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Microreview

Disabling surveillance: bacterial type III secretion system effectors that suppress innate immunity

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Summary

Many Gram-negative bacterial pathogens of plants and animals are dependent on a type III protein secretion system (TTSS). TTSSs translocate effector proteins into host cells and are capable of modifying signal transduction pathways. The innate immune system of eukaryotes detects the presence of pathogens using specific pathogen recognition receptors (PRRs). Plant PRRs include the FLS2 receptor kinase and resistance proteins. Animal PRRs include Toll-like receptors and nucleotide-binding oligomerization domain proteins. PRRs initiate signal transduction pathways that include mitogen-activated protein kinase (MAPK) cascades that activate defence-related transcription factors. This results in induction of proinflammatory cytokines in animals, and hallmarks of defence in plants including the hypersensitive response, callose deposition and the production of pathogenesis-related proteins. Several type III effectors from animal and plant pathogens have evolved to counteract innate immunity. For example, the *Yersinia* YopJ/P cysteine protease and the *Pseudomonas syringae* HopPtoD2 protein tyrosine phosphatase inhibits defence-related MAPK kinase activity in animals and plants respectively. Thus, type III effectors can suppress signal transduction pathways activated by PRR surveillance systems. Understanding targets and activities of type III effectors will reveal much about bacterial pathogenicity and the innate immune system in plants and animals.

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Introduction

A number of Gram-negative bacterial plant and animal pathogens depend on type III secretion systems (TTSSs) to inject virulence effector proteins into host cells (Hueck, 1998; Galán and Collmer, 1999). These pathogens include the animal pathogens *Yersinia* spp., *Salmonella typhimurium*, *Shigella flexneri*, *Escherichia coli* and *Pseudomonas aeruginosa* and the plant pathogens *Pseudomonas syringae*, *Erwinia amylovora*, *Xanthomonas campestris* and *Ralstonia solanacearum*. The diseases they cause run the whole gamut from Black Death in humans (i.e. the bubonic plague) caused by *Yersinia pestis* to fire blight of pear and apple caused by *E. amylovora* (Alfano and Collmer, 1996; Cornelis, 2000). Moreover, TTSSs have also been recently identified in insect pathogens and eukaryotic-associated bacteria (Dale *et al*., 2001; Marie *et al*., 2001). Certain animal pathogens have two distinct TTSSs (three if the flagellar TTSS is included), whereas plant pathogens, excluding the flagellar TTSS, appear to have one (Shea *et al*., 1996; Haller *et al*., 2000).

The TTSSs and the proteins they secrete have been given a variety of names depending on the species of origin. For example, the well-characterized TTSS from *Yersinia* spp. is called the Ysc system (Yop secretion) and the effector proteins it secretes are Yops (*Yersinia* outer protein). In plant pathogens the TTSS is called the Hrp (hypersensitive response and pathogenicity) system because the original mutants lost the ability to elicit the hypersensitive response (HR) (Lam *et al*., 2001), a programmed cell death (PCD) associated with plant defence, as well as their pathogenic ability. We now know these original mutants that displayed Hrp phenotypes were defective in components of the Hrp TTSS apparatus (Alfano and Collmer, 1997). The proteins secreted by Hrp TTSSs have been given a variety of names including Hop (for Hrp outer protein), Xop (*Xanthomonas* outer protein), Pop (*Pseudomonas* outer protein, which actually are *R. solanacearum* proteins based on its earlier genus name) and Avr (for avirulence) because several of these were originally identified based on their property of limiting the host range of the

pathogen (Alfano and Collmer, 1997; Bonas and Lahaye, 2002).

The activities of many of the TTSS effectors have been well characterized in animal pathogens where the majority of them co-opt the host cytoskeleton by either inhibiting or promoting actin polymerization to block or induce phagocytosis by host cells (Cornelis, 2002a; Cossart and Sansonetti, 2004). The TTSS effectors accomplish this by modulating the signal transduction pathways controlling actin polymerization in host cells. There are excellent reviews describing the activities of animal type III effectors (Cornelis, 2002b; Holden, 2002; Zaharik *et al*., 2002). Plant pathogen effectors are less understood. However, this outlook has improved with the recent identification of many effectors from plant pathogens (mostly from *P. syringae*), providing additional insights into their potential activities (Collmer *et al*., 2002; Guttman *et al*., 2002; Petnicki-Ocwieja *et al*., 2002; Greenberg and Vinatzer, 2003; Chang *et al*., 2004). The large increase in the effector inventory has been spurred by genomic investigations and similar studies on other TTSS-containing plant pathogens suggest that the effector list for these microbes will be considerable as well (Salanoubat *et al*., 2002; da Silva *et al*., 2002; Buttner *et al*., 2003). What are these effectors doing inside the plant cell to favour pathogenesis? As we describe below several *P. syringae* effectors have been shown to suppress plant defence responses. Interestingly, several effectors from animal pathogens also suppress the innate immune system in mammals. Thus, an emerging role of type III effectors appears to be suppression of eukaryotic innate immunity.

One of the primary goals of this review is to highlight the plant pathogen effectors that suppress plant defences and compare them with animal pathogen effectors that act in similar ways. Plant defences have been categorized differently based on how the resistance is manifested and a brief introduction to some of these terms is warranted. Induced defences that successfully stop non-pathogenic bacteria (along with premade defences, which are not discussed here) from growing in the intercellular spaces of plants have been referred to as basal defences, localized induced resistance or innate immunity (Nurnberger *et al*., 2004). In addition, plants can protect themselves against pathogens that cause diseases in other plant species, referred to as non-host resistance (Heath, 2000; Thordal-Christensen, 2003). Finally, specific cultivars or lines of a given plant species can be resistant to specific races or strains of a pathogenic microorganism. These defences are referred to as cultivar-specific or gene-for-gene resistance because they result from the presence of Avr signals in the pathogen, which are recognized by resistance (R) proteins in the host plant (Flor, 1971; Keen, 1990). As described below, most of the bacterial Avr signals are type III effectors, which are recognized inside the plant cell by R protein-mediated defence pathways. Here, we will use the terms basal resistance, non-host resistance and cultivar-specific resistance to refer to the different types of plant resistances. Collectively, we will refer to them as the plant innate immune system because they seem to be different layers of the same defence system and have similarities to innate immunity in animals.

