Defining essential processes in plant pathogenesis with *Pseudomonas syringae* pv. *tomato* DC3000 disarmed polymutants and a subset of key type III effectors

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Running title: *P. syringae* effector functions in pathogenesis

Keywords: type III secretion system, effectors, coronatine, bacterial polymutants, minimal pathogenesis requirements

Word count breakdown: Summary 249; Text 6762; Acknowledgements 28; Figure legends 349

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/mpp.12655

SUMMARY

Pseudomonas syringae pv. *tomato* DC3000 and its derivatives cause disease in tomato, Arabidopsis, and *Nicotiana benthamiana*. Primary virulence factors include a repertoire of 29 effector proteins injected into plant cells by the type III secretion system and the phytotoxin coronatine. The complete repertoire of effector genes and key coronatine biosynthesis genes have been progressively deleted and minimally reassembled to reconstitute basic pathogenic ability in *N. benthamiana* and also in Arabidopsis plants that have mutations in target genes that mimic effector actions. This approach and molecular studies of effector activities and plant immune system targets highlight a small subset of effectors that contribute to essential processes in pathogenesis. Most notably, HopM1 and AvrE1 redundantly promote an aqueous apoplastic environment, and AvrPtoB and AvrPto redundantly block early immune responses, two conditions that are sufficient for substantial bacterial growth *in planta*. In addition, disarmed DC3000 polymutants have been used to identify individual effectors responsible for specific activities of the complete repertoire and to more effectively study effector domains, effector interplay, and effector actions on host targets. Such work has revealed that AvrPtoB suppresses cell death elicitation in *N. benthamiana* that is triggered by another effector in the DC3000 repertoire, highlighting an important aspect of effector interplay in native repertoires. Disarmed DC3000 polymutants support natural delivery of test effectors and infection readouts that more accurately reveal effector functions in key pathogenesis processes and enable identification of effectors with similar activities from a broad range of other pathogens that also defeat plants with cytoplasmic effectors.

INTRODUCTION: THE UTILITY OF A DISARMED PATHOGEN IN PROBING THE FUNCTIONS OF TYPE III EFFECTORS

Cytoplasmic effectors delivered by various pathways into plant cells are essential for the virulence of most pathogenic bacteria, fungi, and oomycetes with a biotrophic or hemibiotrophic lifestyle (Win *et al*., 2013). Bacteria, such as *Pseudomonas syringae,* use the type III secretion system (T3SS) to deliver these proteins, which are variously designated Hrp outer proteins (Hops), avirulence (Avr) proteins or, more

broadly, type III effectors (T3Es). Functional genomics has yielded near-complete T3E repertoires for multiple reference strains of *Pseudomonas*, *Xanthomonas*, and *Ralstonia* (Lindeberg *et al.*, 2012). *P. syringae* pv. *tomato* (*Pst*) DC3000, a pathogen of Arabidopsis, tomato, and *Nicotiana benthamiana* (if an avirulence T3E is eliminated), has emerged as a particularly useful model for studying the functions of T3Es in pathogenesis (Lindeberg *et al.*, 2012; Xin and He, 2013). In addition to delivering nearly 30 T3Es, DC3000 produces coronatine (COR), an extensively studied phytotoxin whose functions overlap with those of several T3Es (Geng *et al*., 2014).

Studies of *Pst* DC3000 T3Es are foundational to the current paradigm that plants defend against (hemi)biotrophic pathogens with a two-tiered innate immune system (Jones and Dangl, 2006). AvrPto has been a model in this regard, but AvrPto also highlights discordance between the abilities of T3Es functioning as components of native T3E repertoires and as single agents transgenically expressed in plants. That is, mutations of single genes reveal that neither AvrPto nor any other individual T3E is necessary for more than a quantitative contribution to virulence (Brooks *et al*., 2004; Lin and Martin, 2005; Macho *et al*., 2007), and in general, the cytoplasmic effectors of various pathogens are individually dispensable, although collectively essential (Cunnac *et al*., 2011; Lindeberg *et al*., 2012). However, transgenic expression of AvrPto in Arabidopsis is sufficient to allow substantial multiplication of a *Pst* DC3000 mutant that lacks a functional T3SS and therefore can deliver no T3Es (Hauck *et al*., 2003), and other T3Es typically produce stronger effects when produced transgenically. Findepurthermore, although AvrPto is the smallest T3E in the DC3000 repertoire, it is somewhat like a Swiss Army multi-tool in having two virulence domains and multiple virulence activities (Martin, 2012; Nguyen *et al*., 2010; Yeam *et al*., 2009), and it is found to interact with numerous plant proteins in yeast two-hybrid screens (Bogdanove and Martin, 2000; Mukhtar *et al*., 2011; Singh *et al*., 2014).

Thus, primary functions of T3Es in native bacterial repertoires may be obscured by redundancy, while secondary functions may become prominent when these multi-tools are operating transgenically in 'Sorcerer's Apprentice' mode without the constraints of pinpoint delivery at the pathogen-host cellular interface during natural infections. We explain in this review that model pathosystems involving effector-depleted DC3000 strains are beginning to bridge these two extremes by enabling study of just a few T3Es (or specific domains of each) in native systems. Our guiding premises are that (i) basic pathogenicity can

be informatively reconstituted from small subsets of nonredundant T3Es derived from native repertoires, (ii) these T3Es act on and help define a sequence of interlinked processes that are essential to pathogenesis, (iii) multi-domain T3Es can usefully be considered as having primary and secondary roles in these pathogenic processes, (iv) the consequential activities of individual T3Es and their domains can be more informatively assessed when T3Es are naturally delivered, (v) natural variations in interacting pathogen and host factors provide additional, informative clues to function, (vi) T3E studies using the reference strain *Pst* DC3000 interacting with model hosts benefit from and add to growing systems-level knowledge of these pathosystems, and (vii) a resource toolkit of DC3000 mutants facilitates this systems approach.

We will begin with an update on evidence for a small subset of key T3Es in *Pst* DC3000, introduce the mutant toolkit, and discuss newly discerned processes in pathogenesis that are mediated by the key T3Es and their domains. Table 1 and Figure 1 summarize the series of DC3000 mutants that have proven particularly useful in studying T3E functions in key pathogenic processes. This review emphasizes the biological context in which T3Es operate as much as the functions of the T3Es themselves and is intended to be a complementary update to two previous reviews focusing on *Pst* DC3000 and its T3E repertoire (Lindeberg *et al*., 2012; Xin and He, 2013). Other recent reviews provide more comprehensive coverage of molecular activities of bacterial T3Es (Buttner, 2016; Macho, 2016; Toruno *et al*., 2016).

EFFECTOR-DEPLETED POLYMUTANTS REVEAL A SUBSET OF KEY EFFECTORS

Functional genomics suggests that the pangenome for the *P. syringae* species complex contains at least 68 T3E families (http://www.pseudomonas-syringae.org). However, a single strain usually carries less than a third of the total repertoire, and only a subset of those T3Es may make a detectable contribution to quantitative virulence in a given host (Baltrus *et al*., 2011; Lindeberg *et al*., 2012; O'Brien *et al*., 2011). *Pst* DC3000 has a relatively large repertoire of 29 likely functional T3Es (Chang *et al.*, 2005; Wei *et al*., 2015). The *Pst* DC3000 genome harbors T3E genes that vary widely in their activity. These include several disrupted pseudogenes as well as 34 genes that are intact but range in activity from poorly expressed or poorly translated to robustly expressed, translocated, and demonstrably functional (Figure 1) (Chang *et*

al., 2005; Ferreira *et al*., 2006; Lam *et al*., 2014; Schechter *et al*., 2006; Shan *et al*., 2004; Wei *et al*., 2015).

