive host metabolites through membrane-localized receptors as well as intracellular response mechanisms. Furthermore, I describe how individual strains may coordinate their virulence using multiple distinct host metabolic signals, and how plant signals may positively or negatively regulate virulence responses. I also describe how plant defenses may interfere with the perception of host metabolites as a means to dampen pathogen virulence. The emerging picture is that recognition of host metabolic signals for the purpose of virulence gene regulation represents an important primary layer of interaction between pathogenic bacteria and host plants that shapes infection outcomes.

1. INTRODUCTION

1.1. Overview of Host Metabolites as Virulence-Regulating Signals for Plant-Pathogenic Bacteria

Plants are primary producers of energy-rich nutrients that support microbial life. Although a plant may exude a fraction of its photosynthate into the rhizosphere and phyllosphere (68, 110), in part to support associated microbiota, most nutrients are stored internally and reserved for growth and reproduction. To protect their metabolic resources from opportunistic microbes, plants have evolved formidable preformed and inducible defenses (11, 55). In turn, plant-pathogenic bacteria have evolved traits that allow them to overcome these defensive barriers and gain access to interior stores of plant nutrients to fuel growth (1).

Many of the virulence mechanisms employed by bacterial plant pathogens are complex, requiring the coordinated production of dozens of proteins that, in sum, are energetically costly to produce (88, 104). Furthermore, premature deployment of virulence factors may in some cases betray the presence of a pathogen to the host (83). As a result, most bacterial pathogens limit the production of their virulence factors until needed. Accordingly, pathogenic bacteria must possess the ability to detect the host and deploy their virulence factors at the appropriate location and with appropriate timing to successfully counteract plant defenses and establish a habitable niche. Dosage and coordinated delivery of virulence factors, although still poorly understood, are also likely important for achieving maximal virulence (15). In a similar fashion, plants keep most of their inducible defenses in check until a pathogen is detected to avoid unnecessary draining of resources (50). As a consequence of these mutual constraints, the early stages of infection are necessarily a race in recognition, with the pathogen and host competing to detect and respond to the other as quickly and robustly as possible.

How plants detect invading pathogens is known with atomic-scale resolution (105). By comparison, how bacterial pathogens detect a plant host, although in many ways equally important to infection outcomes, is poorly understood. Accumulating evidence indicates that bacteria rely on specific host metabolites as virulence-regulating signals, and variable levels of these chemical signals in plant tissues can impact disease outcomes (4, 43, 126, 127). Furthermore, host sensing is complex. Sensing of multiple distinct signals may individually or collectively provide information about the presence of potential energy sources and habitable niches as well as provide information on the progress of infection, including the metabolic and defense status of host plants, to coordinate virulence outputs.

Some host metabolites that function as virulence-inducing signals also serve as nutrients for pathogenic bacteria. In these instances, disentangling the importance of a host metabolite as a virulence signal and/or a nutrient during infection is experimentally challenging. As a basic definition, biological signals convey information and instruct the receiver to respond (8). Therefore, to define a metabolite as a bona fide virulence-regulating signal requires careful consideration of whether induced responses are indeed specific to virulence-associated processes. Also, because signal perception mechanisms (e.g., membrane receptors) are often tuned to detect signals at concentrations well below those required for the use of metabolites as growth substrates, it is important to consider the effective concentrations of host metabolites at sites of infection and whether the abundance of available metabolites may change during infection. In this regard, a metabolite may perform both signal and nutrient roles concurrently or may switch between these roles over the course of an infection.

To add to the complexity of this emerging picture of host–pathogen signaling, plants possess both preformed and inducible defenses that can suppress host-sensing mechanisms to negatively impact the deployment of virulence factors by bacteria. These virulence-inhibiting mechanisms

can be viewed as acting in direct opposition to effector proteins and toxins that bacteria deploy to inhibit host immune responses (25, 55, 116). Bacteria may also sense host signals to downregulate the production of virulence factors at certain stages of infection. Therefore, virulence deployment by bacteria within the plant is a highly dynamic process that, at any given stage of infection, reflects the sum total of both positive- and negative-acting signals that are present.

In this review, I highlight the current knowledge on how plant-pathogenic bacteria use host-exuded metabolites as virulence-inducing signals to establish infection. I also discuss recent evidence that plant defenses interfere with the perception of virulence-inducing signals. The broader topic of how phytopathogenic bacteria regulate their virulence genes has been studied for decades, resulting in an expansive body of literature that exceeds the limits of a single review article. Other important aspects of virulence gene regulation, including intercellular communication (i.e., quorum sensing), the response of bacteria to more general environmental features such as temperature, pH, and osmolarity, and the many layers of secondary signaling molecules and pathways that coordinate and influence virulence outputs, have been reviewed in detail elsewhere (9, 13, 22, 65, 84, 87, 106, 111).

1.2. Overview of Bacterial Infection Strategies and Plant Antibacterial Defense

To cause disease, many pathogenic bacteria access and colonize the interior spaces of plant tissues, where they are sheltered from environmental stresses and are in the closest possible proximity to nutrients and water exuded from surrounding host cells (1, 10). There is a range of strategies that plant-pathogenic bacteria employ for colonization (61). At one end of the spectrum, (hemi-)biotrophic pathogens such as *Pseudomonas syringae* and *Xanthomonas* spp. primarily rely on stealth strategies to suppress host immune responses and modify host metabolism to their benefit while at the same time keeping host tissues alive, although cell death and tissue necrosis often occur at later stages of infection. At the other end of the spectrum, necrotrophic pathogens such as *Dickeya dadantii* primarily rely on the brute-force strategy of deploying plant cell wall–macerating enzymes, ultimately disrupting cellular membranes to access otherwise intracellular nutrients. Across this diversity of virulence strategies, bacterial secretion systems play a key role in infection (21). For (hemi-)biotrophic pathogens, a key weapon is the type III secretion system (T3SS), a syringe-like apparatus that delivers into host cells a suite of proteins termed effectors that suppress plant defenses and promote nutrient and water release (16, 38, 49, 96, 118, 125). Genes that encode for the T3SS apparatus, designated *hrp/hrc* [hypersensitive response (HR) and pathogenicity/HR and conserved] genes, are typically clustered within a single pathogenicity island. In contrast to biotrophs, soft-rot pathogens primarily rely on type I and II secretion systems to export cell wall–degrading enzymes (22), although most pathogens employ multiple secretion systems to maximize their virulence. Pathogenic bacteria also produce and secrete a myriad of small molecule toxins, hormones, detoxifying enzymes, and exopolysaccharides, along with other factors, in a coordinated effort to defeat host defenses and establish infection (22, 86, 106, 124).

To counteract bacterial pathogens, plants possess an immune system that surveils for the presence of microbes (55). A key first layer is pattern-triggered immunity (PTI) mediated by pattern recognition receptors (PRRs) that monitor the extracellular environment for conserved microbial features termed pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) (28), exemplified by the peptide flg22 derived from bacterial flagellin that is recognized by the PRR FLAGELLIN SENSING 2 (FLS2) and coreceptor BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1 (BAK1) (28, 39, 44). Recognition of PAMPs by PRRs results in the activation of numerous cellular defense responses, including production of antimicrobial chemicals, reinforcement of plant cell walls, and large-scale

metabolic changes (97). A second layer of plant defense is effector-triggered immunity (ETI), which is mediated by intracellular nucleotide-binding leucine-rich repeat (NLR) proteins that monitor for the presence of pathogen effector proteins (55). ETI generally results in a stronger immune response and often produces localized cell death termed the HR. Both PTI and ETI provide effective resistance against pathogenic bacteria, although, as discussed below, the exact molecular basis of how these immune responses limit bacterial growth is still poorly understood.