The innate immune systems of plants, mammals and insects are dependent on a hardwired sensor-based surveillance system that recognizes indicators of infection by microorganisms (Imler and Hoffmann, 2001; Nurnberger *et al*., 2004). Thus, to succeed as pathogens bacteria must first disable these defences before they gain access to their biochemical loot – the nutrients available in the host. This review will focus primarily on the recent findings that indicate plant pathogens use TTSS effectors to circumvent plant innate immunity. Because TTSS effectors from animal pathogens are also capable of suppressing innate immunity, we will compare these effectors with their plant pathogen counterparts. First, we introduce some of the salient features of the innate immune systems in both plants and animals that constitute the 'alarm' sensors that trigger host defence responses against bacteria.

Tripping the switch: pathogen-associated molecular patterns (PAMPs), general elicitors and Avr proteins activate innate immunity

Plants induce basal defence responses upon sensing conserved molecules produced by microorganisms (Boller, 1995). These include chitin (Felix *et al*., 1993) and ergosterol (Granado *et al*., 1995) from fungi and lipopolysaccharide (LPS) (Dow *et al*., 2000), cold shock protein (Felix and Boller, 2003), and flagellin from bacteria (Gomez-Gomez and Boller, 2002). These molecules have been referred to as general elicitors, but they are conceptually equivalent to pathogen-associated molecular patterns (PAMPs), molecules present in microbes that are recognized by the innate immune system in animals (Boller, 1995; Medzhitov and Janeway, 1997). PAMPs are recognized in animals by specific host pattern recognition receptors (PRRs), which include the Toll-like receptors (TLRs) and nucleotide binding oligomerization domain (NOD) proteins (Medzhitov, 2001; Inohara and Nunez, 2003). TLRs generally sense extracellular PAMPs, whereas NODs recognize PAMPS intracellularly (Girardin *et al*., 2002).

A similar dichotomy is seen with plant recognition proteins. A plant receptor-like kinase (RLK), FLS2, recognizes bacterial flagellin from the outside of plant cells, whereas R proteins recognize bacterial Avr proteins intracellularly. The similarities between these proteins and animal TLRs and NODs have been described (Dangl and Jones, 2001; Medzhitov, 2001; Nurnberger and Brunner,

2002; Inohara and Nunez, 2003; Jones and Takemoto, 2004; Nurnberger *et al*., 2004) and here, we group plant proteins that sense either PAMPs or Avr proteins together with animal proteins that sense PAMPs as PRRs (Fig. 1). FLS2 is a plant PRR that recognizes flagellin PAMP (Gomez-Gomez and Boller, 2000). Bacterial molecules other than flagellin induce similar basal defences, suggesting that other plant PRRs might recognize additional bacterial PAMPs. Moreover, bacterial extracts without elicitor-active flagellin induce basal resistance in *Arabidopsis* implying that plants can recognize other bacterial PAMPs (Zipfel *et al*., 2004). *Arabidopsis* possesses 173 RLKs that contain predicted extracellular domains and 10 candidate RLKs are grouped phylogenetically with FLS2 (Shiu and Bleecker, 2003). Thus, at least a subset of these may encode PRRs. However, RLKs also play a prominent role in plant development and hormone perception (Torii, 2000).

FLS2 has a predicted extracellular leucine-rich repeat (LRR) domain which is probably involved in protein–protein interactions (Kobe and Kajava, 2001) (see Fig. 1). LRR domains are present in plant R proteins as well as in Toll, TLRs and NOD proteins from insects and mammals. Another key domain of FLS2 is a Ser/Thr kinase domain, which transduces the PAMP signal to the cytoplasm (Gomez-Gomez *et al*., 2001). Similar to FLS2, the rice R protein Xa21 possesses an extracellular LRR domain and a cytoplasmic Ser/Thr kinase domain (Fig. 1) (Song *et al*., 1995). Xa21 confers resistance from the bacterial plant pathogen *Xanthomonas oryzae* pv. *oryzae* race 6. This is interesting because known bacterial Avr proteins are recognized inside plant cells by cytoplasmic R proteins. While the Avr determinant, AvrXa21, which is recognized by Xa21 has yet to be identified, *X. o. oryzae* mutants lacking AvrXa21 activity are defective in sulphur assimilation hinting that Xa21 recognizes a sulphated molecule (Shen *et al*., 2002). Moreover, *X. o. oryzae* genes required for AvrXa21 avirulence are similar to *Sinorhizobium meliloti* nodulation genes needed for sulphate decoration of carbohydrate Nod factors. Legume RLKs involved in recognition of rhizobia Nod factors have been identified (Limpens *et al*., 2003; Madsen *et al*., 2003; Radutoiu *et al*., 2003) and they possess a cytoplasmic kinase domain similar to FLS2 and Xa21 (Fig. 1). However, instead of LRR domains they have LysM domains, which are thought to interact with carbohydrates (Spaink, 2004). The similarities between FLS2 and Xa21 suggest AvrXa21 may be more similar to a PAMP than an Avr protein, which blurs the distinction between what constitutes a PAMP and an Avr.