Evidence for the greater importance of a subset of the DC3000 T3Es is based on virulence phenotypes attending disassembly of the complete repertoire and reassembly of a minimal functional repertoire (Table 1 and Figure 1). Disassembly occurred with the following major steps: (i) *hopQ1-1* was deleted, which expanded the host range to the model plant *N. benthamiana* (Wei *et al*., 2007). (ii) 18 T3E genes located in five genomic clusters and plasmid pDC3000A were deleted, as groups in various combinations (Kvitko *et al*., 2009; Wei *et al*., 2007). (iii) The remaining 10 well-expressed T3E genes that reside outside of these clusters were sequentially deleted to produce DC3000D28E (here abbreviated 'D28E') (Cunnac *et al*., 2011). (iv) A reassembly process involving the shuffling of small sets of T3E genes that were assayed for promotion of growth and symptoms in pressure-infiltrated *N. benthamiana* revealed a subset of eight effectors sufficient for disease (Cunnac *et al*., 2011). (v) *hopAD*, which was considered weakly expressed (Chang *et al*., 2005), was subsequently deleted from D28E to produce 'D29E' and thereby eliminate residual elicitation of cell death in *N. benthamiana* at very high levels of inoculum (Wei *et al*., 2015). (vi) Additional weakly-expressed T3E genes were deleted to produce 'D36E' (Wei *et al*., 2015). (vii) Further analysis of combinations of T3Es in the minimal repertoire, using dip inoculations and a D29E derivative restored for COR production (explained below), revealed that just two T3Es — HopM1 and AvrPtoB — were sufficient to produce robust chlorotic spots in *N. benthamiana* (Chakravarthy *et al*., 2017). Finally, it is worth noting that there is no apparent difference in the response of *N. benthamiana* to the effector-depleted polymutants D29E and D36E (deleted of all known T3E genes and several pseudogenes) (Wei *et al*., 2015). Furthermore, analysis of the D36E genome sequence documented all T3E gene deletions and only minor spontaneous changes from the parental DC3000 genome (Wei *et al*., 2015).

It is also important to note that these genetic manipulations included two unbiased interrogations of the system, first for loss-of-function accompanying combinatorial deletions from DC3000 of various T3E gene clusters and second for gain-of-function following combinatorial addition of sets of effectors to D28E. Both interrogations mutually support the importance of a small subset of T3Es in DC3000 interactions with *N. benthamiana*. These are HopM1, AvrE1, AvrPtoB, AvrPto, HopE1, HopG1, and

HopAM1 (inclusion of the appropriate chaperones for HopM1 and AvrE1 in various experiments is implicit in this review).

Of these key T3Es, four can be assigned to two redundant effector groups (REGs) identifiable by the strong phenotype resulting from deletion of all genes in each group (Kvitko *et al*., 2009). The first REG comprises the conserved effector locus (CEL) T3Es HopM1 and AvrE1 (plus the AvrE family member HopR1). The second REG comprises AvrPtoB and AvrPto. We will focus on HopM1 and AvrPtoB as particularly illustrative representatives of each of the REGs. The remaining three T3Es (HopE1, HopG1, HopAM1) emerged from the shuffled reassembly screen as being additionally important for growth or symptoms in *N. benthamiana* (Cunnac *et al*., 2011).

In general, DC3000 and derivatives varying in their production of these key T3Es can be divided into four phenotypic classes (Chakravarthy *et al*., 2017). Class I strains do not grow in *N. benthamiana* and are represented by the effector-depleted polymutants D28E, D29E, and D36E. Class II strains grow significantly better (≤2 logs) in *N. benthamiana*, produce robust chlorotic spots if COR^+ and are epitomized by strains producing just HopM1 plus AvrPtoB. Class III strains grow to nearly wild-type levels (within 1 log), produce some necrotic lesions, and in addition to HopM1 and AvrPtoB, contain either AvrE1 or HopG1/HopE1/HopAM1. DC3000 mutants lacking either of the major REGs or the CEL also broadly align with this phenotypic class in their growth in different hosts (Alfano *et al*., 2000; Badel *et al*., 2006; Kvitko *et al*., 2009; Lin and Martin, 2005). Class IV is defined by DC3000 (if Δ*hopQ1-1* but otherwise wild type). These observations now allow us to focus on a small subset of T3Es as we explore the requirements for DC3000 growth and pathogenesis in *N. benthamiana* and other plants.

DEFENSE SUBVERSION AND OTHER PROCESSES PROMOTING PATHOGENESIS THAT ARE ENABLED BY KEY EFFECTORS

Overview of *P. syringae* interactions with the two-tiered innate immune system of plants and key processes in pathogenesis

P. syringae pathogenesis requires defeat of the plant immune system's two tiers (Abramovitch *et al*., 2006; Boller and He, 2009; Cui *et al*., 2015; Jones and Dangl, 2006). These are (i) recognition by surface-arrayed pattern recognition receptors and co-receptors (PRRs) of microbe-associated molecular patterns (MAMPs) in flagellin and other microbial factors, which leads to pattern-triggered immunity (PTI), and (ii), recognition by cytoplasmic nucleotide-binding leucine-rich repeat proteins (NLRs) of effectors or their activity on targets within plant cells, which leads to effector-triggered immunity (ETI). Recent studies involving disarmed DC3000 derivatives and key T3Es suggest that pathogenic success depends on T3Es defeating a chain of defense processes: (i) stomatal immunity, (ii) apparent defense triggered by the T3SS machinery, (iii) PTI perception and implementation processes, (iv) apoplastic water insufficiencies, (v) ETI otherwise triggered in the host by T3Es in the native repertoire, (vi) compensatory networks in the immune system that can thwart T3E virulence-promoting activities, and (vii) ultimately, the production of disease symptoms. Table 2 summarizes recent insights into the impacts of the subset of key T3Es on these processes.

Natural variations among closely related *P. syringae* strains and their T3E repertoires are also informative. Strains in the *P. syringae* species complex are divided into seven primary phylogenetic groups, over 60 host-specific pathovars, and in some cases races that are avirulent on certain cultivars of the host (Baltrus *et al*., 2017). For example, DC3000 is a member of phylogenetic group I, of pathovar *tomato* (causing bacterial speck disease), and race 0 (producing AvrPto and AvrPtoB, either of which trigger ETI in tomato cultivars expressing Pto/Prf-mediated speck resistance). DC3000-like strains were largely replaced in the 1960s in tomato fields around the world by T1-like strains of both races, which have significantly different T3E repertoires (notably, lacking HopE1, HopG1, HopAM1), vary in their ability to produce COR, and have polymorphic flagellin MAMPs (Almeida *et al*., 2009; Cai *et al*., 2011; Clarke *et al*., 2013). Interestingly, T1 strains are not virulent on Arabidopsis, whereas DC3000 can cause disease in Arabidopsis and some edible brassicas (Elizabeth and Bender, 2007).

Thus, DC3000 and its polymutant derivatives and close relatives can be used to address fundamental questions about T3E-mediated pathogenesis in the face of alternative hosts and pathogen evolution. For example, what is the relative importance of suppressing PTI and ETI in different plants, and what is the significance of individual T3Es targeting multiple defense processes? The following sections will present

an overview of the major defense processes and the role of the key T3Es in defeating them and promoting symptoms. Figure 2 depicts the primary molecular activities of key T3Es in the context of these processes.