2. POSITIVE REGULATION OF VIRULENCE GENES BY PLANT-EXUDED METABOLITES

All pathogens possess specific virulence traits that allow them to colonize their host and cause disease. Although what constitutes a bona fide virulence trait and what distinguishes pathogen from nonpathogen are matters for debate (18), it is clear that specific genes (e.g., the T3SS-encoding *hrp/hrc* genes) are critical for many pathogenic bacteria to colonize their hosts and cause disease (16). Across all plant pathosystems, numerous host signals are known to induce or repress the expression of genes required for virulence, although in most cases the mechanistic basis for their action has yet to be discovered. Here, I highlight examples of virulence gene regulation by plant metabolic signals, organized based on whether signal perception occurs via cell surface–localized receptors or through intracellular perception mechanisms.

2.1. Host Metabolite Sensing by Bacterial Surface-Localized Receptors

Bacteria often rely on cell surface receptors such as two-component system (TCS) sensor kinases (62) to detect environmental stimuli and alter the expression of genes involved in nutrient uptake and metabolism, among other cellular functions. Given this generality, it is not surprising that pathogenic bacteria also employ these same types of membrane-localized receptors to perceive host signals and coordinate virulence functions. However, identifying receptors important for host infection has been hampered by the large number of predicted receptors encoded in the genomes of most plant-pathogenic bacteria, along with the challenges of defining specific host-derived ligands for candidate receptors. Here, I discuss the current knowledge on specific membrane-localized receptors that positively regulate virulence gene expression in response to host metabolite signals.

2.1.1. Regulation of *Agrobacterium vir* genes by plant phenolics and sugars. *Agrobacterium tumefaciens* (*Agrobacterium*) are Gram-negative α-proteobacteria that can cause crown gall disease on a wide range of plant hosts (73). Often described as nature's genetic engineers, *Agrobacterium* modify the physiology and metabolism of hosts to their benefit through direct transfer and integration of a transferred DNA (T-DNA) fragment into the host cell genome (78). Successful T-DNA transfer requires a suite of tumor-inducing (Ti) plasmid-encoded *vir* genes and chromosomal *chv* genes that code for proteins involved in host sensing as well as for processing and transfer of the T-DNA (78, 132).

Agrobacterium vir genes are environmentally responsive and regulated by signals associated with plant cells. Plant-derived phenolic compounds such as acetosyringone are essential *vir*-inducing signals (103), whereas monosaccharides (5), low pH (48, 67, 70), and low phosphate (117) are additional signals that induce amplification of phenolic compound–induced *vir* gene expression (78). Host sugars and phenolics are perceived by VirA-VirG, a TCS that, together with a periplasm-localized protein, ChvE, integrates information from these distinct host signals to regulate the levels of *vir* gene expression (13, 78). VirA is a membrane-localized hybrid histidine kinase comprising an extracellular periplasmic domain as well as intracellular linker, kinase, and receiver

domains. In response to plant signals, VirA is activated in similar fashion to many other TCSs, with the autophosphorylated kinase domain transferring a phosphate group to the response regulator protein VirG, which in turn activates *vir* genes by directly binding to defined *vir* box motifs within their promoters.

VirA detects plant-derived phenolics and sugars through two distinct mechanisms. The intracellular linker domain is required for *vir* induction, and current models propose direct binding of phenolic signals to this domain (20, 73, 107). In contrast, sugar-induced expression of *vir* genes requires the periplasmic domain of VirA as well as ChvE, a chromosomally encoded protein that belongs to a large class of periplasmic substrate-binding proteins (SBPs) that function in nutrient uptake and chemotaxis (78, 98). ChvE binds to a wide range of aldose monosaccharides as well as sugar acids such as galacturonic acid, and direct binding of a sugar-ChvE complex with the periplasmic domain of VirA is likely the inducing signal that promotes *vir* induction (43). A remarkable feature of this sugar perception system is that, in addition to interacting with VirA, ChvE also functions in nutrient uptake by delivering sugars to the outer face of the sugar uptake transporter MmsAB (43, 131). Owing to these dual roles of ChvE, VirA and MmsAB likely compete for sugar-bound ChvE in the periplasm, and this competition influences the levels of *vir* induction, as evidenced by higher sugar-induced *vir* expression in an *mmsAB* mutant (48). This dampening effect of MmsAB on *vir* induction may be due to MmsAB transport activity lowering the concentration of sugars with the periplasm and is proposed to be a mechanism to maintain high-affinity perception of sugars by VirA across the wide range of μM to mM sugar concentrations that *Agrobacterium* may encounter between soil and plant environments (48).

Studies of ChvE also provide important evidence that the relative abundance of virulence-inducing metabolites in plant tissues can shape the host range of a pathogen. Through an elegant combination of biochemical and genetic assays to manipulate and test the sugar-binding properties of ChvE, Hu et al. (48) identified an amino acid–substituted derivative of ChvE that maintained normal binding affinity to neutral sugars, but no longer bound to sugar acids. Intriguingly, a *chvE* mutant expressing this ChvE derivative was avirulent on *Kalanchoe* leaves but retained full virulence on tobacco explants. These data indicate that sugar acids are critical inducers of *vir* genes in *Kalanchoe* but not in tobacco and suggest that the unusually broad binding capacity of ChvE toward a diversity of sugars may contribute to the ability of some *Agrobacterium* to infect a wider range of hosts. Furthermore, by examining the in planta phenotypes of additional ChvE derivatives with attenuated sugar binding and measuring the EC_{50} of *vir* induction by strains expressing these derivatives, it was possible to estimate the concentrations of neutral sugars present at infection sites in tobacco tissues. Hu and colleagues propose that manipulating the ligand binding properties of ChvE will be useful for defining the host sugar signal landscape encountered by *Agrobacterium* on different hosts (48, 73). More broadly, this type of ligand-receptor manipulation, akin to a biosensor, could be potentially applied to other pathosystems as a way to interrogate the in planta distribution and abundance of virulence-inducing metabolites across many distinct host species or microenvironments within a single host plant.

2.1.2. Regulation of *Pseudomonas syringae* type III secretion by plant-exuded organic acids and amino acids. *Pseudomonas syringae* are Gram-negative bacteria that can infect most aerial plant tissues, although most isolates are primarily studied for their ability to infect leaves (47). T3SS deployment by *P. syringae* is regulated by a core signaling cascade comprising HrpL, HrpR, and HrpS. This regulatory pathway has been reviewed extensively (84, 123) and is briefly covered here. HrpL is an extracytoplasmic function (ECF)-type alternative transcription factor that functions as a positive regulator of T3SS gene expression by binding to a *hrp* box motif found within the promoter regions of nearly all *hrp/hrc* and effector genes (37, 100, 120). Transcription

of *hrpL* requires the alternative sigma factor RpoN (σ^{54}) and is regulated by HrpR and HrpS, a pair of transcriptional activators expressed by a *hrpRS* operon (27, 46, 51). HrpR and HrpS form a hetero-multimeric complex that binds to the *hrpL* promoter and activates *hrpL* transcription through interactions with the σ^{54}-RNA polymerase holoenzyme (56, 84).

Both *hrpL* and *hrpRS* are induced within the first hours of *P. syringae* infection (69, 121). Therefore, the HrpR–HrpS–HrpL pathway is responsive to the host environment. However, defining the host signals and corresponding signaling pathways that feed into and activate this core pathway is challenging for several reasons. First, although HrpR and HrpS possess domains that function in oligomerization and DNA binding, they both lack any additional signal receiver domains, suggesting their function as transcriptional regulators is not directly regulated by small molecule signals (40, 56). Second, aside from *hrpL* and *hrpRS*, *P. syringae hrp/hrc* islands lack genes with predicted roles in bacterial signal transduction and environment sensing (e.g., TCSs). Lastly, although synthetic media conditions that induce *hrp/hrc* genes are known (52) and the discovery of these media greatly facilitated the elucidation of *hrpRS* and *hrpL* regulons (34, 64), the relevance of these culture conditions to infection is not clear.