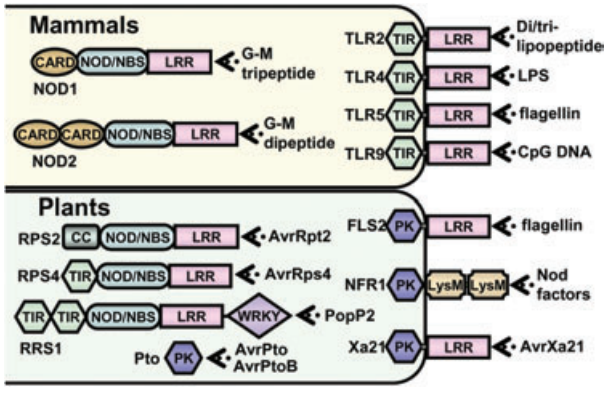

Fig. 1. Representative mammalian and plant pattern recognition receptors that recognize bacterial molecules. In mammals, TLR2 recognizes several different microbial PAMPs including diacyl and triacyl lipopeptides with the assistance of TLR1 and TLR6, respectively, which are not shown (Takeda and Akira, 2003). TLR4 recognizes the lipopolysaccaride component of the outer membrane of Gram-negative bacteria in conjunction with CD14 (not shown). TLR5 recognizes bacterial flagellin and TLR9 recognizes bacterial CpG DNA. NOD1 and NOD2 both recognize components of peptidoglycan. NOD1 recognizes *N*-acetylglucosamine-*N*-acetylmuramic acid (G-M) with a tripeptide containing mesodiaminopimelate as the terminal amino acid, which is found mainly in Gram-negative bacteria (Chamaillard et al., 2003). NOD2 recognizes G-M dipeptide, which is found in all bacteria containing peptidoglycan. In plants, FLS2 recognizes flagellin, receptor kinases such as NFR1 recognize carbohydrate NOD factors from rhizobia, and the Xa21 receptor kinase recognizes AvrXa21, an undefined Avr signal. Representative R proteins that recognize different Avr proteins are shown (see text for details). LPS, lipopolysaccharide; LRR, leucine-rich repeats; TIR, toll and IL-receptor; PK, serine/threonine protein kinase; LysM, lysin motif; NOD/NBS, nucleotide-binding oligomerization domain/nucleotide-binding site; CARD, amino terminal caspase recruitment effector domain; CC, coiled-coil; WRKY, tryptophan-arginine-lysine-tyrosine domain. This figure was modified from Jones and Takemoto (2004) and Takeda and Akira (2003).

Plant R proteins that perceive bacterial Avr proteins can be categorized into four different classes (Fig. 1). The largest, referred to as CC-NBS-LRR R proteins, have a N-terminal coiled-coil (CC) domain, a central nucleotide binding site (NBS) domain (equivalent to NOD domains in animal PRRs) and a C-terminal LRR domain (Dangl and Jones, 2001; Jones and Takemoto, 2004). A second class, TIR-NBS-LRR, possesses a different N-terminal domain, TIR (for Toll and IL-1 receptor) instead of a CC domain. Only one member of this class is known to recognize a bacterial Avr, AvrRps4 (Gassmann *et al*., 1999), and several other members recognize fungal and viral Avr proteins (Nimchuk *et al*., 2003). A third type of NBS-LRR R protein, RRS1, is a TIR-NBS-LRR R protein that has a C-terminal tryptophan-arginine-lysine-tyrosine (WRKY) domain. WRKY domains are predicted to act as transcription factors (Eulgem *et al*., 2000; Deslandes *et al*., 2002). Another R protein is the tomato Pto protein, which is a Ser/Thr protein kinase required for the recognition of the *P. syringae* Avr proteins AvrPto and AvrPtoB (Pedley and Martin, 2003). Pto is not an NBS-LRR type R protein. However, Pto is dependent on Prf, a CC-NBS-LRR type R protein, for recognition of Avr proteins. Thus, Pto is not a 'classic' PRR like FLS2 and NBS-LRR R

proteins. Thus far, all the R proteins that recognize identified bacterial Avr proteins are located inside the plant cell with several of them localized to the plant plasma membrane (Boyes *et al*., 1998; Nimchuk *et al*., 2001). RRS1 is unusual because it becomes nuclear-localized only in the presence of its cognate Avr protein, PopP2, which contains nuclear localization signals (Deslandes *et al*., 2003). The long standing model for how R proteins recognize their cognate Avr proteins is by directly interacting with them (Gabriel and Rolfe, 1990). However, recent evidence, at least for specific bacterial Avr proteins, supports another model called the guard hypothesis, in which R proteins sense an Avr protein indirectly by detecting its enzymatic activity (Schneider, 2002; Innes, 2004). Because the activities of the majority of plant pathogen effectors are unknown, it remains possible that some are not enzymes. Thus, some bacterial Avr proteins may still be recognized directly by their cognate R proteins.

Letting out the dogs: outputs of innate immunity

Signal transduction pathways are triggered once animal or plant PRRs perceive PAMPs or Avr proteins and defence-related outputs are deployed. The signal transduction components acting downstream of Avr protein recognition have been recently reviewed (Martin *et al*., 2003; Nimchuk *et al*., 2003). Many of these are dependent on salicylic acid (SA) and are also important in systemic acquired resistance (SAR) (Durrant and Dong, 2004), a form of resistance that is triggered in plants away from the local site of infection. The emerging picture is that a complex signal network is triggered upon perception of an Avr protein. One distinction that can be made is that CC-NBS-LRR R proteins are dependent on NDR1 (Century *et al*., 1997), whereas TIR-NBS-LRR R proteins are dependent on EDS1 (Aarts *et al*., 1998; Falk *et al*., 1999).