Suppressing stomatal immunity

Epiphytic growth on plant surfaces to threshold population levels is an important initial process in pathogenesis for some *P. syringae* strains, such as *P. syringae* pv. *syringae* B728a (Yu *et al*., 2013). However, for *Pst* DC3000, entry into the apoplast via stomata appears to be the initial pathogenic challenge (Xin and He, 2013). MAMPs trigger stomatal closure as part of the PTI response, and the virulence in Arabidopsis of surface-inoculated *Pst* DC3000 strongly benefits from the phytotoxin COR, a jasmonic acid-isoleucine (JA-Ile) analog that reverses stomatal closure (Melotto *et al*., 2006 and 2008). COR also promotes symptoms in *Pst* DC3000 hosts (discussed further below), but it appears less important for DC3000 entry into *N. benthamiana* leaves, and it is produced by only a subset of *P. syringae* pathovars (Chakravarthy *et al*., 2017; O'Brien *et al*., 2011). Some *Pst* DC3000 T3Es, including HopM1, HopF2, and HopX1, also have the potential to block stomatal immunity (Gimenez-Ibanez *et al*., 2014; Hurley *et al*., 2014; Lozano-Duran *et al*., 2014). Heterologous production of HopM1 in both Arabidopsis and *N. benthamiana* blocks two early PTI responses: a burst of reactive oxygen species (ROS) and stomata closure in response to flg22 or to a *Pst* DC3000 COR$^-$ mutant (Lozano-Duran *et al*., 2014). In considering more broadly the functions of COR and the T3E repertoire, it is noteworthy that substantial overlap was observed in transcriptome responses of Arabidopsis to *Pst* DC3000 mutants differentially producing the complete T3E repertoire or COR (Thilmony *et al*., 2006).

Preventing the T3SS machinery from interfering with a basal ability of *Pst* DC3000 to multiply in the apoplast

As noted above when comparing the growth of various DC3000 polymutants, T3SS$^-$ mutants of *Pst* DC3000 and D28E have a significant basal growth ability in *N. benthamiana* and can even produce COR-dependent chlorosis (Chakravarthy *et al*., 2017; Worley *et al*., 2013). The ability of DC3000 mutants and phylogenetic subgroup 2c strains of *P. syringae* to grow in a T3SS-independent manner in *N. benthamiana* has been previously observed (Clarke *et al*., 2010; Hann and Rathjen, 2007). In contrast, the growth in *N. benthamiana* of D28E and other effector-depleted, T3SS-proficient polymutants is substantially less than that of T3SS$^-$ derivatives of D28E or of DC3000 (Chakravarthy *et al*., 2017; Cunnac *et al*., 2011). We presently have no explanation for the basal growth of some *P. syringae* strains in *N. benthamiana* or why production of the T3SS interferes with it. However, observation that D29E elicits a stronger PTI transcriptome response in tomato than T3SS$^-$ DC3000 is consistent with an immune response to the T3SS machinery in solanaceous plants (Worley *et al*., 2016).

The *P. syringae* T3SS machinery has 12 extracellular components that are capable of being injected into plant cells, and some may be recognized by immune receptors (Wei and Collmer, 2012). Among these extracellular components are four harpins (Kvitko *et al*., 2007), which are known to elicit plant defenses (Choi *et al*., 2013). Also, HrpJ, a multifunctional regulator of T3SS assembly, suppresses PTI markers when transgenically expressed in Arabidopsis, which raises the possibility that such effector-like activity could be under immune surveillance in some plants (Crabill *et al*., 2012). It is also important to note that HopAD1 (discussed further below) is not responsible for the T3SS-dependent growth inhibition of D28E (Chakravarthy *et al*., 2017). However, the observation that AvrPtoB, but not HopM1, restores growth of D28E to levels equivalent to T3SS$^-$ mutants suggests a role for AvrPtoB in suppressing this novel defense (Cunnac *et al*., 2011). As will be discussed below, AvrPtoB has multiple domains that suppress both PTI and ETI. Finally, a variety of DC3000 polymutants altered in production of COR, flagellin, T3SS components, and T3Es have made interplay among these factors and the plant immune system more accessible to genetic interrogation (Table 1 and Figure 1).

Blocking PTI perception processes

The ability of multiple DC3000 MAMPs to elicit PTI highlights immune system redundancy. Also, MAMP perception varies among plants according to their PRR repertoires, but flagellin is broadly recognized (Boller and Felix, 2009; Boutrot and Zipfel, 2017; Couto and Zipfel, 2016). Most plant families produce the FLS2 PRR, which along with co-receptor BAK1 recognizes the flg22 peptide in flagellin (Boller and Felix, 2009). Most tomato accessions also produce FLS3 (Hind *et al*., 2016), which recognizes the flagellin flgII-28 peptide (Cai *et al*., 2011). Additional DC3000 MAMPs for which matching PRRs have been identified include the elf18 peptide in elongation factor Tu (Zipfel *et al*., 2006), csp22 in cold shock protein (Wang *et al*., 2016), peptidoglycan (Willmann *et al*., 2011), and lipopolysaccharide (Ranf *et al*., 2015). The EFR PRR (elf18 perception) is present in Arabidopsis but not tomato, whereas the CORE PRR (csp22 perception), is present in tomato but not Arabidopsis, and reciprocal expression of EFR and CORE produces stronger gain-of-function resistance against DC3000 than is typically observed in loss-of-function genetic tests involving these and other taxonomically restricted PRRs (Boutrot and Zipfel, 2017; Lacombe *et al*., 2010; Wang *et al*., 2016).

Loss-of-function tests and natural variation in the field indicate a particularly important role for flagellin perception in interactions of *P. syringae* with solanaceous plants. For example, virus-induced gene silencing (VIGS) of FLS2 in *N. benthamiana* enhances growth of T3SS$^-$ *Pst* DC3000 and strongly compromises indicators of functional PTI (Chakravarthy *et al*., 2010; Hann and Rathjen, 2007). Deletion of the DC3000 flagellin *fliC* gene rescues growth of a DC3000 Δ*hopQ1-1*Δ*avrPto*/Δ*avrPtoB* mutant in *N. benthamiana* and abolishes a 15-h ROS response to bacteria that is associated with PTI (Kvitko *et al*., 2009; Wei *et al*., 2013).

Regarding natural variation in flg22 perception in plants, substantial differences have been observed within Arabidopsis genotypes and heirloom tomato accessions, which may involve downstream signaling components as well as PRR polymorphisms (Veluchamy *et al*., 2014; Vicente and Holub, 2013). There is also relevant natural variation in *Pst* strains isolated from tomato fields worldwide over many years. Notably, an ancestral *fliC* allele, which is carried by *Pst* DC3000, has been replaced by *fliC* alleles that elicit weaker PTI responses and lack a functional flgII-28 MAMP (Cai *et al*., 2011).

The mechanisms by which the key T3Es AvrPto and AvrPtoB interfere with PRRs and PTI perception have been extensively studied, as summarized in Table 2 and reviewed elsewhere (Martin, 2012).

However, domain modularity and redundancy in these proteins warrant highlighting in the context of the themes of this review. The proteins are sequence-unrelated but share an ability to inhibit the kinase activity of specific PRRs, with AvrPto and AvrPtoB having one and two PRR kinase-interacting domains, respectively. AvrPto has an additional virulence domain, and AvrPtoB has at least two other virulence domains, which are discussed below in the context of ETI suppression. Both T3Es have additional virulence activities, for example, promoting cell death symptoms in tomato in an ethylene-dependent manner (Cohn and Martin, 2005).

Recent transcriptional profiling experiments provide striking evidence for the importance of flagellin perception in tomato interactions with *Pst* DC3000 and for the efficacy of AvrPto and AvrPtoB interference with this perception (Rosli *et al*., 2013). Specifically, RNA-seq was used to profile tomato responses to flgII-28 and DC3000 derivatives carrying mutations variously affecting the T3SS, flagellin, and AvrPto/AvrPtoB. Remarkably, inoculation of a Δ*fliC* T3SS$^-$ mutant (producing all MAMPs beyond flagellin but unable to suppress PTI with T3Es) increased expression of only 223 tomato genes, whereas flgII-28 treatment increased expression of 2,268 genes. Further analysis of these 2,268 genes in the context of the differing responses to DC3000 and the Δ*avrPto*/Δ*avrPtoB* mutant revealed 622 *FIRE* (flagellin-induced, repressed by effectors) genes. One of the *FIRE* genes, *SlWAK1,* encoding a plant cell wall-associated kinase, was silenced with VIGS, which severely compromised PTI (Rosli *et al*., 2013). These observations support a model in which increased numbers of SlWAK1 receptors in the plasma membrane enhance perception of oligogalacturonides released from the plant cell wall that function as damage-associated molecular patterns to amplify PTI signaling in response to flgII-28 and possibly other MAMPs (Rosli *et al*., 2013).

