

MicroCommentary

Domain switching and host recognition

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Summary

The collective function of secreted pathogen effector molecules is to enhance the virulence and avirulence activity of the pathogen during the infection of its host. While the activity of a majority of pathogen effectors is unknown, several classes of effector molecules have been well characterized. Among these include proteins which function to modulate host defences either through proteolysis, post-translational modifications, or by directly manipulating the host transcriptional machinery that regulates the induction of defence responses. In recent years, several key advances have been made in the characterization of the latter class of effector molecules. Among these include research characterizing the processes associated with host nuclear import and the targeting of host transcriptional defences. While current research is beginning to reveal the biochemical and genetic mechanisms controlling the induction of host resistance, the signalling events that control host specificity remain largely unknown. In this issue of *Molecular Microbiology*, work by Nissan *et al.* sheds light onto the molecular-genetic patterns involved in determining host specificity and pathogen virulence in the *Pantoea*–*gypsophila* interaction.

Plant pathogens deliver a diverse array of effector proteins into the plant cell during the infection process (Chisholm *et al.*, 2006). Most Gram-negative bacterial pathogens require the function of a specialized, multiprotein conduit known as the type III secretion system (T3SS) to deliver effector proteins into the host cell (He and Jin, 2003). Conversely, plant pathogenic fungi and oomycetes transport secreted proteins through unknown mechanisms (Birch *et al.*, 2006). Once inside the target cell, these effector proteins play dual roles as both virulence

and avirulence factors, fulfilling a variety of functions related to both pathology and pathogen fitness (Alfano and Collmer, 2004). While the activity of most pathogen effector proteins is unknown, it is believed that the majority of them disable host basal defences and/or release nutrients from the cell, rendering the host susceptible to pathogen proliferation and ultimately, death.

Recent work has characterized the activity of a number of pathogen effector molecules (Alfano and Collmer, 2004). Within the broad class of pathogen effectors that have been identified from bacteria, fungi, oomycetes and nematodes, several are targeted to the plant nucleus, where they appear modulate host gene transcription to disable host defences (Fig. 1; Szurek *et al.*, 2001; Yang and White, 2004; Gu *et al.*, 2005). The largest class of effector proteins from bacterial plant pathogens is represented by the AvrBs3/pthA family, found in many strains of *Xanthomonas* species (Yang and White, 2004). While the mechanisms of the AvrBs3/pthA effector family action are largely unknown, several key structural features indicate that they are potential transcription factors that regulate the avirulence and virulence functions of the pathogen. First, all members of the AvrBs3/pthA family contain a functional nuclear localization signal (NLS) motif that is followed by an acidic transcriptional activation domain (AD) within the C-terminal region (Zhu *et al.*, 1998; Yang *et al.*, 2000). Second, there is emerging evidence that, while the NLS and AD are critical for both virulence and avirulence, a central repetitive region within the protein regulates host specificity (Yang *et al.*, 2000; Yang and White, 2004; Gu *et al.*, 2005). Experimental evidence for this phenomenon came from a number of studies, including a domain swapping experiment involving AvrXa10 and AvrXa27, two effector proteins within the AvrBs3/pthA family from *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial blight of rice. Replacement of the repeat coding region in *avrXa10* with that of *avrXa27* converts *avrXa10* to a gene with avirulence specificity for Xa27, resulting in a loss of activity towards Xa10 (Gu *et al.*, 2005). Similar studies have demonstrated that exchanging the repeat domains of *avr6* from *Xanthomonas campestris* pv. *malvacearum* and *pthA* from *Xanthomonas citri* alters the virulence of the bacterium that produces them on the respective hosts, demonstrating that specificity for virulence is also controlled by the repeat domain (Yang *et al.*, 1994).

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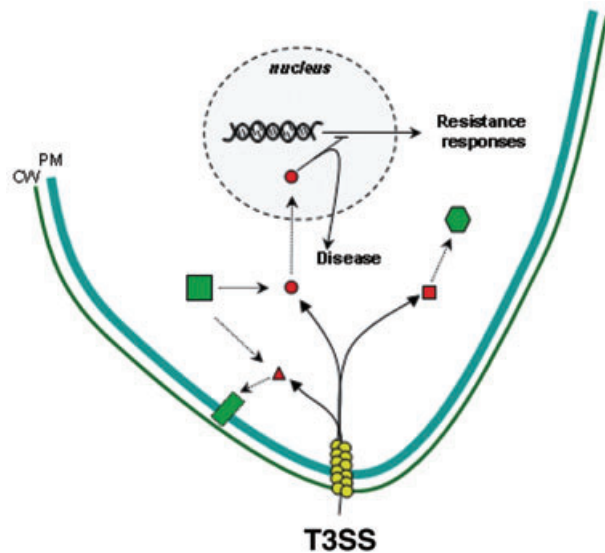