A recent advance was the discovery of specific plant-exuded metabolites that induce T3SS-encoding genes in *P. syringae* pv. *tomato* DC3000 (herein DC3000), a pathogen of both tomato and *Arabidopsis* (4). The initial observation was that *Arabidopsis* seedling exudates contained bioactive compounds that strongly upregulated *hrpL* expression. By a metabolomics approach, a set of organic acids and amino acids, including aspartic acid, citric acid, and 4-hydroxybenzoic acid, was identified as bioactive metabolites (4). An important caveat is that *hrp* induction by these compounds required the presence of a simple sugar or sugar alcohol, such as fructose or mannitol, in the culture medium (4). The molecular basis for this synergistic action of sugars and acidic compounds is currently unknown, although sugars are able to induce *hrp/hrc* genes on their own (see below), suggesting that their recognition may occur through a mechanism distinct from induction by amino acids and organic acids.

Aspartic acid (Asp) was among the most potent *hrp*-inducing metabolites identified from plant exudates. Both Asp and glutamic acid (Glu) are amino acids abundant in leaf tissues across a wide diversity of plant species and, along with other amino acids and organic acids, are growth substrates for *P. syringae* (63, 94). Asp and Glu also function as chemotactic signals for DC3000, suggesting the same plant signals regulate motility and T3SS deployment (19). Efficient uptake of Asp and Glu by *P. syringae* requires genes within the *amino acid transport/utilization* (*aat/aau*) locus that encodes for a predicted ATP-binding cassette (ABC) transporter and an associated TCS (101, 102). The *aat/aau* locus is conserved across all pseudomonads (101). Based on knowledge of ABC transporters, AatQ and AatM are predicted to be membrane-spanning permeases, AatP is an intracellular ATPase that powers transport, and AatJ is a SBP that directly binds and delivers transport substrates to the periplasmic face of AatQM for uptake (101). The *aat/aau* locus also includes *aauS* and *aauR* that encode for a predicted TCS sensor kinase and response regulator, respectively. AauS and AauR positively regulate *aat/aau* expression in response to Asp and Glu signals (102).

A direct role for *aat/aau* genes in regulating T3SS-encoding genes in *P. syringae* was recently reported (127). From a random mutagenesis screen, mutants of DC3000 with individual Tn*5* transposon insertions in *aatJ*, *aauS*, and *aauR* were identified as having partially compromised *hrp/hrc* gene expression in response to Asp and Glu, suggesting a possible role for amino acid transport in T3SS induction. However, no mutants with Tn*5* insertions in *aatQ*, *aatM*, or *aatP* were identified. Furthermore, deletion of *aatP* had no impact on Asp/Glu induction of *hrpL*, indicating that active transport of these amino acids is not a prerequisite for T3SS induction. In *Arabidopsis* infection assays, Δ*aatJ*, Δ*aauS*, and Δ*aauR* mutants were partially compromised in

T3SS induction and virulence. Therefore, Asp and Glu are likely virulence-inducing signals encountered by DC3000 during infection. A remaining question was how AatJ and AauSR regulate *hrp/hrc* expression. The answer came with the discovery of an AauR-binding motif (Rbm) within the *hrpRS* promoter (127). Deletion of this Rbm attenuated *hrpRS* and *hrpL* induction by Asp and Glu, as well as DC3000 virulence on *Arabidopsis*, to levels observed with a Δ*aauR* mutant. Based on these data, the Rbm upstream of *hrpRS* is sufficient to explain the role of AauSR in *hrp/hrc* regulation.

2.1.3. Co-option of periplasmic substrate-binding proteins as a common path for evolving host-signal-dependent regulation of virulence. The discovery of AatJ-AauSR regulation of T3SS-encoding genes provides several interesting insights into the evolution of host perception by bacterial pathogens. First, the Rbm upstream of *hrpRS* is conserved across the full phylogeny of the *P. syringae* species (127), indicating that all *P. syringae*, irrespective of their host range and evolutionary relationships, rely on Asp and Glu as signals to regulate deployment of their T3SS. From an evolutionary perspective, the conserved regulation of *hrpRS* by AauSR suggests that, following horizontal acquisition of the *hrp/hrc* pathogenicity island, the Rbm upstream of *hrpRS* was acquired as a mechanism to co-opt an existing housekeeping AauSR signaling pathway for virulence functions. Alternatively, AauSR regulation of *hrpRS* may have existed prior to *hrp/hrc* acquisition. This relatively simple path for evolving novel inputs for virulence gene regulation by repurposing existing host-responsive signaling pathways and promoter elements has been noted in animal pathogens (53). As more pathosystems are characterized, this type of repurposing of promoter elements may be found to be a common mechanism for connecting horizontally transferred virulence genes to existing environmental response pathways.

A second evolutionary insight is the common role for ABC transporter–associated proteins in regulating virulence gene expression in both *P. syringae* and *Agrobacterium* (**Figure 1**). In both pathogens, the metabolite-binding functions of SBPs, specifically AatJ and ChvE, provide the means for detecting virulence-regulating signals. This common feature of virulence regulation, shared by two pathogens with very distinct pathogenic lifestyles, suggests that SBPs may be particularly amenable to repurposing for virulence functions. This could be due to the inherent modularity of ABC transporter systems, with the metabolite-binding functions of AatJ and ChvE genetically and biochemically separable from transport functions of AttQMP and MmsAB, allowing for repurposing of SBP functions for metabolite sensing while maintaining metabolite uptake. Additionally, SBPs are also involved in perceiving chemotactic signals, exemplified by the role of ChvE in chemotaxis toward sugars (17). As such, a single host metabolite signal can potentially regulate motility, nutrient uptake, and virulence gene regulation through binding to a single periplasmic protein.

2.1.4. Regulation of *Ralstonia solanacearum* type III secretion by physical contact with plant cell wall components. *Ralstonia solanacearum* (*Rs*) are soil-dwelling Gram-negative bacteria that invade root tissues, enter xylem tissues, and spread systemically throughout the plant vasculature (95). *Rs* rely on the T3SS to deliver defense- and metabolism-altering effectors in host cells (86, 118). In *Rs*, expression of *hrp/hrc* and effector genes are regulated by HrpB, an AraC-type regulator that likely directly binds to a hrp_{II} box motif found upstream of HrpB-regulated genes (60, 76). Key upstream regulators are HrpG and PrhG, two OmpR-type regulators (most commonly associated with TCSs) that direct the transcription of *hrpB* (75, 86).