Blocking PTI implementation

The PTI-induced changes that protect plants from *P. syringae* and other pathogens are less understood than PTI perception processes. Pre-inoculation of *N. benthamiana*, tomato, or Arabidopsis leaves with a nonpathogen induces within 6 h a PTI-protected condition in which challenge DC3000 derivatives no longer multiply, cause disease, elicit ETI-associated cell death, or translocate T3Es (Crabill *et al*., 2010;

Klement *et al*., 2003; Oh *et al*., 2010). The question is what explains these many deficits in pathogen performance.

A VIGS forward screen in *N. benthamiana* for PTI genes revealed surprisingly few essential genes and no silver-bullet candidates for the PTI-protected state (Chakravarthy *et al*., 2010). However, RNA-seq transcription profiling aimed at distinguishing PTI and ETI responses of tomato to DC3000 derivatives revealed enrichment for genes associated with the phenylpropanoid pathway in the PTI-specific set (Pombo *et al*., 2014). Phenylpropanoid pathway genes were similarly found in a transcriptome study of PTI genes in tobacco (Szatmari *et al*., 2014), and the *N. benthamiana* VIGS screen identified the CA4H gene which plays a role in phenylpropanoid biosynthesis (Chakravarthy *et al*., 2010). Histochemical study of a DC3000 T3SS$^-$ mutant in Arabidopsis highlighted localized production of H_2O_2 by chloroplasts as a prominent response that could promote plant cell wall strengthening and bacterial agglutination (Mitchell *et al*., 2015). Consistent with this observation, a triple-catalase deficient DC3000 polymutant is less virulent in Arabidopsis (Guo *et al*., 2012). Finally, callose deposition in papillae between the plasma membrane and plant cell wall is a factor that demonstrably contributes to PTI-based defense against DC3000 (Kim *et al*., 2005; Mitchell *et al*., 2015). Collectively, these observations point to localized, plant cell wall-associated defenses as an important component of PTI implementation.

Changes in apoplastic fluids may also contribute to PTI, particularly by altering expression of the T3SS. *Pst* DC3000 utilizes nutrients that are prevalent in the tomato apoplast, and expression of the T3SS Hrp regulon is supported by tomato apoplastic fluids (Rico and Preston, 2008). Among apoplastic sugars, fructose optimally induces expression, apparently because it supports slower growth than other inducing carbon sources (Stauber *et al*., 2012). One mechanism by which PTI may downregulate *Pst* DC3000 T3SS expression in Arabidopsis is through phosphorylation-dependent regulation of sugar transport protein 13 (STP13) (Yamada *et al*., 2016). BAKI-mediated phosphorylation of STP13, which is activated by flg22, enhances depletion from the apoplast of fructose and other monosaccharides and reduces T3SS-dependent T3E translocation (Yamada *et al*., 2016). Consistent with this model, *Pst* DC3000 T3Es may promote sugar release to the apoplast, as indicated by the failure of a T3SS$^-$ mutant to induce three of the seven *AtSWEET* (sugar efflux transporter) genes that are induced by the wild-type strain (Chen *et al*., 2010), although specific T3Es responsible for this effect are undefined.

A potentially complementary mechanism for downregulating T3SS gene expression involves MKP1, a negative regulator of PTI in Arabidopsis (Anderson *et al*., 2011). The *Pst* DC3000 T3SS is less expressed in exudates from a *mkp1* mutant relative to wild type plants, and three organic acids (citric, 4-hydroxybenzoic, and aspartic) are less abundant in mutant exudates. Exogenous supplementation of these organic acids increases T3SS expression in culture (especially if fructose is added) and also increases T3E delivery *in planta* (Anderson *et al*., 2014).

Gamma-amino butyric acid (GABA) represents another defense-associated apoplastic molecule that could affect expression of the T3SS during pathogenesis (Mirabella *et al*., 2007; O'Leary *et al*., 2016). The use of Arabidopsis and tobacco plants altered in GABA metabolism and *Pst* DC3000 mutants altered in GABA uptake and metabolism that collectively promote higher levels of GABA within bacteria suggest that GABA also can reduce T3E delivery (McCraw *et al*., 2016; Park *et al*., 2010).

In assessing the importance of changes in T3SS gene expression and function during pathogenesis, it is unfortunate that we still poorly understand the contact-dependent regulation of T3SS assembly in *P. syringae*, particularly the switch from the export of extracellular T3SS components (such as pilus and translocon proteins) to the translocation of T3Es that occurs *in planta* (Ji and Dong, 2015; Wei and Collmer, 2012). Given that some extracellular components, such as HrpJ and HrpH, also have effector-like properties (Crabill *et al*., 2012; Oh *et al*., 2010), the T3SS machinery and its assembly warrant more attention in the context of both PTI and ETI.

Another critical regulatory switch involving the T3SS that occurs with *P. syringae* strains in the apoplast is the AlgW/AlgU-dependent upregulation of the T3SS, alginate synthesis, and osmotic and oxidative stress tolerance factors and the downregulation of flagellin production (Markel *et al*., 2016; Schreiber and Desveaux, 2011). The reciprocal regulation of FliC and the T3SS is consistent with the opposing role of these factors in PTI induction and suppression (as well as motility and contact-dependent T3E translocation).

Also, the T3SS of *Pst* DC3000, like that of many mammalian pathogens, can translocate flagellin into host cells via pathway cross-trafficking (Rossez *et al*., 2015; Wei *et al*., 2013). Flagellin inside mammalian cells triggers NLR-dependent programmed cell death (Broz and Monack, 2011), but no death response is observed in *N. benthamiana* cells presented with cytosolic flagellin, as indicated by the use of various

T3SS mutants in the *Pst* DC3000 toolkit (Table 1) (Wei *et al*., 2013). One explanation for this major difference in the innate immune systems of plants and mammals is that a death response to translocated flagellin could confer susceptibility to necrotrophs that benefit from ETI cell death (Mengiste, 2012). On this point, it is noteworthy that *Pst* DC3000 derivatives have been used to study mechanisms by which Arabidopsis regulates the potentially conflicting defense responses to necrotrophs and biotrophs (Liu *et al*., 2016; Spoel *et al*., 2007).

The apparent foundational role of the AvrPto/AvrPtoB REG in *Pst* DC3000 virulence is consistent with the need to rapidly block PTI perception processes in a dynamic race against defenses that can compromise the T3SS and other microbial functions. However, disruption of cell wall-associated PTI implementation defenses also appears important in basic pathogenesis given that three of the key T3Es, HopM1, AvrE1, and HopE1, can reduce these defenses (Table 2). HopM1 targets for proteasomal destruction the Arabidopsis MIN7 ADP ribosylation factor–guanine nucleotide exchange factor protein, which is involved in vesicle trafficking (Nomura *et al*., 2006). AvrE1 does not destabilize MIN7 but appears to also disrupt vesicle trafficking (Nomura *et al*., 2006). HopE1 co-opts calmodulin and causes dissociation of MAP65 from microtubules, and Arabidopsis transgenically expressing HopE1 is reduced in secretion to the apoplast of PR-1, a marker for a variety of secreted antimicrobial proteins (Guo *et al*., 2016). Furthermore, all three of these key T3Es interfere with callose deposition.

Promoting an aqueous apoplastic environment

Transient water soaking is commonly observed before development of the necrotic and chlorotic lesions that characterize the foliar diseases caused by many *P. syringae* strains, including *Pst* DC3000. Restoration of the 28 well-expressed T3Es individually to D28E and analysis of their ability to promote water soaking in Arabidopsis under conditions of high humidity (which are associated with disease incidence in the field) identified two T3Es: HopM1 and AvrE1 (Xin *et al*., 2016). The water soaking activity of HopM1 is dependent on its ability to destroy MIN7 (discussed above). Remarkably, conditions supporting growth of D28E in Arabidopsis could be reconstituted by inoculating *min7* plants with D28E+*avrPto* or PTI-compromised *fls2 efr cerk1* plants with D28E+*hopM1*. Furthermore, *min7 fls2 efr*

cerk1 quadruple mutant plants maintained at 95% relative humidity displayed significant susceptibility to D28E (Xin *et al*., 2016).