Fig. 1. Schematic overview of the type III secretion, translocation and mode of action of bacterial pathogen effector molecules. More than 50 validated and potential T3SS effector molecules that are delivered into the plant cell during pathogen infection have been identified. Once delivered into the host cell, pathogen effectors disarm host defence mechanisms by interfering with their ability to initiate a defence response. Among the various activities described for pathogen effectors, a subset is potential transcriptional activators. Among them are, AvrBs3, AvrXa7 and AvrXa27, as well as HsvG and HsvB that are described by Nissan *et al.* in this issue of *Molecular Microbiology*. CW, cell wall; PM, plasma membrane; T3SS, type III secretion system. Pathogen effectors are indicated in red. Host proteins are indicated in green.

In this issue of *Molecular Microbiology*, Nissan *et al.* describe two effector proteins, HsvG and HsvB, from *Pantoea agglomerans* that function as pathogenicity factors. Interestingly, the activity of HsvG and HsvB not only dictate pathogen–host specificity but also likely function as transcription activators. HsvG and HsvB represent a novel type of effector proteins that differ from the AvrBs3/pthA family. HsvG and HsvB contain five helix–turn–helix motifs, indicative of potential DNA-binding properties, followed by one or two direct amino acid repeats. As shown by Nissan *et al.* these amino acid repeats determine host specificity. Previous work from the Barash laboratory has shown that HsvG is a virulence factor that determines the host specificity of *Pantoea agglomerans* pathovars *gypsophilae* (*Pag*) and *betae* (*Pab*) on gypsophila (Valinsky *et al.*, 1998). In the study by Nissan *et al.* the authors identified an HsvG homologue, HsvB, which determines host specificity of *Pag* and *Pab* on beet. While HsvG requires two direct amino acid repeats for pathogenicity on gypsophila, one repeat in HsvB is sufficient for pathogenicity on beet. Interestingly, exchanging repeats between HsvG-*Pag* and HsvB-*Pab* caused a host specificity switch. The work presented by Nissan *et al.* in this issue clearly

demonstrates that either of the repeats is capable of activating the expression of the reporter gene in the yeast one-hybrid experiment. The transcription activation domain also resides within this amino acid repeat region. It is this feature of HsvG that distinguishes it from the AvrBs3/PthA family of effectors, in which the acidic activation domain is separated from the amino acid repeat region (Yang *et al.*, 2000). Moreover, the amino acid repeat regions of HsvG and HsvB are dramatically different from that of AvrBs3/PthA effectors. In the case of Hsv-*Pag*, this region includes two-tandem direct repeats, R1 (74 amino acids) and RII (71 amino acids) that are 88% identical. Interestingly, the R1 repeat is truncated in Hsv-*Pag* and completely absent from Hsv-*Pab*. In contrast to HsvG/HsvB, the repetitive region of the AvrBs3/PthA family of effectors consists of a variable number of near-identical direct repeats of 34 amino acids (Yang *et al.*, 2000; Yang and White, 2004). In addition, unlike the AvrBs3/PthA family of effectors, HsvG and HsvB do not have a known or predictable NLS.

Previous work has demonstrated that members of the AvrBs3/pthA family of effectors are potential nuclear-localized transcriptional activators. Nissan *et al.* used green fluorescent protein-tagging to demonstrate that HsvG and HsvB are also nuclear-localized. This finding, together with the yeast one-hybrid data, strengthens the argument that *Pab* and *Pag* might use HsvB to target the plant transcriptional machinery, thereby manipulating host defences. However, the potential host targets of HsvG and HsvB have not yet been identified. Based on previous work in this area, the authors hypothesize that HsvG and HsvB might modulate the signalling networks associated with host phytohormones that control gall formation. In support of this hypothesis, previous work has demonstrated the simultaneous inactivation of the pathways for biosynthesis of indole acetic acid (IAA) and cytokinins in *Pag*, leading to a significant reduction in gall size (Manulis *et al.*, 1998). Interestingly, however, T3SS mutants of *Pag* or *Pab* completely eliminate gall formation (Nizan *et al.*, 1997; Nizan-Koren *et al.*, 2003). These findings seem to indicate that *P. agglomerans* not only uses its own hormone synthesis machinery to promote gall development but also, more importantly, utilizes effector proteins to target the transcription of host genes involved in hormone production. The complexity of the molecular, genetic and biochemical events required for resistance signalling in plants is further complicated by the interplay between numerous, converging signalling pathways, such as hormone biosynthesis. Clearly, the next steps forward in this area of research will be to define the specific activity of the pathogen effector molecules and, as in the case of the work described by Nissan *et al.* to identify the specific genetic elements targeted, and regulated, by the effector proteins.

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