Rs hrp/hrc genes are induced by multiple distinct host signals. Like many phytopathogenic bacteria, culturing *Rs* in minimal media that lack a complex nitrogen source and contain a single carbon source such as sucrose or glucose is sufficient to induce *hrp/hrc* expression (7), and this signaling occurs through the PhrG-*hrpB* cascade described above (86, 89). The *hrp/hrc* genes of

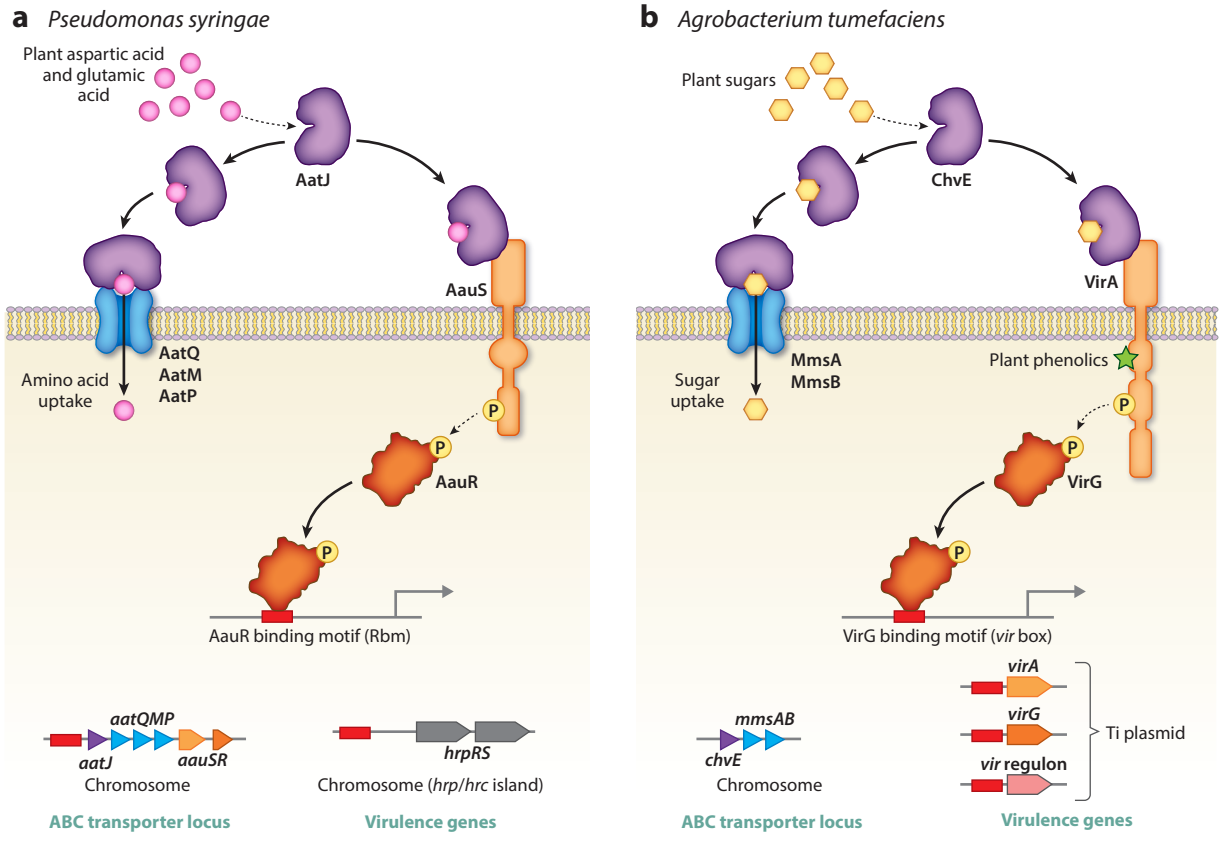

Figure 1

Co-option of ABC transporter-associated proteins as a common evolutionary path for host-signal-dependent regulation of virulence in plant-pathogenic bacteria. The diagram highlights similar roles for ABC transporter-associated substrate-binding proteins (ChvE and AatJ) as receptors for virulence-inducing signals in both (*a*) *Pseudomonas syringae* and (*b*) *Agrobacterium tumefaciens*.

Rs are also highly induced when cells are cocultured with plant cells, and this response requires PrhA, a protein embedded within the outer membrane (2). PrhA is homologous to known TonB-dependent siderophore receptors and senses yet unknown components of the plant cell wall (2). PrhA-dependent signaling requires PrhR and PrhI, a membrane-localized antisigma factor and an ECF-type sigma factor, respectively (14). Based on models proposed for similar signal transfer systems, binding of plant signals to PrhA likely stimulates PrhR to release PrhI into the cytoplasm, allowing PrhI to trigger a signaling cascade through transcriptional regulation of downstream *prhJ* and *hrpG* genes that ultimately stimulates *hrpB* expression and T3SS production (14, 86).

2.1.5. Regulation of *Xanthomonas* virulence by plant cytokinins. Gram-negative *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causative agent of black-rot diseases of crucifers, has ~50 predicted TCSs encoded in its genome, most with unknown functions (113). By taking a direct biochemical approach to identifying receptor–ligand pairs, Wang et al. (112) identified the cytokinin 2-isopentenyladenine (2iP) as a ligand for plant cytokinin receptor kinase (PcrK). 2iP bound to the periplasmic domain of PcrK with nanomolar affinity and decreased the kinase activity of PcrK, resulting in decreased phosphorylation of the cognate response regulator PcrR. Rather than a DNA-binding domain, PcrR possesses an HD-GYP phosphodiesterase domain, and

dephosphorylation of PcrR increased its activity to degrade the bacterial secondary messenger c-di-GMP. Importantly, loss of *pcrK* or *pcrR* significantly decreased *Xcc* growth and disease symptoms during infection of host cabbage leaves, thus validating this biochemical approach to identifying host signal receptors important for virulence. Follow-up biochemical and genetic experiments linked PcrR-mediated decrease in c-di-GMP levels to increased expression of a cytokinin-related TonB-dependent receptor gene (*ctrA*), possibly through c-di-GMP regulation of transcription factor Clp (CAP-like protein) (112). Overexpression of *ctrA* or *clp* was sufficient to restore virulence to a Δ*pcrR* mutant, suggesting that altered signaling through Clp-CtrA is causal for 2iP-dependent oxidative stress tolerance, although the mechanism of this tolerance remains to be elucidated. Cytokinins play important roles in regulating plant growth and nutrient assimilation as well as interactions with pathogenic and beneficial microbes (74). Where and when *Xcc* may encounter 2iP during infection, and how PcrKR detection of 2iP may allow *Xcc* to adapt to defense-associated oxidative stress, are interesting questions for future experiments.

2.1.6. Plant-exuded mineral nutrients as virulence-regulating signals. Although not metabolites by definition, essential nutrients such as calcium (35, 36), iron (114), and magnesium (42) also function as virulence-inducing signals for plant pathogens. In DC3000, *hrp/hrc* genes are regulated by calcium through the action of a two-component system CvsSR (35). Expression of *cvsSR* is induced when DC3000 is cultured in a *hrp/hrc*-inducing, calcium-containing minimal medium. Similarly, apoplastic wash fluid extracted from tomato leaves also induces *cvsSR* expression, and this induction is mitigated by the addition of calcium-chelating EGTA, suggesting calcium is indeed a bona fide host signal recognized by DC3000 during infection. A direct connection between CvsSR and *hrp/hrc* genes was revealed through ChIP-seq and EMSA/DNase I footprinting experiments, with CvsR binding to two distinct sites within the *hrpRS* promoter (35). Consistent with direct calcium-dependent regulation of *hrpRS* by CvsSR, addition of calcium to DC3000 cultures induced *hrpRS* and *hrpL* transcription, and this response was significantly reduced in Δ*cvsS* and Δ*cvsR* mutants. Furthermore, DC3000 Δ*cvsS* and Δ*cvsR* mutants were less virulent on host tomato and *Arabidopsis* plants. However, whether CvsSR regulation of *hrpRS* occurs during infection is not yet clear, as there was no difference in the HR caused by effectors delivered by the Δ*cvsR* strain or the Δ*cvsS* strain in nonhost *N. benthamiana*. This lack of an HR phenotype suggests that CvsSR may not regulate T3SS deployment during growth in planta. CvsR binding sites were also identified upstream of several type III effector–encoding genes, and loss of regulation of these genes may contribute to Δ*cvsR* and Δ*cvsS* phenotypes. Alternatively, more than two hundred non-T3SS genes were differentially expressed in Δ*cvsR*, including the global regulator *algU*, and both Δ*cvsS* and Δ*cvsR* strains had altered cellulose production and swarming motility. Therefore, virulence defects of these strains could be due to T3SS-independent phenotypes. Additional experiments to define the in planta CvsSR regulon and assess whether calcium perception by CvsSR induces *hrpRS* during infection will be valuable in distinguishing between these possibilities.