Another plant protein known to play a prominent role in R protein-mediated defence pathways is NPR1/NIM1, which was first identified for its role in SAR (Cao *et al*., 1994; Delaney *et al*., 1995). NPR1 interacts with several members of the TGA family of basic leucine zipper transcription factors, is nuclear-localized when activated and is a positive regulator of defence genes (Durrant and Dong, 2004). NPR1 shares some similarity with IκB, a negative regulator of the NF-κB transcription factor utilized in signal transduction pathways in animals (Ryals *et al*., 1997). The key role that NPR1 plays in local resistance is highlighted by the fact that overexpression of NPR1 in *Arabidopsis* enhances resistance to *P. syringae*, the oomycete *Peronospora parasitica*, and fungus *Erysiphe cichoracearum* (Friedrich *et al*. 2001; Cao *et al*., 1998) and in rice conferred enhanced resistance to *X. o. oryzae* (Chern *et al*., 2001). As NPR1 activation requires SA, this emphasizes the importance of SA-dependent defences at infection sites.

Components of signal transduction pathways operating downstream of PAMP perception in both plants and animals are not well understood. However, there are common features shared by both, which have been noted (Cohn *et al*., 2001; Nurnberger and Brunner, 2002; Holt *et al*., 2003; Nurnberger *et al*., 2004). For example, mitogen-activated protein kinase (MAPK) cascades are activated in plants and animals after PAMP perception (Nurnberger *et al*., 2004). In both hosts, transcription factors are activated after activation of MAPK – the NF-κB transcription factor in animals and WRKY transcription factors in plants are good examples (Lee *et al*., 1997; Asai *et al*., 2002; Kim and Zhang, 2004; Zipfel *et al*., 2004). In plants, MAPK pathways appear to be points of convergence between multiple elicitors of plant defence including PAMPs and Avr proteins. For example in tobacco, the MAPKs SIPK (salicylate-inducible protein kinase) and WIPK (wound-inducible protein kinase) are activated by fungal, viral and bacterial elicitors (Zhang and Klessig, 2001). Silencing of a tobacco MAPK kinase kinase (MAPKKK), NPK1, stops the plant from responding to several Avr proteins, including the *X. c. vesicatoria* Avr protein AvrBs2, consistent with a requirement of MAPK cascades in these R protein-mediated responses (Jin *et al*., 2002). A MAPKKK that acts upstream of SIPK in *Nicotiana benthamiana* and tomato, MAPKKKα, has recently been identified. This MAPKKK is triggered by different elicitors including AvrPto (del Pozo *et al*., 2004). A complete MAPK cascade [MKK1 (MAPKKK), MKK4/5 (MAPK kinases) and MPK3/6 (MAPKs)] has been identified in *Arabidopsis*. Among these, MPK6, a SIPK MAPK orthologue, is activated by the flagellin PAMP (Asai *et al*., 2002; Nuhse *et al*., 2000). Interestingly, this MAPK cascade results in the activation of several WRKY transcription factors and at least one of these factors protects *Arabidopsis* from fungal and bacterial infections (Asai *et al*., 2002; Navarro *et al*., 2004; Zipfel *et al*., 2004).

There is much overlap between the outputs of both PAMP-triggered and Avr-triggered signal transduction pathways in plants. Some of these physiological responses include generation of reactive oxygen species (ROS) and nitric oxide (NO), changes in cytoplasmic Ca^{2+} levels, production of pathogen-related gene expression, phytoalexin production and callose deposition in the cell wall (Heath, 2000; Dangl and Jones, 2001; Gomez-Gomez and Boller, 2002; Jones and Takemoto, 2004). Among the more dramatic defence responses triggered is the aforementioned HR, more frequently associated with non-host and cultivar-specific resistance than with basal resistance. Even though we know many of the outputs of a successful defence response, it remains unclear which outputs are effective against bacteria. The failure of

PAMPs to trigger an HR may be more of a threshold effect than differences in signal transduction as most of the other responses are shared between cultivar-specific and basal resistances. Supporting this, the non-specific oxidative burst triggered by both saprophytes and pathogens is less substantial than the one that is specifically induced by the presence of Avr proteins (Baker and Orlandi, 1995).

Clearly, a better understanding of the differences in R protein pathways and PAMP-triggered pathways is needed. Currently, one way to separate plant defence outputs is to test whether the output in question is dependent on SA. This can be easily done by determining whether the response is produced in *Arabidopsis* mutant plants impaired in SA biosynthesis (Wildermuth *et al*., 2001). Alternatively, *nahG* plants could be utilized to test whether a plant response is dependent on SA. These plants carry the *P. putida nahG* gene that encodes salicylate hydroxylase (Gaffney *et al*., 1993), an enzyme that degrades SA, which is generally required for R protein-dependent defences (Dangl and Jones, 2001; Bonas and Lahaye, 2002). Nevertheless, experiments using *nahG* plants should be interpreted with caution because a recent report suggests that the loss of non-host resistance to *P. syringae* by *nahG*-containing *Arabidopsis* plants results from SA degradation products and not from the inhibition of SA-dependent defences (van Wees and Glazebrook, 2003).

In animals, PAMPs are recognized by TLRs and NOD PRRs, which activate signalling pathways that induce innate proinflammatory responses (Barton and Medzhitov, 2003; Inohara and Nunez, 2003). Several of these responses, once over a threshold, will activate the adaptive immune system in animals. Identifying signal transduction components downstream of animal PRRs is an active area of research (Barton and Medzhitov, 2004). All known TLRs utilize MyD88 TIR-domain adaptor proteins. Recently, certain TLRs have been shown to utilize other TIR-domain-containing adaptor proteins called Trif (or TICAM-1) (Yamamoto *et al*., 2003) and TIRAP (Horng *et al*., 2002). MyD88 recruits members of the IL-1 receptor-associated kinases IRAK-1 and IRAK-4 (Takeda *et al*., 2003). The plant R protein Pto shares some similarities with IRAKs and Pelle, a kinase involved in innate immunity in *Drosophilia* highlighting another similarity in plant and animal innate immunity (Cohn *et al*., 2001). After IRAK is activated by phosphorylation, it associates with TRAF6, an E3 ligase, which in turn activates MAP kinases. This kinase cascade results in the activation of both the Ap-1 and NF-κB transcription factors, which activate a large number of proinflammatory genes (Pasare and Medzhitov, 2003; Takeda *et al*., 2003).