These observations led to a new paradigm for bacterium-plant interactions, which is that an aqueous apoplastic environment and PTI suppression are necessary and sufficient conditions for basic pathogenesis (Xin *et al*., 2016). Independent support for this conclusion is found in the ability of D28E expressing just HopM1 and AvrPtoB, which targets MIN7 FLS2 CERK1/BTI9 in *N. benthamiana*, to multiply 2 logs and produce chlorotic spots in *N. benthamiana* leaves if the bacteria are COR^+ and therefore able to display chlorosis (Chakravarthy *et al*., 2017; Cunnac *et al*., 2011).

Disrupted vesicle trafficking resulting from the loss of MIN7 activity is apparently responsible for the water soaking effect of HopM1, and uninfected Arabidopsis *min7* plants show spotty water soaking at high humidity (Xin *et al*., 2016). Interestingly, AvrE1 expressed in D28E also promotes water soaking but in a MIN7-independent manner (Xin *et al*., 2016). HopAM1 also may promote apoplastic water availability, particularly during drought stress, but through the different mechanism of ABA sensitivity (Goel *et al*., 2008).

Suppressing ETI

Among the key T3Es, AvrPto, AvrPtoB, HopM1, and HopAM1 can elicit apparent ETI in resistant plants, although responses involving HopM1 and HopAM1 are not well understood (Baltrus *et al*., 2012; Iakovidis *et al*., 2016; Velasquez *et al*., 2017) (Table 2). When delivered individually by a *P. syringae* T3SS or expressed transgenically in plants, HopM1, AvrE1, and HopAM1 also have a capacity to elicit cell death in susceptible hosts, but the relationship of this response to ETI also is unclear (Cunnac *et al*., 2011; Lindeberg *et al*., 2012; Wei *et al*., 2007; Wroblewski *et al*., 2009). Two other T3Es in the complete DC3000 repertoire, HopQ1-1 and HopAD1, have the remarkable property of mutually contributing to the avirulence of wild-type DC3000 in *N. benthamiana* (that is, deleting either of these genes confers virulence) (Wei *et al*., 2007; Wei *et al*., 2015). A few additional DC3000 T3Es, such as HopAA1 and HopK1, also show some ability to elicit cell death when delivered by *P. fluorescens* expressing a *P.*

syringae T3SS into *N. benthamiana* or when transgenically expressed in diverse plant accessions (Wei *et al*., 2007; Wroblewski *et al*., 2009).

ETI processes have been extensively studied using *Pst* DC3000 derivatives deploying AvrPto or AvrPtoB in tomato accessions expressing the Pto/Prf decoy/NLR proteins or deploying effectors like AvrRpt2 that are from different *P. syringae* strains and confer NLR-dependent avirulence in appropriate Arabidopsis accessions (Cui *et al*., 2015; Jones *et al*., 2016; Khan *et al*., 2016, Martin, 2012). A cognate NLR has recently been identified for HopQ1 in *N. benthamiana* (Schultink *et al*., 2017), but none has been found in any plant for HopAD1, HopM1, AvrE1, or HopAM1. The plethora of T3Es in the DC3000 repertoire that show some capacity to elicit cell death in *N. benthamiana* suggests that ETI suppression may be an important function of native repertoires. Indeed, the majority of DC3000 T3Es have some ability to suppress ETI triggered in *N. benthamiana* by HopA1 (from *P. syringae* pv. *syringae* 61) (Guo *et al*., 2009; Jamir *et al*., 2004), and AvrPtoB has the remarkable ability to suppress ETI elicited by another domain in the same protein (Abramovitch *et al*., 2003; Martin, 2012).

AvrPtoB, in addition to its self-domain ETI suppression activity, has recently been shown to suppress ETI elicited by other effectors, such as HopAD1 (Wei *et al*., 2015). HopAD1, a weakly expressed T3E, was not eliminated in the construction of D28E and is solely responsible for the cell death caused by this strain in *N. benthamiana* at high levels of inoculum. HopAD1-dependent cell death is partially reduced when *avrPtoB* is restored to the D28E genome. The C-terminal E3 ubiquitin ligase domain of AvrPtoB is responsible for suppressing self-domain ETI in tomato accessions that express Fen, a decoy kinase that otherwise interacts with one of the two known kinase interaction domains in AvrPtoB, specifically the domain targeted by the E3 ligase (Mathieu *et al*., 2014; Rosebrock *et al*., 2007). Surprisingly, E3 ligase-deficient mutants of AvrPtoB are better able to suppress the HopAD1-dependent cell death elicited by D28E. Furthermore, an $AvrPtoB_{M3}$ mutant, which is deficient in the activity of both of the known kinase interaction domains and the E3 domain, interacted with MKK2, an immunity kinase that is required for ETI elicitation by HopAD1 and for ETI elicitation by a subset of cytoplasmic effectors from various other pathogens (Ekengren *et al*., 2003; Oh and Martin, 2011; Wei *et al*., 2015). The prevalence of an E3 domain functional polymorphism naturally occurring in AvrPtoB homologs in several other *P. syringae* pathovars suggests a mechanism for rapid adaptation to differences in ETI surveillance in different plants

and provides further evidence for the fundamental importance of ETI suppression in pathogen success (Wei *et al*., 2015).

Negotiating interactions between PTI and ETI response networks in plants

Although there is considerable overlap in PTI and ETI transcriptional profiles (Navarro *et al*., 2004; Pombo *et al*., 2014), these immune responses are distinct and can interfere with each other, as exemplified by differences in apoplastic water potentials and MIN7 stability during Arabidopsis interactions with *Pst* DC3000 variants that variously lead to PTI, susceptibility, or ETI. The rapid onset of water stress is a significant factor restricting DC3000 growth during ETI (Wright and Beattie, 2004). However, the presence of HopM1 does not interfere with the ability of DC3000 expressing various Arabidopsis-recognized avirulence T3Es derived from other *P. syringae* strains to implement effective ETI in Arabidopsis. In fact, analysis of MIN7 levels during activation of ETI by three different avirulence T3Es revealed increased levels and stability of the protein despite the presence of HopM1 (Nomura *et al*., 2011). Thus, Arabidopsis has a mechanism for protecting MIN7 from HopM1 and thereby allowing apoplastic water stress during ETI. Differences in the signaling networks for PTI and ETI contribute to immunity robustness (Tsuda and Katagiri, 2010), and Arabidopsis quadruple signaling sector mutants were recently shown to respond with ETI cell death to AvrRpt2 expressed *in planta* but not to AvrRpt2 delivered by PTI-inducing D36E (Hatsugai *et al*., 2017).

The role of apoplastic water stress in PTI is unclear. Vascular dyes show blocked vascular flow into leaf zones expressing PTI or ETI (Freeman and Beattie, 2009; Oh and Collmer, 2005). However, the use of a bacterial water potential-sensing promoter/reporter system did not indicate strong water stress during PTI, in contrast to what was observed during ETI (Wright and Beattie, 2004).

Promoting the development of bacterial colonies in the apoplast and the production of chlorotic and necrotic disease symptoms

Analogous to T3Es with multiple domains, COR contains two independently synthesized moieties: coronafacic acid (CFA) and coronamic acid (CMA) (Gross and Loper, 2009). CFA is a JA mimic, whereas COR is a mimic of the more potent JA-Ile. COR is also similar to T3Es in having multiple activities that vary in importance in different plants (Geng *et al*., 2014). As noted in Table 1 and Figure 1, a gene encoding a previously unknown step in CMA production was lost with deletion of T3E gene cluster IX in the construction of D28E (Munkvold *et al*., 2009; Worley *et al*., 2013). The reduced chlorosis linked to the cluster IX deletion led to discovery of *cmaL*, which directs production of the L-*allo*-isoleucine precursor of CMA (Worley *et al*., 2013).