2.2. Regulation of Virulence Genes Through Intracellular Perception of Host Metabolic Signals

In contrast to host signal detection by membrane-localized receptors described above, host-derived metabolites in some instances must first be transported into bacterial cells to function as virulence-inducing signals. In this section, I review the current understanding of how plant-derived metabolites regulate virulence genes in phytopathogenic bacteria through intracellular perception mechanisms.

2.2.1. Positive regulation of *Pseudomonas syringae* type III secretion by plant sugar signals. Bacteria encounter sugars on the surface of plant tissues (66) as well as in interstitial spaces and vasculature tissue (12), making sugars ideal signals for coordinating virulence gene regulation. In this regard, culturing bacteria in a sugar-containing minimal medium is usually sufficient to induce *hrp/hrc* gene expression in most T3SS-carrying phytopathogenic bacteria (7, 84, 121). In *P. syringae*, multiple genes involved in sugar uptake and catabolism have been associated with *hrp/hrc* gene expression (**Figure 2**). For instance, a gene encoding a predicted enzyme IIBC (EIIBC) component of a fructose-specific phosphoenolpyruvate (PEP)-dependent phosphotransferase system is required for fructose-induced *hrp/hrc* induction in DC3000 (S.E. Turner, Y.Y. Pang, J.C. Anderson, unpublished results; 30, 108), indicating that uptake of fructose into cells is a necessary prerequisite for *hrp/hrc* induction. Similarly, multiple genes encoding sugar catabolic

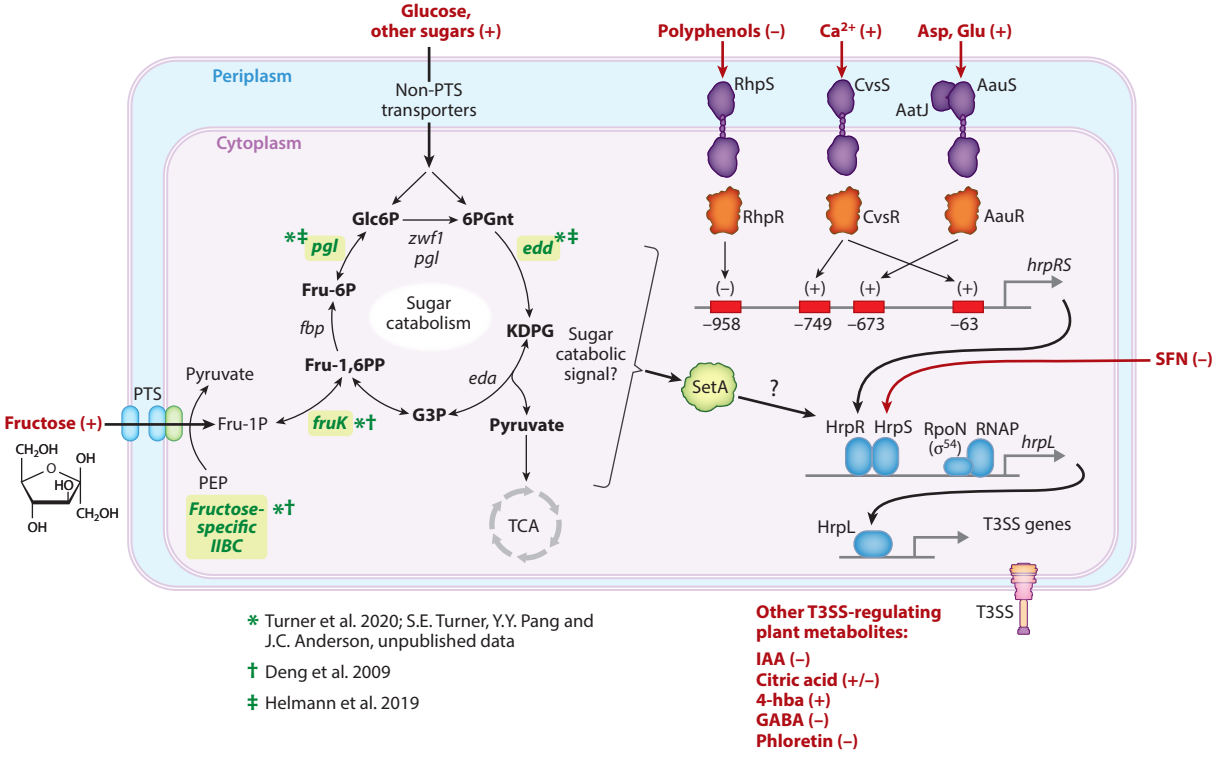

Figure 2

Model of plant metabolite–dependent regulation of type III secretion system (T3SS)-encoding genes in *Pseudomonas syringae*. Highlighted in green are sugar uptake–related and catabolism-related genes required for maximal *hrp/hrc* induction in fructose-containing minimal media or for fitness in planta. Red boxes upstream of *hrpRS* are known binding sites of AauR, RhpR, and CvsSR. Negative numbers indicate binding site positions relative to the HrpR start codon. Red arrows are direct metabolite–protein interactions. Parentheses with + or – indicate positive or negative regulation effects on *hrp/hrc* expression, respectively. Plant-derived metabolites that alter *hrp/hrc* expression are listed in red. Abbreviations: 4-hba, 4-hydroxybenzoic acid; 6PGnt, 6-phosphogluconate; Asp, aspartic acid; *eda*, 2-keto-3-deoxy-6-phosphogluconate aldolase; *edd*, 6-phosphogluconate dehydratase; *fbp*, fructose-1,6-bisphophatase; Fru-1,6PP, fructose-1,6-bisphosphate; Fru-1P, fructose-1-phosphate; Fru-6P, fructose-6-phosphate; *fruK*, 1-phosphofructokinase; G3P, glyceraldehyde-3-phosphate; GABA, γ-aminobutyric acid; Glc6P, glucose-6-phosphate; Glu, glutamic acid; IAA, indole-3-acetic acid; KDPG, 2-keto-3-deoxy-6-phosphogluconate; PEP, phosphoenolpyruvate; *pgi*, glucose-6-phosphate isomerase; *pgl*, 6-phosphogluconolactonase; PTS, phosphoenolpyruvate:sugar phosphotransferase system; RNAP, RNA polymerase; SFN, sulforaphane; TCA, tricarboxylic acid; *zwf1*, glucose 6-P dehydrogenase.

enzymes are required for maximal fructose-induced *hrp/hrc* expression in DC3000 and are necessary for the full fitness of *P. syringae* pv. *syringae* B728a during leaf infection (30, 45, 108). If sugars must be inside the bacterial cell to function as a virulence signal, what then are the possible regulatory connections of these molecules to *hrp/hrc* expression? It may be that the sugar itself directly functions as an inducing signal (similar to direct detection of Asp/Glu by AauSR described above), although this does not explain the need for intact catabolic pathways. Alternatively, the signal could be a product of sugar catabolism that is perceived by a metabolite-sensing protein, or perhaps sugars are perceived indirectly through their impact on metabolic enzyme activity and/or abundance.