Signalling pathways acting downstream of NOD PRRs are less understood than TLRs probably because they have only recently been associated with PAMP perception. However, activation of both NOD1 and NOD2 results in NF-κB and caspase activation (Inohara and Nunez, 2003). In addition, both also associate with RICK (also known as RIP2), a CARD-containing protein kinase, which in turn associates with NEMO, a regulatory subunit of the IKK complex (Carneiro *et al*., 2004). Ultimately, triggering NOD1 and NOD2 results in the secretion of cytokines (e.g. IL-8) from antigen-presenting cells and the expression of other costimulatory molecules that activate antigen-specific effector T cells of the adaptive immune system (Inohara and Nunez, 2003).

A myriad of responses occur after triggering either animal or plant PRRs. The identification of these responses has been greatly facilitated by mRNA expression profiling after exposure to a PAMP or pathogen. For example, Tao *et al*. (2003) profiled the changes in mRNA expression in *Arabidopsis* infected with a virulent *P. syringae* strain versus *P. syringae* strains containing Avr proteins recognized by R protein PRRs present in the test plant. The presence of specific Avr proteins in *P. syringae* (i.e. making *P. syringae* avirulent) produced a similar mRNA pattern as the virulent pathogen except that the expression of defence genes was earlier and increased. This supports the hypothesis that bacterial plant pathogens have strategies to successfully delay and/or suppress defence responses. Recent gene profiling experiments found that many genes are activated upon challenging *Arabidopsis* plants with flagellin (Navarro *et al*., 2004; Zipfel *et al*., 2004). These experiments revealed important observations and new directions for experimentation. One particularly interesting result is that the recognition of flagellin by FLS2 induces the expression of many other RLKs suggesting that recognition of the flagellin PAMP potentiates the plant to recognize other PAMPs. Studies such as these should continue to reveal the global changes that occur upon PAMP recognition.

Disabling surveillance: type III effectors that act as suppressors of innate immunity

Given the elaborate surveillance strategies plants and animals use to defend against microorganisms, it is not surprising that microorganisms have countered with their own strategies to thwart their hosts. It has become increasingly clear within the last couple of years that plant pathogen type III effectors suppress plant defence responses (Alfano and Collmer, 2004). Indeed, there were clues in the early 1990s that the TTSSs of plant pathogens were capable of suppressing plant defences. For example, *X. c.* pv. *campestris hrp* mutants induced an HR-like response in the vascular system of crucifers (called the vascular HR), which the compatible wild-type strain did not elicit, suggesting that wild-type strains suppressed the vascular HR (Kamoun *et al*., 1992). In pioneering

research, Jakobek *et al*. (1993) showed that a compatible *P. syringae* pathovar, *P. s.* pv. *phaseolicola*, suppressed induction of defence-related mRNA and phytoalexins in bean that were separately induced by an incompatible *P. syringae* pathovar and non-pathogenic *E. coli*. In retrospect, the induction of defence responses by *E. coli* probably resulted from PAMP-triggered defences. Another study, used electron microscopy to show that *X. c.* pv. *vesicatoria* suppressed papillae formation in plants and this ability was dependent on *hrp* genes (Brown *et al*., 1995).

These early studies suggested the TTSS of bacterial plant pathogens was involved in defence suppression. More recent studies suggested that specific type III effectors may be altering defence pathways in plants. For example, VirPphA, a type III effector from *P. s.* pv. *phaseolicola*, blocked other 'masked' Avr proteins from eliciting the HR (Jackson *et al*., 1999). *P. s.* pv. *phaseolicola virPphA* mutants elicited HRs on certain bean cultivars that were normally infected by the wild-type strain. Thus, the *virPphA* mutation effectively converted a virulent pathogen to an avirulent one. Similar phenotypes were observed for *P. s.* pv. *phaseolicola* mutants defective in AvrPphC and AvrPphF (Tsiamis *et al*., 2000). Another phenomenon that now makes more sense in the context of effectors acting as suppressors is the observation that AvrRpt2 blocked the ability of AvrRpm1 to elicit an RPM1-dependent HR in *Arabidopsis* plants (Ritter and Dangl, 1996). AvrRpm1 elicits an HR in *Arabidopsis* more quickly than AvrRpt2. However, when both Avr proteins are present in *P. syringae* and infiltrated into *Arabidopsis* Col-0, which contains R proteins that recognize both Avrs, only the slower AvrRpt2-dependent HR develops. This suggested that the AvrRpt2 somehow interfered with AvrRpm1 recognition. This effect appears to result from the AvrRpt2-dependent elimination of a plant protein called RIN4, which is monitored by RPM1 such that when RIN4 is eliminated the RPM1 surveillance system is disabled (Mackey *et al*., 2002; 2003; Axtell and Staskawicz, 2003).

Recently, several *P. syringae* effectors have been identified as suppressors of the HR and other plant responses associated with defence. The HR elicited by several different Avr proteins were suppressed by the *P. syringae* effectors $AvrPphE_{Pto}$, $AvrPpiB1_{Pto}$, AvrPtoB, AvrRpt2, HopPtoD2, HopPtoE, HopPtoF (an AvrPphF homologue) and HopPtoN (Lopez-Solanilla *et al*., 2004; Abramovitch *et al*., 2003; Axtell and Staskawicz, 2003; Bretz *et al*., 2003; Espinosa *et al*., 2003; Mackey *et al*., 2003; Jamir *et al*., 2004). A subset of these also has been shown to suppress other hallmarks of plant defence (Bretz *et al*., 2003; Chen *et al*., 2004; Jamir *et al*., 2004). Moreover, $AvrPphE_{Pto}$, HopPtoE, AvrPtoB, HopPtoF and HopPtoG suppressed Bax-induced PCD in yeast and plants, and induction of the PR transcript *PR1a* (Jamir *et al*., 2004). AvrPtoB also suppressed the Bax-dependent PCD in plants and as well as stress-induced PCD in yeast (Abramovitch *et al*., 2003). While the mechanism of suppression is unknown, suppression of PCD pathways in yeast suggests that these effectors may act on conserved pathways in eukaryotes.