D28E produces only CFA, but restoring *cmaL* to the genome enables production of COR (Chakravarthy *et al*., 2017). The construction of D28E derivatives genetically modified to produce CFA, CMA, or COR and then inoculated at low levels revealed that COR^+ D28E+2 (HopM1+AvrPtoB) is capable of producing robust chlorotic spots in *N. benthamiana* leaves from individual input bacteria that develop into individual colonies in the apoplast (visible with confocal microscopy of fluorescent protein-labeled bacteria). D28E+8 (*avrPtoB+hopM1+avrE1+hopAA1-1+hopN1+hopE1+hopG1+hopAM1*) can produce chlorotic spots in *N. benthamiana* leaves if CFA^+ but produces more chlorosis if COR^+. Interestingly, COR^+ strains of D28E+2 and D28E+8 produce similar numbers of chlorotic spots, but the size of the spots (and bacterial populations) are higher for D28E+8, suggesting that the primary impact of the additional T3Es in D28E+8 is to prolong the growth of pathogen colonies in the apoplast (Chakravarthy *et al*., 2017).

COR also can enhance T3E-dependent disease-associated necrosis (Geng *et al*., 2014). As noted above, several of the key T3Es, including AvrPtoB, HopM1, AvrE1, and HopAM1, can contribute to disease necrosis in various host plants, and the reassembly intermediates used for construction of D28E+8 highlighted the potential for HopAM1 to do this in the *N. benthamiana* pathosystem (Cunnac *et al*., 2011). Restoration of *cmaL* to D28E+8 revealed that COR strongly enhances necrosis (Chakravarthy *et al*., 2017). Necrotic lesions are a common feature of *P. syringae* diseases, and various pathovars produce diagnostic differences in these symptoms, as reflected in disease terms like speck, shot-hole, and angular leaf spot. Several *P. syringae* pathovars that are adapted for plants other than tomato (nonhost pathovars) can multiply and produce symptoms in the leaves of tomato plants that lack Pto or

Prf. Remarkably, these symptoms vary greatly and reflect those caused on natural hosts, which highlights the role of pathogen factors in controlling symptom production (Lin and Martin, 2007).

The challenge of fitting countless T3E puzzle pieces into a broader picture of plant pathogenesis

As described above and summarized in Table 2, HopM1 and AvrPtoB target multiple host proteins and defense processes, and they also act interdependently with other T3Es in the DC3000 repertoire. For example, the ability of HopM1 to promote D28E growth is redundant with AvrE1 but dependent upon AvrPtoB, but AvrPtoB acting in D28E also suppresses HopAD1-dependent cell death. Further complicating explanation of what these key T3Es may be doing are the potentially complex downstream effects of MIN7 degradation and the possibility that the extensive regions of intrinsic disorder in AvrPtoB enable interaction with yet more host proteins (Marin and Ott, 2014). Still further thwarting broader understanding of effector-mediated pathogen interactions with a given crop, such as tomato, is that significantly different repertories are used by T1-like *Pst* strains, *Xanthomonas euvesicatoria* (bacterial spot) and various other (hemi)biotrophic pathogens, such as *Phytophthora infestans* (late blight) (Haas *et al*., 2009; Lindeberg, 2012).

Given the bewildering complexity of T3E repertoires, it is surprising that just two T3Es — HopM1 and AvrPtoB — are sufficient to confer basic plant pathogenicity through their ability to promote an aqueous apoplast and suppress immunity, respectively. Thus, effector-depleted DC3000 derivatives can usefully simplify the study of T3Es and enable the interplay of T3Es and their domains to be studied at the level of key disease processes in natural systems. In this regard, it is noteworthy that the recent advances with HopM1 were guided by 'disease triangle' effects of humidity on infections and those with AvrPtoB were guided by subtle differences in tissue collapse observed in inoculated leaves (Wei *et al*., 2015; Xin *et al*., 2016).

Pst DC3000 derivatives lacking specific REGs or all T3Es already have been used to assess the virulence activity of effectors from pathogens as diverse as *Hyaloperonospora arabidopsidis* and *Pseudomonas savastanoi* pv. *savastanoi* (Table 1). We propose that the interactions of *Pst* DC3000 and possibly all (hemi)biotrophic pathogens with plants may be informatively reduced to the actions of a small

number of effector groups that act on key plant processes, that these groups will feature internal redundancy and may allow informative substitutions from repertoires of other pathogens, and that these effector groups and their targets may represent a fundamentally 'modular' interaction system that has repeatedly arisen in the evolution of diverse pathosystems involving cytoplasmic effectors.

ACKNOWLEDGEMENTS

This work is supported by United States National Science Foundation grants IOS-1025642 and IOS-1546625. We are grateful for helpful comments from Gregory B. Martin and Brian H. Kvitko. The authors have no conflicts of interest.

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FIGURE LEGENDS

Fig. 1 T3SS, T3E, flagellar, and COR genes, as arranged in the *Pst* DC3000 genome and cross-referenced with the genetic manipulations described in Table 1. The first column shows designations for the genomic clusters in which a subset of the T3E genes occur. In the second column, gene names are colored by function: well-expressed T3Es (pink), extracellular 'helper' T3SS components (light green), T3SS core components (dark green), flagellar biogenesis (blue), weakly-expressed T3Es (yellow), T3E pseudogenes (tan), COR biosynthesis (orange). The numbers in loss-of-function (LOF) column A refer to mutants described in Table 1. Additional LOF columns represent CUCPB5500 (B), D28E (C), D29E (D), and D36E (E), with shaded boxes denoting deleted genes. The numbers in the gain-of-function (GOF) column indicate genes restored to D28E, D29E, and (or) D36E as described in Table 1. Green numbers indicate that the respective T3E function was achieved directly *in planta*, through mutation of the T3E target (5) or transgenic expression of the T3E (9). Not shown are genes for T3E or helper candidates in clusters III, V, and VII or elsewhere in the genome that appear nonfunctional: *hopAS*' PSPTO_474, *hopAJ1* PSPTO_852, *hopAT*' PSPTO_5618, *hopAG1*' PSPTO_901, *hopAH1* PSPTO_905, *hopAI1* PSPTO_906, *hopAH2-1* PSPTO_3292, *hopAH2-2* PSPTO_3293, *hopAQ1* PSPTO_4703, and *hopAN1* PSPTO_5061 (Chang *et al*., 2005; Lam *et al*., 2014; Schechter *et al*., 2006). Additional information on the *Pst* DC3000 T3E repertoire is available at http://www.pseudomonas-syringae.org/.

Fig. 2 Primary virulence activities of a subset of key, nonredundant *Pst* DC3000 T3Es: AvrPtoB, HopM1, HopE1, HopG1, and HopAM1. The arrangement of the T3Es from upper left to lower right connotes the progression of pathogenic processes from blocking PTI perception to promoting lesion necrosis. AvrPtoB has three alternative primary activities depending on combinations of pathogen and host ETI factors. The arrows immediately below AvrPtoB denote the Pto and Fen kinase-interaction domains. The AvrPtoB E3 ubiquitin ligase domain '($E3^+$)' eliminates kinases at the Fen-interaction domain (Mathieu *et al*., 2014). The figure shows primary interactors (orange boxes) and pathogenesis process (green arrows) for each T3E. Additional interactors, activities, and references for all of these T3Es are presented in Table 2.