A potential inroad toward uncovering the link between sugar catabolism and *hrp/hrc* expression is provided by the gene *sugar expression of type III secretion A* (*setA*) in DC3000 (108). Mutants that lack a functional *setA* gene have decreased *hrpL* expression when cultured in a minimal medium supplemented with individual sugars, including fructose, glucose, and mannitol, as the sole carbon source. Consistent with the important role of sugars as host signals, a *setA* mutant was also impaired in T3SS deployment and virulence during infection of *Arabidopsis*. Notably, the *setA* mutant did not show any growth defects in culture when provided with sugars as the sole nutrient source. Therefore, decreased T3SS deployment in the absence of *setA* is not likely because of perturbations in sugar uptake, sugar catabolism, or general defects that decrease bacterial fitness. The *setA* gene encodes for a predicted DeoR-type transcriptional regulator with a metabolite-sensing domain and a DNA-binding domain. In *E. coli*, DeoR functions as a repressor of the *deo* operon that encodes deoxyribonucleotide catabolic enzymes, and this repression is relieved by the binding of deoxyribose-5-phosphate to the metabolite-sensing domain (26). Whether SetA similarly functions as a transcriptional repressor and what promoters may be regulated by SetA are still unknown. Loss of *setA* did not alter the expression of upstream regulators *hrpRS* or *rpoN*. Therefore, SetA regulation likely occurs at the level of *hrpL* transcription or through post-transcriptional regulation of HrpR, HrpS, and/or RpoN (**Figure 2**). Overexpression of either SetA domain is not sufficient to restore *hrpL* expression in a *setA* mutant background (Y.Y. Pang & J.C. Anderson, unpublished results), suggesting both metabolite-sensing and DNA-binding functions of SetA are necessary for virulence regulation. Based on the model of DeoR function, it is tempting to speculate that SetA may provide a direct signaling link between sugar metabolism and virulence in *P. syringae*, perhaps by regulating gene expression in response to increased levels of phosphorylated metabolic intermediates such as 2-keto-3-deoxy-6-phosphogluconate that likely accumulate in cells during catabolism of sugars (see **Figure 2**).

2.2.2. Host-signal-dependent production of pectinolytic enzymes by soft-rot bacterial pathogens. Similar to regulation of the *P. syringae* T3SS by host sugars, virulence genes of soft-rot pathogens *Dickeya* and *Pectobacterium* spp. are also induced by plant-derived carbohydrate signals (22, 65, 93). A key virulence determinant for these pathogens is the *pel* genes that encode for enzymes that degrade plant cell wall pectin, resulting in the maceration of plant tissues and release of sugar derivatives that can be used by bacteria as an energy source. The regulation of *pel* genes is complex, involving at least twelve different transcription factors and multiple input signals (65). Key among these regulators is KdgR, a repressor of *pel* gene expression that responds directly to metabolite by-products of pectin degradation (77). In the absence of pectin signals, *pel* expression is maintained at a low level due to constitutive binding of KdgR to *pel* promoters. When pectin is first encountered during infection, a low level of secreted Pels cleaves pectin to produce smaller oligogalacturonides that are imported into the bacterial cell and catabolized as an energy source (58). Several intracellular intermediates of pectin catabolism can induce *pel* genes, and one, 2-keto-3-deoxygluconate, has been shown to directly bind to KdgR and relieve KdgR repression of *pel* expression (77). Maximal induction of *pel* genes also requires activation by the

transcriptional regulator cAMP receptor protein (CRP) that responds to increases in intracellular cAMP produced as a consequence of the metabolic shift from glucose to pectin catabolism (92). Recent modeling suggests that the combined action of both KdgR derepression and CRP activation at *pel* promoters may be responsible for the massive upregulation of Pels during infection (58).

3. INHIBITION OF BACTERIAL VIRULENCE BY PLANT-DERIVED METABOLITES

Bacterial pathogens produce and deploy virulence factors to colonize host tissues and cause disease. As such, the signaling pathways and regulatory processes that bacteria use to initiate virulence factor production represent a vulnerability that can be targeted by plant defenses. At the level of metabolic signaling between bacterial pathogens and plants, several distinct mechanisms of virulence gene suppression are known. First, plants produce chemical inhibitors that interfere with virulence gene induction. In this regard, plants produce an array of defense-associated secondary metabolites (11), and many of these compounds can inhibit the expression of virulence genes without impacting bacterial viability in culture (90). Although it is currently unknown whether most inhibitory compounds contribute to plant defense in situ, the production of such antivirulence metabolites (91) may be a way for plants to specifically hinder bacteria from inducing virulence while avoiding off-target effects on other nonpathogenic, plant-associated bacteria. Second, plants may respond to pathogen attack by decreasing the abundance of virulence-inducing metabolites available to bacteria at sites of infection and thereby limiting the production of virulence factors (4, 126). Because different pathogens use the same general types of plant metabolites (e.g., sugars, amino acids) as virulence-inducing cues and many virulence-inducing metabolites are nutrients for bacteria as well, metabolite depletion may be broadly effective against many pathogenic microbes.

In regard to the antivirulence strategy described above, it is important to note that bacteria may sense specific host metabolites as a means to downregulate virulence gene expression during infection, perhaps to avoid the unnecessary production of virulence factors after successful colonization. Therefore, careful consideration of where and when inhibitory metabolites are encountered during infection, their effective concentrations and mode(s) of action, and whether their effects benefit host or pathogen is essential to differentiate between these possibilities.

The T3SS is a critical virulence determinant for many bacterial pathogens and, as such, there is considerable interest in identifying host factors that impede its production during infection (90). A diversity of plant-derived metabolites is reported to inhibit the expression of T3SS-encoding *hrp/hrc* genes in *P. syringae* (**Figure 2**) (31, 57, 72, 109, 119). In this final section, I highlight studies of T3SS deployment by *P. syringae* that provide evidence for each of the distinct mechanisms of virulence gene suppression described above.

3.1. Inhibition of Type III Secretion System Deployment by Plant-Derived Metabolites

In this section, I describe the current understanding of how plant-derived metabolites inhibit signaling pathways that regulate the expression of T3SS-encoding genes in *P. syringae*.

3.1.1. Polyphenol suppression of type III secretion system genes through inhibition of RhpSR. Plant metabolites can inhibit virulence by targeting TCSs that regulate T3SS-encoding genes. One such example is the RhpSR TCS. RhpS is a membrane-localized sensor kinase, whereas RhpR is a response regulator that negatively regulates the expression of *hrpRS* (**Figure 2**) (29, 122). RhpS has dual kinase/phosphatase activity and can directly phosphorylate or dephosphorylate

RhpR (29). RhpR binding to the *hrpRS* promoter is phosphorylation dependent. Current models predict that under T3SS-inducing conditions, RhpR accumulates in its unphosphorylated form through increased RhpS phosphatase activity, allowing for *hrpRS* expression to occur (122).

Recently, several plant polyphenols were shown to inhibit *P. syringae* T3SS genes by repressing the activity of RhpSR (122). To identify host signals detected by RhpS, Xie et al. (122) screened a chemical library for compounds that could affect RhpSR signaling in *Pseudomonas savastanoi* pv. *phaseolicola* (*Psph*), a species belonging to the broader *P. syringae* species complex. Three polyphenols (tannic acid, 1,2,3,4,6-pentagalloylglucose, and epigallocatechin gallate) known to be present in plants were identified that repressed *hrpRS* expression in an RhpS-dependent manner. Importantly, none of the compounds had adverse effects on *Psph* growth at concentrations that inhibited *hrpRS* expression, indicating that phenotypes were not due to general toxicity. All three polyphenols directly bound to the extracellular domain of RhpS, and a single residue of RhpS, Pro 40, was identified as a potential binding site. In addition, all three polyphenols inhibited the phosphatase activity of recombinant RhpS. Based on these data, Xie and colleagues proposed that polyphenol binding to RhpS results in increased intracellular accumulation of phosphorylated RhpR, which in turn represses *hrpRS* expression and decreases T3SS deployment (122). Consistent with this model, *Psph* was less virulent when coinfiltrated with individual polyphenols into leaves of host bean plants, and this phenotype could be partially rescued by overexpression of *hrpL*. Furthermore, polyphenols were less effective at repressing the virulence of a *Psph* strain expressing RhpS(P40A), suggesting direct binding to RhpS underlies their inhibitory effects in planta. Despite clear evidence that polyphenols can inhibit *Psph* virulence through an RhpSR pathway, it is not known whether *Psph* encounters these metabolites during infection of plant tissues and, if so, what effects they may have on infection outcomes.