Some type III effectors are able to suppress basal defences triggered by PAMPs. For example, Hauck *et al*. (2003) found that transgenically expressed AvrPto in *Arabidopsis* suppressed the expression of a set of genes predicted to encode proteins that are secreted cell wall and defence proteins, which are normally expressed in a manner independent of SA. Moreover, when *P. syringae* TTSS-defective mutants were infected into AvrPto- or AvrRpt2-expressing plants these mutants grew to significantly higher levels than control strains suggesting that plant defences induced by these mutants were suppressed by both AvrRpt2 and AvrPto (Hauck *et al*., 2003; Chen *et al*., 2004). Recently, DebRoy *et al*. (2004) have shown that the *P. syringae* effectors AvrE and HopPtoM suppress SA-dependent basal defences. Characterization of other type III effectors to determine whether they suppress SA-dependent or -independent defence responses (or both) will help identify the plant targets of these effectors. A listing of plant pathogen type III effectors that appear to have a role in suppression of innate immunity has been recently published (Alfano and Collmer, 2004).

Several caveats should be noted regarding effectors that possess suppressor activity: many of the experiments were carried out in heterologous systems (e.g. yeast); or in conditions where the effector displaying the suppressing activity was overexpressed; and, finally, many of the mutants defective in effectors that suppress the HR do not obviously alter their host range (although, in many cases this was not tested), which would be predicted if individual suppressors were required for growth on a specific host plant. Another paradox associated with several of the effectors that suppress defence response is that they can also form necrotic lesions in compatible hosts (Badel *et al*., 2003; DebRoy *et al*., 2004; Lopez-Solanilla *et al*., 2004). These lesions may represent a late HR resulting from latent recognition of these effectors by the plant's surveillance system. A recent report demonstrates that both the HR and necrotic lesions caused by *P. syringae* require a MAPK pathway suggesting that they may be related responses (del Pozo *et al*., 2004).

There has been substantial progress on the identification of enzymatic activities of plant pathogen type III effectors (Innes, 2003; Alfano and Collmer, 2004; Chang *et al*., 2004). A growing number of these are active or predicted cysteine proteases (Shao *et al*., 2002; Axtell *et al*., 2003; Hotson *et al*., 2003; Lopez-Solanilla *et al*., 2004). While the roles that many of these cysteine proteases play in

virulence are unknown, there is evidence that one role for this group of effectors is defence suppression. For example, the *P. s.* pv. *phaseolicola* AvrPphB cysteine protease targets the *Arabidopsis* PBS1 kinase, a protein involved in R protein-mediated defences (Shao *et al.*, 2003). The *P. s. tomato* HopPtoN effector, a cysteine protease belonging to the same clad as AvrPphB, suppresses an Avr-triggered HR (Lopez-Solanilla *et al.*, 2004).

One plant pathogen type III effector that is particularly noteworthy is HopPtoD2 from *P. s.* pv. *tomato* DC3000. When HopPtoD2 is expressed in *P. s.* pv. *phaseolicola*, it suppresses the non-host HR, PR1 gene expression and the oxidative burst (Bretz *et al.*, 2003; Espinosa *et al.*, 2003). Importantly, HopPtoD2 was also demonstrated to possess protein tyrosine phosphatase (PTP) activity. We are not aware of any reports of tyrosine phosphorylated proteins in plants other than the MAPKs (Zhang and Klessig, 2001), which are phosphorylated at both threonine and tyrosine. As noted above, MAPK pathways play important roles in signal transduction pathways controlling plant defence (Zhang and Klessig, 2001). An HR-like response is produced when a constitutively active tobacco MAPKK ($NtMEK2^{DD}$) is transiently expressed in plants (Yang *et al.*, 2001). Interestingly, when HopPtoD2 is coexpressed in plants with $NtMEK2^{DD}$ the HR-like response is suppressed suggesting that the host cell target(s) of HopPtoD2 is downstream of this MAPKK (Espinosa *et al.*, 2003). This MAPK pathway is activated by a number of Avr proteins and the *Arabidopsis* MAPK homologues AtMPK3/AtMPK6 are also involved in the response to the PAMP flagellin (Zhang and Klessig, 2001; Asai *et al.*, 2002). Thus, it is plausible that HopPtoD2 acts at a point of convergence of signal transduction pathways that are utilized by both Avr-triggered and PAMP-triggered defence responses. Figure 2 shows possible sites of action in the plant defence signal transduction pathways for type III effectors that suppress innate immunity.

Animal pathogen type III effectors have also been shown to suppress innate immunity. Two type III effectors, SptP and YopH, from *S. typhimurium* and *Yersinia* spp., respectively, are also PTPs similar to HopPtoD2 (Guan and Dixon, 1990; Kaniga *et al.*, 1996). Both of these effectors, like many animal pathogen type III effectors, suppress actin polymerization, which inhibits phagocytosis and prevents bacterial uptake by animal cells (Andersson *et al.*, 1996; Fu and Galán, 1998). YopH is also known to downregulate the proinflammatory response (Boland and Cornelis, 1998; Viboud *et al.*, 2003), which is probably at least partially due to the suppression of the synthesis of the monocyte chemoattractant protein 1, a chemokine involved in macrophage recruitment to the sites of infection (Sauvonnet *et al.*, 2002a). YopH is also responsible for the ability of *Yersinia* to prevent T and B cell activation and therefore inhibiting adaptive immunity as well (Yao *et al.*, 1999). Recently, the Lck kinase, a protein involved in T cell receptor signalling, was shown to be a substrate of YopH (Alonso *et al.*, 2004). This is consistent with suppression of innate and adaptive immunity by preventing T cell activation.