Cluster	Gene	PSPTO_	Genetic manipulations from Table 1					
			LOF: A	B	C	D	E	GOF
	hopK1	44						
	hopY1	61						
I	*hopU1*	501						
I	*hopF2*	502						
II	*hopH1*	588	13					
II	*hopC1*	589	13					
IV	*hopD1*	876	10					
IV	*hopQ1-1*	877	9,10,11,12					
IV	*HopR1*	883	10					
	hopAM1-1	1022						2,8
VI = CEL	*hopN1*	1370	6					2
VI = CEL	*hopAA1-1*	1372	6					2
VI = CEL	*hrpW1*	1373	5,6					2
VI = CEL	*hopM1*	1375	6,7					1, 2,4,5
VI = CEL	*avrE*	1377	6,7					2
	hrpH	1378						
	hrpZ1	1382	5					
	T3SS	many	4					
	hrpK1	1405	5					
	hopB1	1406						
	hopAF1	1568						9
	Flagellar	many	3					
	fliC	1949	2,12					
	hopP1	2678						
	avrPtoB	3087	8,11,12					1, 2,4,6
	avrPto	4001	8,11,12					7
	hopAK1	4101	5					
	hopE1	4331						2
VIII	*hopS2*	4588						
VIII	*hopT2*	4590						
VIII	*hopO1-3'*	4592						
VIII	*hopT1-2*	4593						
VIII	*hopO1-2*	4594						
VIII	*hopS1'*	4597						
	hopAD1	4691						
	COR	many						
IX	*hopAA1-2*	4718						
IX	*hopV1*	4720						
IX	*hopAO1*	4722						
IX	*cmaL*	4723	1					3,4
IX	*hopD'*	4724						
IX	*hopG1*	4727						2
IX	*hopH'*	5623						
IX	*hopQ1-2*	4732						
	hopI1	4776						
	hopA1	5354						
	hopBM1	5633						
X=pDC3000A	*hopAM1-2*	A0005						
X=pDC3000A	*hopX1*	A0012						
X=pDC3000A	*hopO1-1*	A0018						
X=pDC3000A	*hopT1-1*	A0019						

Figure 1

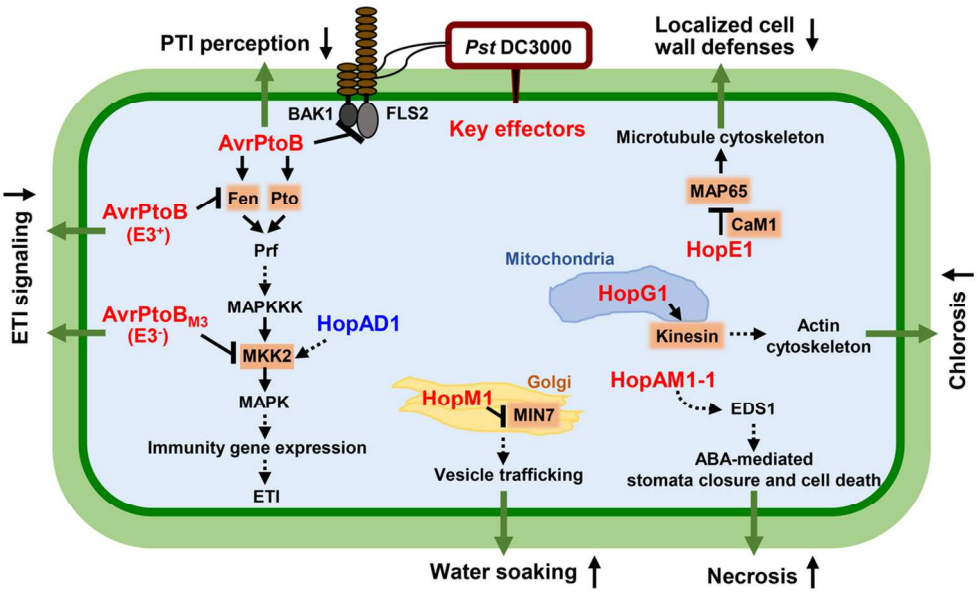

Figure 2

Table 1 Representative *Pst* DC3000 derivatives that have disassembled and reassembled T3E gene repertoires or mutations affecting COR biosynthesis, flagellum production, and T3SS secretion and translocation functions (all cross-referenced to Figure 1) and their uses in studying plant-pathogen interactions.

Genotypes and key features	Figure 1	Sources	Representative uses and lessons
Δ*cmaL* (or ΔIX T3E cluster) = COR$^-$	LOF: A 1	(Munkvold *et al*., 2009)	Does not produce coronamic acid (CMA) precursor to COR (Worley *et al*., 2013)
Δ*fliC* = no flagellin	LOF: A 2	(Kvitko *et al*., 2009)	Deficient in primary DC3000 MAMP; tool for studying PTI (Rosli *et al*., 2013; Wei *et al*., 2013)
Δ*flgGHI* = no flagellar secretion pathway	LOF: A 3	(Wei *et al*., 2013)	Flagellin cross-secreted and translocated through T3SS (Wei *et al*., 2013)
Δ*hrcQ$_B$-U* = no T3SS inner membrane complex	LOF: A 4	(Badel *et al*., 2006)	T3SS$^-$ hence no secretion of Hops or secretion of flagellin independent of the flagellar pathway (Wei *et al*., 2013)
Δ*hrpK1*Δ*hrpZ1*Δ*hrpW1*Δ*hopAK1* = no T3SS translocators	LOF: A 5	(Kvitko *et al*., 2007)	Secretion but no translocation of Hops or flagellin (Kvitko *et al*., 2007; Wei *et al*., 2013)
Δ*avrE1*Δ*hopM1*Δ*hopAA1-1*Δ*hopN1* = ΔCEL	LOF: A 6	(Alfano *et al*., 2000)	CEL = Conserved Effector Locus of Hrp pathogenicity island; reduced-virulence mutant used for studies of HopM1, AvrE1, and virulence assays of *Hyaloperonospora arabidopsidis* effectors (Baltrus *et al*., 2011; Fabro *et al*., 2011; Jin *et al*., 2016, Nomura *et al*., 2006; Ustun *et al*., 2016)
Δ*avrE1*Δ*hopM1*	LOF: A 7	(Badel *et al*., 2006)	Lacking key T3Es that redundantly promote lesions in tomato, growth in *N. benthamiana*, and water soaking in Arabidopsis (Badel *et al*., 2003; Kvitko *et al*., 2009; Xin *et al*., 2016)
Δ*avrPto*Δ*avrPtoB*	LOF: A 8	(Lin and Martin, 2005)	Widely used reduced-virulence mutant impaired in PTI suppression (Liu *et al*., 2014; Rosli *et al*., 2013)

Δ*hopQ1-1* (or ΔIV T3E cluster)	LOF: A 9	(Wei *et al*., 2007)	Deletion of avirulence determinant, thus allowing disease in *N. benthamiana* (Wei *et al*., 2007)
ΔIVΔCEL (cluster IV = *hopD1*, *hopQ1-1*, *hopR1*)	LOF: A 10	(Kvitko *et al*., 2009)	Used to define HopM1/AvrE1/HopR1 redundant effector group (REG) in *N. benthamiana* (Kvitko *et al*., 2009)
Δ*hopQ1-1*Δ*avrPto*Δ*avrPtoB*	LOF: A 11	(Kvitko *et al*., 2009)	Used to investigate AvrPto/AvrPtoB REG in *N. benthamiana* (Kvitko *et al*., 2009)
Δ*hopQ1-1*Δ*avrPto*Δ*avrPtoB*Δ*fliC*	LOF: A 12	(Kvitko *et al*., 2009)	Δ*fliC* restores growth in *N. benthamiana* to AvrPto/AvrPtoB REG mutant (Kvitko *et al*., 2009)
ΔIVΔII (*hopH1-hopC1* = T3E cluster II)	LOF: A 13	(Wei *et al*., 2007)	Strong reduction in Arabidopsis virulence accompanying ΔII suggests a HopC1/HopH1 REG (Wei *et al*., 2007)
ΔIΔIIΔIVΔCELΔIXΔX = 'ΔT3E clusters' = CUCPB5500	LOF: B	(Kvitko *et al*., 2009; Wei *et al*., 2007)	Various combinations of cluster deletions assist identification of T3Es contributing to phenotypes detectable with wild-type versus T3SS$^-$ comparisons; e.g., actin cytoskeletal changes caused by HopG1 (Shimono *et al*., 2016)
ΔT3E clusters Δ*hopI1…hopY1* = DC3000D28E = 'D28E'	LOF: C	(Cunnac *et al*., 2011)	Deficient in 28 well-expressed T3Es but has native T3SS (D28E is COR$^-$ due to loss of *cmaL* with ΔIX) (Cunnac *et al*., 2011; Worley *et al*., 2013)
D28EΔ*hopAD1* = DC3000D29E = 'D29E'	LOF: D	(Wei *et al*., 2015)	HopAD1 responsible for cell death in *N. benthamiana* elicited by D28E at high levels of inoculum but does not limit growth of D28E with low levels of inoculum (Wei *et al*., 2015)
D29EΔVIIIΔ*hopBM1* = DC300036E = 'D36E'	LOF: E	(Wei *et al*., 2015)	All known T3E genes deleted and sequence confirmed (Bao *et al*., 2014, Wei *et al*., 2015)
D28E+*hopM1*+*avrPtoB* = 'D28E+2'	GOF 1	(Cunnac *et al*., 2011)	Grows ≤2 logs better than D28E but produces no symptoms in *N.*