3.1.2. Sulforaphane suppression of type III secretion system through direct modification of HrpS. The plant-derived metabolite sulforaphane (SFN) was also recently identified as an inhibitor of the *P. syringae* T3SS (116). SFN is the breakdown product of an aliphatic glucosinolate, and it contains a reactive isothiocyanate group. In plants, glucosinolates are defense-associated compounds that in response to tissue wounding are converted into bioactive compounds that are toxic to pests and microbes (41). SFN can covalently modify the side chains of cysteine residues in proteins. Wang and colleagues (116) discovered that *Arabidopsis* leaf extracts inhibited T3SS-encoding genes in *P. syringae* and, using a bioassay-guided purification strategy, identified SFN as the responsible bioactive compound. To identify *P. syringae* proteins modified by SFN, a chemoproteomic method was employed, and two cysteine residues of HrpS were identified as targets of SFN. An alanine substitution of one SFN-modified residue, Cys_{209}, abolished the ability of HrpS to oligomerize with HrpR. Because HrpR–HrpS association is a necessary prerequisite for *hrpL* induction (56), these data provide a mechanistic explanation for the observed inhibitory activity of SFN.

The contribution of SFN modification of HrpS to plant disease resistance was investigated using an *Arabidopsis myb28/29* mutant that does not produce aliphatic glucosinolates (116). DC3000 possesses a suite of *sax* genes that can effectively detoxify SFN (33). Therefore, to exclude the influence of *sax*-mediated detoxification on phenotypes, infection assays were done using a DC3000Δ*sax* strain. In *myb28/29* compared to wild-type Col-0 plants, DC3000Δ*sax* grew to higher levels and had higher levels of T3SS expression, and these enhanced phenotypes were suppressed by coinfiltration with 20 μM SFN, which mirrors amounts of SFN present within the apoplast of uninfected Col-0 leaves. Importantly, a DC3000Δ*sax*Δ*hrpS* strain expressing $hrpS^{C209A}$ showed equal growth in Col-0 and *myb28/29*, suggesting that SFN targeting of HrpS is responsible for the increased resistance in Col-0 compared to *myb28/29*. Although it cannot be excluded

that the absence of other glucosinolates or other unknown factors in *myb28/29* contributes to phenotypes, these data strongly support that SFN within the leaf apoplast can provide effective resistance against pathogen attack. In line with SFN functioning as a specific antivirulence inhibitor, concentrations of SFN in the apoplast were not bactericidal, and 16S RNA sequencing revealed no differences in leaf microbiota between Col-0 and *myb28/29*. Levels of SFN did not change in flg22-elicited *Arabidopsis* leaves; therefore, it is unlikely to contribute to PTI-associated inhibition of T3SS deployment described below.

3.1.3. GABA and auxin suppression of type III secretion system genes. The nonproteinaceous amino acid γ-aminobutyric acid (GABA) is among the most abundant metabolites within the leaf apoplast of many plants, and it accumulates more in plant tissues in response to various biotic and abiotic stresses (85, 94). GABA has important roles in plant development and metabolism, although its roles in biotic stress responses are not yet clearly defined (99). Although GABA can be used as a growth substrate by *P. syringae*, it can also have inhibitory effects on the expression of T3SS genes and virulence. In a report by Park et al. (85), addition of GABA to cultures of *P. syringae* DC3000 inhibited *hrpL* expression. Consistent with this result, an *Arabidopsis pop2–1* mutant that accumulates higher levels of GABA was more resistant to DC3000 infection. Interestingly, a DC3000 Δ*gabT1/T2/T3* mutant, lacking three putative transaminases required for GABA catabolism, was hypersensitive to GABA treatment, showing both enhanced repression of *hrpL* and decreased virulence in *Arabidopsis*. These phenotypes of Δ*gabT1/T2/T3* were further exacerbated in *pop2–1* leaves, indicating that elevated GABA levels combined with an inability to catabolize GABA have the greatest repressive effect on T3SS deployment. How GABA inhibits *hrpL* expression is not known. GABA uptake primarily occurs through a GabP permease, and a Δ*gabP* strain was insensitive to GABA-mediated inhibition of *hrpL* expression (72). Therefore, GABA perception leading to *hrpL* repression likely occurs intracellularly.

It is not clear whether inhibition of T3SS genes by GABA is a mechanism that benefits pathogen or host. On one hand, GABA may have yet undefined antivirulence properties similar to SFN described above, although it is challenging to see how producing large quantities of a metabolite that can be used as a nutrient by *P. syringae* would be advantageous. On the other hand, because GABA accumulates in leaf tissue during *P. syringae* infection (85), GABA may be a signal for *P. syringae* to downregulate its T3SS at later stages of infection (72). In this manner, GABA functions as an inhibitory signal for *Agrobacterium* by negatively impacting quorum signal–dependent conjugation of Ti plasmids (24). It may be that the relatively high abundance of GABA in plant tissues, combined with its accumulation in stressed tissues, makes it a robust host signal for fine-tuning virulence processes in many phytopathogens.

Similar to models proposed for GABA signaling, Djami-Tchatchou and colleagues (31) hypothesized that DC3000 relies on elevated levels of the plant auxin hormone indole-3-acetic acid (IAA) as a signal to downregulate virulence genes at later stages of infection. In support of this possibility, IAA accumulates during DC3000 infection of susceptible hosts (23), and IAA represses *hrpL* expression in DC3000 and in *P. syringae* pv. *savastanoi* (6, 31). Auxins are key regulators of both plant development and defense. Their roles in regulating plant–bacteria interactions are complex, in part due to the fact that many plant-associated bacteria, including DC3000, can synthesize IAA during infection (71). Consistent with a role for IAA regulation of the T3SS during infection, *hrpL* expression by DC3000 was attenuated during infection of an *Arabidopsis tir1 afb1/4/5* mutant that has elevated levels of IAA (31). In addition to inhibiting *hrpL*, IAA treatment of DC3000 altered the expression of more than 700 additional genes, including stimulating the expression of approximately 30 known and putative transcription factors and transcriptional regulators, suggesting that IAA has global effects on *P. syringae* that extend beyond the T3SS (32).

3.2. Depletion of Extracellular Type III Secretion System–Inducing Metabolites as a Potential Plant Defense Mechanism Against *Pseudomonas syringae*

Induced plant defenses restrict the ability of bacteria to deliver type III effectors (T3Es) into host cells (4, 25, 130), and accumulating evidence suggests that depletion of extracellular T3SS-inducing signals may contribute to this repressive effect (4, 126). Early studies reported that treating leaves with defense elicitors could inhibit a plant's ability to effectively mount an ETI-associated HR against avirulent *P. syringae* strains (59, 79, 82). The molecular basis of this inhibition became apparent with the discovery that pretreating leaf tissue with PAMPs restricts the ability of *P. syringae* to deliver T3Es into cells, thereby preventing T3Es from triggering the HR (25). In work by Crabill et al. (25), pretreatment of tobacco leaves with a flg21 peptide restricted the ability of DC3000 to deliver the T3E HopU1. The block in effector delivery was observed as early as 1 h post flg21 treatment, suggesting that T3E delivery restriction can be rapidly established following PAMP perception. Based on these data, it was hypothesized that the restriction in T3E delivery could be due to cell wall reinforcements that physically block the T3SS and/or effects on bacterial physiology (25).

