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Lessons learned from type III effector transgenic plants

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The Gram negative bacterial phytopathogen *Pseudomonas syringae* employs a molecular syringe termed the type III secretion system (TTSS) to deliver an array of type III secreted effector (TTSE) proteins into plant cells. The major function ascribed to type III effectors of *P. syringae* is their ability to suppress plant immunity. Because individual pathovars of *P. syringae* can possess over 30 TTSEs, functional redundancy can provide a hurdle to ascribing functions by TTSE-deletion or -overexpression in such TTSE-rich backgrounds. Approaches to overcome functional redundancy have included the deletion of multiple TTSEs from individual pathovars as well as engineering the plant commensal *P. fluorescens* strain to express the *P. syringae* TTSS and deliver *P. syringae* TTSEs. As we describe here, transgenic Arabidopsis plants expressing individual TTSEs have also been used to overcome problems of functional redundancy and provide invaluable insights into TTSE virulence functions.

Key words: pathogen, virulence, effector, plant immunity, HopF2$_{Pto}$, RIN4

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Functional Insights from TTSE Transgenic Plants

Plant immunity can be triggered by two major classes of pathogen molecules. PAMP-triggered immunity (PTI) is induced by conserved microbial features termed pathogen/microbe associated molecular patterns (PAMPS or MAMPS). Effector-triggered immunity (ETI) induced by pathogen effector proteins is mediated by plant resistance (R) proteins and is often associated with localized cell death termed the hypersensitive response (HR).[1,2] TTSE transgenic plants have demonstrated that individual TTSEs can interfere with both branches of plant immunity.

The first Arabidopsis TTSE transgenic plants expressing AvrB or AvrRpt2 demonstrated that TTSE's can function as avirulence and virulence factors inside plant cells.[3,4,5] Since then Arabidopsis TTSE transgenic plants have provided invaluable insights into TTSE functions. In a landmark study, Hauck et al. (2003) demonstrated that transgenic expression of AvrPto in Arabidopsis can suppress callose deposition associated with PTI.[6] Furthermore, AvrPto can single-handedly promote the growth of non-virulent *Pto* DC3000 Δ*hrcC* (lacking a functional TTSS) to levels comparable to wild-type *Pto* DC3000. Since this study, numerous effectors have been demonstrated to suppress PTI when expressed transgenically, including AvrRpm1, AvrRpt2, AvrB, AvrPtoB, HopAI1, HopF2, HopAO1 and HopG1.[7-13] In addition to PTI suppression, TTSE transgenic plants have demonstrated that individual TTSEs can alter plant hormone levels and hormone sensitivity[14-16] as well as manipulate miRNA pathways.[17] A forward genetic screen was conducted on AvrB transgenic plants to identify potential targets of AvrB operation (*TAO* genes).[18] *TAO1* was mapped to a TIR-NB-LRR resistance gene that contributes to AvrB ETI in Arabidopsis.[19]

We recently investigated the ETI-suppression ability of HopF2$_{Pto}$ using transgenic plants in an attempt to provide clues about its host targets.[18] We found that transgenic HopF2$_{Pto}$ differentially inhibited the ETI-associated hypersensitive response induced by various TTSEs in Arabidopsis (ecotype Col-0). HopF2$_{Pto}$ expression compromised AvrRpt2-mediated HR but not the HR induced by AvrRpm1, AvrB or

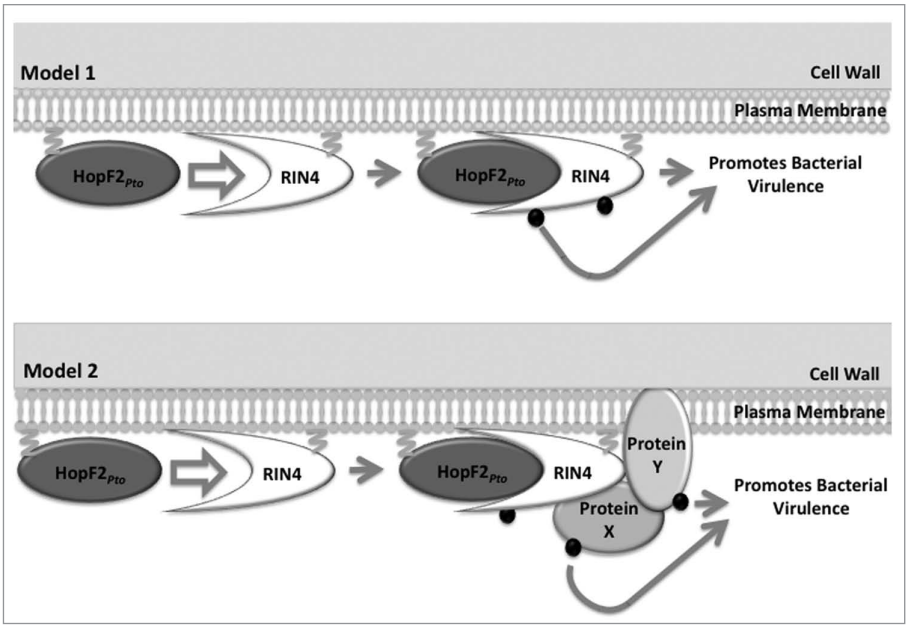

Figure 1. Two models of RIN4-dependent HopF2$_{Pto}$ action. In Model 1 HopF2$_{Pto}$ directly binds and modifies RIN4 and this RIN4-modification directly promotes bacterial virulence. In Model 2, HopF2$_{Pto}$ uses RIN4 as a scaffold to modify RIN4-associated proteins (hypothetical proteins X and Y) to promote bacterial virulence. Both RIN4 and HopF2$_{Pto}$ are membrane localized by prenylation and myristoylation, respectively.[24,26]