			benthamiana (Chakravarthy *et al*., 2017; Cunnac *et al*., 2011)
D28E+2+CEL+*hopE1+hopG1+hopAM1* = 'D28E+8'	GOF 2	(Cunnac *et al*., 2011)	Grows to within 1 log of wild type and produces symptoms in *N. benthamiana* (Chakravarthy *et al*., 2017; Cunnac *et al*., 2011)
D28E+*cmaL*	GOF 3	(Chakravarthy *et al*., 2017)	Growth in *N. benthamiana* same as D28E but produces faint chlorotic spots with dip inoculation (Chakravarthy *et al*., 2017)
D28E+2+*cmaL*	GOF 4	(Chakravarthy *et al*., 2017)	Growth in *N. benthamiana* same as D28E+2 but produces robust chlorotic spots from single bacteria and resultant colonies in 1:1:1 manner (Chakravarthy *et al*., 2017)
D28E+*hopM1* or D28E in *min7* Arabidopsis	GOF 5	(Xin *et al*., 2016)	HopM1 or mutation of its MIN7 target promote water soaking in Arabidopsis (Xin *et al*., 2016)
D28E or D29E +(*avrPtoB*$_{M3}$ or avrPtoB$_{1\text{-}307}$ or…)	GOF 6	(Wei *et al*., 2015)	An AvrPtoB mutant deficient in the three known domains strongly suppresses elicitation of cell death in *N. benthamiana* by HopAD1 (Wei *et al*., 2015)
D29E+(*avrPto* with core domain and/or C-terminal domain mutations)	GOF 7	(Worley *et al*., 2016)	Two AvrPto subdomains multiplicatively suppress PTI and affect transcriptomic changes (Worley *et al*., 2016)
D28E+(various T3E genes from *P. savastanoi* pv. *savastanoi*)	-	(Castaneda-Ojeda *et al*., 2017; Matas *et al*., 2014)	Multiple T3Es show an ability to modulate plant immune responses and increase D28E competitiveness in *N. benthamiana* (Castaneda-Ojeda *et al*., 2017; Matas *et al*., 2014)
D28E+*hopAM1*	GOF 8	(Iakovidis *et al*., 2016)	HopAM1 elicits variable cell death symptoms in Arabidopsis accessions indicative of multigenic resistance (Iakovidis *et al*., 2016)
D28E + Arabidopsis transgenic *hopAF1*	GOF 9	(Washington *et al*., 2016)	Transgenic expression of HopAF1 suppresses PTI induced by D28E

			(Washington *et al*., 2016)
D36E+(*avrRpt2* from *P. syringae* pv. *tomato* JL1065)	-	(Hatsugai *et al*., 2017)	Arabidopsis quadruple signaling sector mutants respond with ETI cell death to AvrRpt2 expressed *in planta* but not to AvrRpt2 delivered by PTI-inducing D36E (Hatsugai *et al*. 2017)

Table 2 Activities of seven key *Pst* DC3000 type III effectors (T3Es) in disease processes.

Effector	sPTI	sETI	ETI	Virulence subprocesses	Subcellular locations	Plant interactors	Biochemical activities	Cellular processes affected
HopM1	Y		Y	Apoplast water soaking↑; callose deposition↓; ROS burst↓; stomatal immunity↓; necrotic symptoms↑	Trans-Golgi network/ endosome	MIN7; MIN10/GRF8	Target MIN7 to proteasome; inhibit proteasome; disrupt GRF8 14-3-3 protein; down-regulate NHL13	Vesicle trafficking and possibly membrane stability; immune signaling
AvrE1	Y			Apoplast water soaking↑; callose deposition↓; ROS burst↓; necrotic symptoms↑	Plasma membrane and associated vesicles	Protein phosphatase PP2A	Down-regulate NHL13; interfere with PTI signaling functions of PP2A	Vesicle trafficking; immune signaling
AvrPto	Y		Y	PTI perception↓; ethylene-dependent necrotic symptoms↑	Plasma membrane	FLS2/BAK1; EFR/BKK1; Pto; Fen	Kinase inhibitor interacting with PTI PRRs and ETI decoys	Immunity-associated kinase signaling in PTI and ETI
AvrPtoB	Y	Y	Y	PTI perception↓, ETI signaling↓, ethylene-dependent necrotic	Cytosol	FLS2/BAK1; CERK1/Bti9; Pto; Fen; MKK2	Kinase inhibitor interacting with PTI PRRs, ETI decoys, and kinase	Immunity-associated kinase signaling in PTI, ETI, and sETI

				symptoms↑			cascade protein	
HopE1	Y	Y		Cell wall-based extracellular immunity↓	n.d.	CaM/MAP65	Dissociation of MAP65 from microtubules in the presence of calmodulin	Defense protein secretion
HopG1	Y			Chlorotic symptoms↑	Mitochondria	Mitochondrial-localized kinesin motor protein	Induce actin filament bundling	Actin remodeling
HopAM1			Y	Necrosis↑; osmotic stress↓; meristem chlorosis↑	n.d.	n.d.	n.d.	ABA signaling; apoplastic water status; senescence

Column headings: sPTI denotes ability to suppress PTI; sETI denotes ability to suppress ETI; ETI denotes evidence of avirulence in at least one resistant plant; for T3E processes and activities, the primary function based on interaction phenotypes is listed first. References:
HopM1 (Baltrus *et al*., 2011 and 2012; Cai *et al*., 2011; Chakravarthy *et al*., 2017; DebRoy *et al*., 2004; Lozano-Duran *et al*., 2014, Nomura *et al*., 2006 and 2011; Ustun *et al*., 2016; Xin *et al*., 2016);
AvrE1 (Degrave *et al*., 2015; Jin *et al*., 2016; Xin *et al*., 2015 and 2016);
AvrPto (Martin, 2012; Shan *et al*., 2008; Tang *et al*., 1996; Xiang *et al*., 2008);
AvrPtoB (Gimenez-Ibanez *et al*., 2009; Henry *et al*., 2017; Martin, 2012; Mathieu *et al*., 2014; Rosebrock *et al*., 2007; Shan *et al*., 2008; de Vries *et al*., 2006; Wei *et al*., 2015);
HopE1 (Guo *et al*., 2009 and 2016; Jamir *et al*., 2004);

HopG1 (Block *et al*., 2010; Mukhtar *et al*., 2011; Shimono *et al*., 2016; Ustun *et al*., 2016);
HopAM1 (Cunnac *et al*. 2011; Goel *et al*., 2008; Iakovidis *et al*., 2016; Velasquez *et al*., 2017)