More recent in planta transcriptomic analyses have found that many T3SS-encoding genes, including *hrpL*, are repressed when DC3000 is infiltrated into flg22-elicited *Arabidopsis* leaves (69, 81). These data support the hypothesis that restriction of the T3SS during PTI occurs by altering bacterial physiology (25). Although T3SS genes were among those repressed based on gene ontology (GO) analysis, GO terms that are not directly associated with DC3000 virulence, such as translation (ribosomal proteins) and siderophore function (54), were also enriched within the flg22-inhibited gene set. Therefore, PTI effects on DC3000 are likely not limited to repressing T3SS-associated genes.

A possible role for plant-exuded metabolites in T3SS repression during PTI was revealed by studies of an *Arabidopsis* mutant lacking *MAP KINASE PHOSPHATASE 1* (*MKP1*) (3, 4). The *mkp1* mutant has enhanced PAMP-induced responses and is more resistant to DC3000 infection (3). A key observation was that during infection of *mkp1* seedlings, DC3000 expressed lower levels of the T3E gene *avrPto*, and lower amounts of AvrPto were detected in plant cells. These data suggested that enhanced resistance of *mkp1* may be due to an inability of DC3000 to fully express its T3SS-encoding genes, perhaps due to a lack of proper inducing signals in *mkp1* tissues. Indeed, exudate collected from *mkp1* seedlings had reduced *hrpL*-inducing activity, and a metabolomics analysis of *mkp1* exudate revealed reduced levels of several T3SS-inducing metabolites, including aspartic acid, citric acid, and 4-hydroxybenzoic acid (4-hba). Exogenous addition of these bioactive metabolites during DC3000 infection of *mkp1* seedlings restored AvrPto delivery into plant cells, as well as DC3000 growth, to levels observed in wild-type plants. This work demonstrated that the abundance of virulence-inducing metabolites exuded by plants is genetically regulated and that decreased abundance of these signals provides effective resistance against a virulent bacterial pathogen.

The association between heightened PAMP-induced signaling and decreased exudation of T3SS-inducing metabolites in *mkp1* seedlings suggested that similar changes in metabolites may occur during PTI as well. In support of this possibility, pretreating *Arabidopsis* seedlings with PAMPs prior to infection restricted DC3000 delivery of T3Es and growth, and addition of a mixture of aspartic acid, citric acid, and 4-hba restored these phenotypes back to levels observed in mock-treated seedlings (4). These data revealed that PTI effects on T3SS deployment are rapidly reversible and unlikely to be due to changes to the physical barriers of host cells. In support of a metabolite depletion defense strategy, a recent study reported that flg22-treated *Arabidopsis* seedlings exuded lower amounts of most proteinaceous amino acids (129). Furthermore, decreased

exudation of amino acids required a specific amino acid transporter gene *LYSINE HISTIDINE TRANSPORTER 1* (*LHT1*) (129). Surprisingly, flg22-treated *lht1* seedlings were even more resistant to infection by *P. syringae* pv. *maculicola* ES4326, suggesting that amino acid depletion does not contribute to flg22-induced impairment of *P. syringae* growth. However, loss of *LHT1* may have pleiotropic effects, perhaps on the accumulation of other T3SS-inducing or -inhibiting signals, that might alter these flg22-induced phenotypes.

During PTI there may also be active mechanisms to decrease the abundance of extracellular sugars, thereby limiting their use as T3SS-inducing signals (126). In this regard, flg22-treated *Arabidopsis* seedlings had enhanced uptake of glucose and fructose from the growth medium (126). This increased uptake required a specific gene, *SUGAR TRANSPORT PROTEIN 13* (*STP13*), which encodes for a transporter that functions in sugar absorption into plant cells. Consistent with a role for sugar transport in defense, DC3000 grew to higher levels and delivered increased amounts of the T3E AvrPto when spray-inoculated onto leaves of an *Arabidopsis* mutant lacking both *STP13* and a related transporter gene, *STP1*. However, there is mixed evidence regarding whether PAMP-induced sugar depletion occurs within the apoplast of adult leaves. The *stp1 stp13* mutant accumulated higher levels of apoplastic glucose in response to flg22, yet flg22 treatment did not alter glucose levels within the apoplast of wild-type leaves at the 24-h time point (126). In our own experiments, the abundance of glucose, fructose, and sucrose was not altered in apoplast wash fluid from *Arabidopsis* leaves treated with flg22 for eight hours (C.J. Rogan & J.C. Anderson, unpublished results). It may be that PAMP-induced sugar uptake within the apoplast occurs in a spatially or temporally restricted manner, such that differences may be obscured by isolating total apoplast contents or by single time points. Alternatively, PAMP perception may have differential effects on metabolites exuded from whole seedlings versus the metabolic changes that occur within the leaf apoplast.

Lastly, there is evidence to suggest that ETI also inhibits the delivery of T3Es into plant cells (130) and that coactivation of PTI and ETI enhances the magnitude of this restriction (25). However, in planta transcriptome profiling of DC3000 revealed that ETI does not decrease the expression of T3SS genes similar to PTI (81). In fact, a reanalysis of this data set showed T3SS genes were hyperexpressed by DC3000 during ETI interactions triggered by T3E AvrRps4 (115). These data indicate that ETI may not inhibit T3SS gene expression but instead may inhibit T3E delivery, possibly by interfering with later stages of T3SS deployment. The possibility of distinct T3SS-inhibitory mechanisms is intriguing given recent evidence of extensive overlap in PTI and ETI signaling pathways (80, 128).

4. CONCLUSION

Plant bacterial pathogens detect specific host metabolites to coordinate the expression of genes required for virulence. Host sensing is complex, as shown by the multiple distinct plant metabolites that positively or negatively regulate the *P. syringae* T3SS (**Figure 2**). The abundance of virulence-inducing metabolites in plant tissues is important for disease outcomes, as exemplified by the enhanced resistance of *mkp1* plants to *P. syringae* infection. Plant defenses interfere with virulence gene induction through production of antivirulence metabolites and possibly through depletion of virulence-inducing signals at sites of infection. These insights open the possibility of engineering plants to be more disease resistant by altering the availability of specific metabolites to pathogens or enhancing existing plant defense responses that interfere with metabolite perception.

In the context of a coevolutionary arms race, it is not surprising that plants would evolve defenses that specifically interfere with the deployment of pathogen virulence factors. In turn, adapted pathogens can overcome these defenses by detoxifying antivirulence metabolites (e.g.,

sax genes) (33) or deploying effectors and toxins with sufficient speed and/or potency to suppress immune responses. However, bacteria may have other ways to counter these defenses, as recent reports show that T3Es manipulate plant cells to exude higher amounts of metabolites that fuel bacterial growth (38, 118). A fascinating question is whether these T3E-manipulated metabolites are virulence-regulating signals and whether they may function in a feedback loop to promote further virulence factor deployment and tip the scales toward successful infection.

FUTURE ISSUES

1. Experiments are needed to determine whether the spatial distribution and abundance of virulence-regulated metabolite signals in plant tissues influence when and where virulence genes are expressed.
2. It remains unclear why individual strains use multiple distinct host metabolite signals to coordinate their virulence.
3. An unresolved question is whether all members of a bacterial species rely on the same host signals to regulate their virulence genes.
4. It is important to establish whether natural variation exists in the abundance of virulence-regulating metabolites within plant populations and whether this variation contributes to differences in resistance phenotypes and possibly host range of pathogens.
5. Plant disease resistance is influenced by plant growth conditions and developmental stage. Future studies should investigate whether changes in the abundance of virulence-regulating metabolites in plant tissues contribute to these phenomena.
6. Determining what changes in the leaf apoplast are causal for type III secretion system inhibition by pattern-triggered immunity and effector-triggered immunity is crucial for understanding how plants defend against bacterial pathogens.

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