HopZ1a. Interestingly, HopF2$_{Pto}$ also compromised the depletion of RIN4 protein that is normally associated with AvrRpt2-HR suggesting that RIN4 could be a target of HopF2$_{Pto}$.[20,21] In support of this, HopF2$_{Pto}$ interacted with RIN4 both in vitro and in vivo, leading us to investigate whether RIN4 is a virulence target of bacterially delivered HopF2$_{Pto}$. *Pseudomonas syringae* growth in Arabidopsis was enhanced by overexpressing HopF2$_{Pto}$ in *P. syringae* pv. *tomato* DC3000 (*Pto*$_{DC3000}$) lacking endogenous HopF2$_{Pto}$. This virulence enhancement was not observed in Arabidopsis plants lacking RIN4, confirming that RIN4 is a virulence target of bacterially delivered HopF2$_{Pto}$.

The crystal structure of HopF1$_{Pph7}$ displays limited structural similarity to the catalytic domain of the ADP-ribosyltransferase diphtheria toxin.[21] Although HopF2$_{Pto}$ is predicted to adopt a similar structure, no HopF2$_{Pto}$ ADP-RT activity could be detected using RIN4 as a substrate in vitro nor from plant extracts of HopF2$_{Pto}$-expressing plants.[19] Nevertheless, a structurally predicted potential catalytic residue (D175) was required for suppression of AvrRpt2–ETI and associated RIN4 depletion, as well as HopF2$_{Pto}$-enhanced bacterial virulence indicating that HopF2$_{Pto}$ may modify RIN4 in order to promote bacterial virulence. However, the affinity of HopF2$_{Pto}^{D175A}$ for RIN4 has yet to be assessed.

Based on the aforementioned data, we propose two possible models of RIN4-dependent HopF2$_{Pto}$ action (**Fig. 1**). In the first model HopF2$_{Pto}$ binds and modifies RIN4, and this RIN4-modification directly promotes bacterial virulence. In the second model, HopF2$_{Pto}$ utilizes RIN4 as a scaffold to modify RIN4-associated proteins thereby promoting bacterial virulence. It is important to note that HopF2$_{Pto}$ may have RIN4-independent virulence targets. In support of this, HopF2$_{Pto}$-mediated PTI suppression is maintained in HopF2$_{Pto}$ transgenic plants lacking RIN4 (Wilton M and Desveaux D, unpublished).[11] However, since HopF2$_{Pto}$-enhanced *Pto*$_{DC3000}$ virulence was RIN4-dependent, these targets must be functionally redundant to those of endogenous *Pto*$_{DC3000}$ TTSEs.

ETI-Suppression in TTSE Transgenic Plants—Learning from Specificity

Our results with HopF2$_{Pto}$ emphasize the potential advantage of using TTSE-transgenics to investigate TTSE functions as well as ETI-signaling pathways. We hypothesize that ETI-suppression by TTSEs can occur by targeting three broad categories of ETI-signaling proteins: (1) R proteins or R protein monitored TTSE targets, (2) R protein signaling components that are differentially required by various R protein classes, or (3) R protein signaling components that are required by most or all R proteins (**Fig. 2**). In the first two cases, ETI-suppression will be specific to certain R protein classes and is exemplified by the AvrRpt2-ETI suppression by HopF2$_{Pto}$ and also by AvrB- and AvrRpm1-ETI suppression by AvrRpt2.[19,23,24] In the third case, ETI-suppression will be effective against a broad range of R protein classes. This may be the case for TTSEs that can suppress both ETI and Bax-induced programmed cell death.[25] Therefore, important insights into TTSE function can be gained by investigating their specificity of ETI-suppression in transgenic plants. This specificity can also potentially be used to dissect R protein signaling pathways. A continual challenge of TTSE-transgenic plant work will be to confirm that what a TTSE can do when expressed in transgenic plants is actually relevant to the function of that TTSE when delivered from the bacteria.

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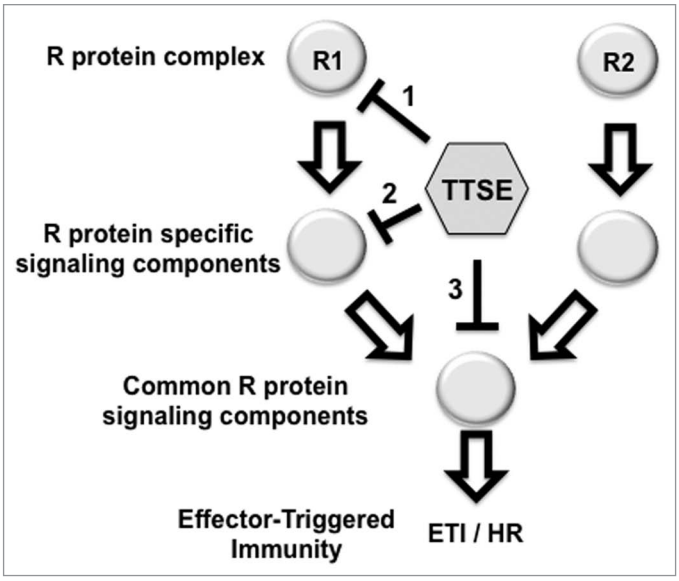

Figure 2. ETI-suppression in TTSE transgenic plants. Individual TTSE can potentially suppress ETI by targeting various components of R protein signaling pathways including: (1) R proteins or R protein-associated proteins, (2) ETI signaling components that are differentially required by R proteins, or (3) ETI signaling components required by most or all R proteins. These possible scenarios can be investigated using TTSE transgenic plants in order gain insight into TTSE functions as well as R protein signaling pathways.

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