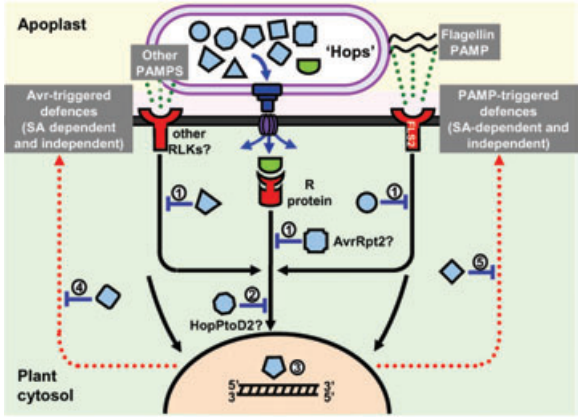

Fig. 2. Potential sites of action of plant pathogen type III effectors in innate immunity signal transduction pathways in plants. Bacterial plant pathogens inject many different type III effectors (i.e. Hops) into plant cells. Some type III effectors (i.e. Avr proteins depicted here as a green object) are recognized by plant R proteins triggering SA-dependent and SA-independent defences. The plant cell is capable of recognizing the flagellin PAMP with the receptor-like kinase (RLK) FLS2. Other PAMPs are probably recognized by other plant RLKs. Type III effectors that act as defence suppressors can act at unique parts of the innate immunity signal transduction pathways (e.g. AvrRpt2 suppression of the AvrRpm1-triggered defences) (1); or others may act at convergence point of different signal transduction pathways (e.g. HopPtoD2 suppression of a MAPK pathway) (2); certain type III effectors such as AvrBs3 are localized to the nucleus and modify eukaryotic transcription (Buttner and Bonas, 2003) (3); type III effectors may also act post-transcriptionally to suppress Avr-triggered (4) and PAMP-triggered outputs (5), both of which result from SA-dependent and SA-independent responses. See text for additional details. This figure was modified from Alfano and Collmer (2004).

SptP is a multifunctional effector with a GTPase activating protein domain in its N-terminal half and a PTP domain in its C-terminal half (Kaniga *et al.*, 1996). Murli *et al.* (2001) showed that *S. typhimurium sptP* mutants activated Erk MAPK pathways to higher levels suggesting that SptP modulated this MAPK pathway. SptP appears to inhibit the Erk MAPK pathway by interfering with Raf kinase activation (Lin *et al.*, 2003). *Salmonella* is known to trigger proinflammatory cytokines in animal cells through the activation of Erk, Jnk and p38 MAPK pathways (Hobbie *et al.*, 1997). SptP also downregulates secretion of the proinflammatory cytokine IL-8 (Haraga and Miller, 2003), which is consistent with it acting as a suppressor of animal innate immunity. Interestingly, SptP also inhibits the p38 MAPK pathway in *Caenorhabditis elegans* and a *Salmonella sptP* mutant is reduced in its ability to kill *C. elegans* suggesting a role in virulence in this heterologous pathosystem (Tenor *et al.*, 2004). These

results indicate that SptP targets MAPK pathways involved in innate immunity (Kim *et al*., 2002; Aballay *et al*., 2003).

The *Yersinia* YopJ/P type III effector also suppresses animal innate immunity. YopJ belongs to a large family of type III effectors that are cysteine proteases that are well distributed in animal and plant pathogens (Orth, 2002). The first clue that YopJ suppressed innate immunity was that *Yersinia* was capable of suppressing the proinflammatory cytokine tumour necrosis factor α (TNF-α) that was induced by several MAPK pathways activated after infection (Ruckdeschel *et al*., 1997). YopJ/P was shown by several groups to be capable of suppressing both NF-κB and TNF-α in macrophages (Boland and Cornelis, 1998; Palmer *et al*., 1998; Ruckdeschel *et al*., 1998; Schesser *et al*., 1998). YopJ also blocks several signal transduction pathways that activate the CREB transcription factor (Meijer *et al*., 2000). This transcription factor is activated by the LPS PAMP indicating that YopJ suppresses PAMP-triggered innate immunity. How does YopJ cause these effects? YopJ blocks members of a superfamily of MAPKKs needed to activate MAPK pathways induced in mammalian cells by pathogens, and the IκB complex, which is required for activation of NF-κB (Orth *et al*., 1999). YopJ is an isopeptidase that can use proteins conjugated to small ubiquitin-related modifier (SUMO) as substrates cleaving the isopeptide bond that links SUMO to the target protein (Orth *et al*., 2000). Cleaving a SUMO group from a protein can alter the protein's activity. However, why YopJ/P's cysteine protease activity is required for MAPK and NF-κB signalling and how this contributes to its ability to suppress the mammalian immune system is currently unknown.

There are other animal pathogen type III effectors that are capable of suppressing innate immunity. Recently, the *Salmonella* SspH 1 type III effector was shown to localize to the nucleus where it inhibits NF-κB resulting in down-regulation of IL-8 production (Haraga and Miller, 2003). SspH 1 belongs to a family of type III effectors that contain LRR predicted to be involved in protein–protein interactions. Several of these, including the *Yersinia* YopM and the *Shigella* IpaH9.8, have also been shown to localize the nucleus (Skrzypek *et al*., 1998; Toyotome *et al*., 2001). YopM's LRRs are required for localization to the nucleus (Benabdillah *et al*., 2004). IpaH9.8, like SspH 1, inhibits NF-κB suggesting that this group of effectors may modify eukaryotic transcription (Haraga and Miller, 2003). Consistent with this idea is the observation that a *Yersinia yopM* mutant alters the transcription profile of macrophages compared with the transcription profile of macrophages challenged with wild-type bacteria (Sauvonnet *et al*., 2002b). Type III effectors that act as eukaryotic defence suppressors do not appear to be limited to pathogens. Recently, NopL, a type III effector from the symbiont *Rhizobium* sp. NGR234, was found to suppress the induction of PR genes in tobacco suggesting that symbionts may also benefit from suppressing plant innate immunity (Bartsev *et al*., 2004).

Concluding remarks

The ability to avoid eukaryotic surveillance systems of the innate immune system was probably a critical development in the evolution of bacterial pathogenicity. In the animal world, this was made doubly complex by an adaptive immune system that could recognize a bewildering array of molecules and specific immune cells (i.e. macrophages) that have the capacity to engulf microorganisms. At first glance, plants seem to be an easier mark because their defence systems rely only on premade defences and a surveillance system that recognizes both conserved molecules on microorganisms (PAMPs) and specific products of pathogens (Avr proteins). However, this surveillance system is surprisingly complex and successful, which may explain why there are relatively few bacterial species that can invade plants. It seems logical to assume that the ability to recognize PAMPs came before Avr recognition in the evolution of plant immunity. Figure 3 shows a possible sequence of events that allowed Gram-negative bacterial pathogens to evolve centred on the horizontal acquisition of a TTSS. In this model, acquisition of the TTSS and type III effectors that disable the PAMP-based innate immune system is the central event that allowed these bacteria to evade plant defences. It seems likely that after pathogens were successful in disabling PAMP-triggered surveillance systems, the plant countered by evolving the ability to recognize Avr proteins of pathogens. To counteract, the pathogen acquired many type III effectors, which are probably functionally redundant, and the plant evolved many PRRs that could recognized as many possible 'looks' the pathogen gives the plant. Understanding plant surveillance systems and type III effectors that disable it is imperative if we are to understand the pathogenicity of plants.

In a larger context, the study of bacterial pathogenicity of plants and animals has benefited from a comparative pathobiology approach. For example, the similarity of NODs to NBS-LRR R proteins probably aided the discovery that NODs were involved in pathogen recognition. Further comparative analyses of the protein complexes involved in PRR perception and their downstream signal transduction pathways will probably increase our understanding of both pathosystems. And many other points of comparison remain. For example, is the YopJ/P-induced apoptosis of macrophages (Mills *et al*., 1997; Monack *et al*., 1997) equivalent to the Avr-induced PCD (i.e. HR) in plants? That is, is the PCD of a macrophage a form of defence or a virulence strategy of the pathogen? The

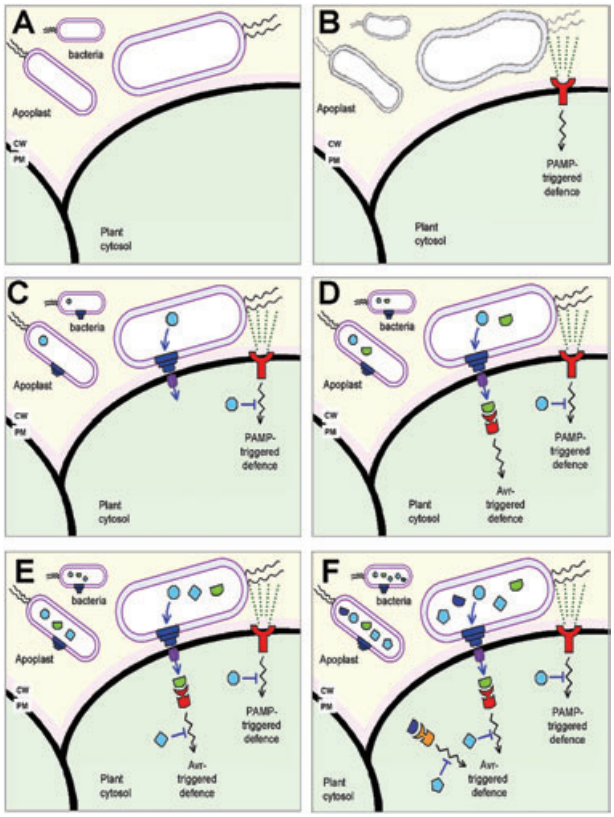

Fig. 3. Scenario for the evolution of bacterial plant pathogens based on suppression of plant innate immunity with type III effectors.
A. A possible scenario for the evolution of Gram-negative bacterial plant pathogenicity predicts a stage in the evolution of plants that allowed non-pathogens to grow in the apoplast unperturbed because plants lacked an innate immune system.
B. Plants that evolved or possessed an innate immune system that allowed for the detection of PAMPs were successful in stopping the growth of non-pathogens.
C. Bacteria that acquired a TTSS through horizontal transfer or evolved a specialized TTSS from the flagellar TTSS and delivered type III effectors that suppressed the PAMP-triggered defence responses were successful in growing in the intercellular spaces of plants.
D. Plants responded to this biotic stress by evolving R proteins that could recognize certain type III effects as Avr protein to trigger a rapid intense defence response which included the HR.
E. The acquisition of additional type III effectors conferred the ability to suppress Avr-triggered defences.
F. This put additional evolutionary pressure on plants to expand their R protein surveillance system to recognize the activity of other type III effectors.
The scenario predicts that the first targets of type III effectors were plant components that were involved in the PAMP-triggered defences (i.e. basal defences). Continued co-evolution of plant PRRs and type III effector suppressors resulted in a highly polymorphic collection of effectors and plant PRRs.
CW, plant cell wall; PM, plant plasma membrane.

Yersinia YopB type III translocator does trigger proinflammatory responses in host cells similar to PAMPs (Viboud *et al*., 2003), and potentially analogous to Avr proteins. Plant and animal bacterial pathogens now have groups of type III effectors that have similar activities (e.g. cysteine proteases and tyrosine phosphatases) and a subset of defence suppressors. Thus, comparing the activities and targets of type III suppressors in plant and animal pathosystems should lead to a greater understanding of the innate immune system in eukaryotes.

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