



Common infection strategies of plant and animal pathogenic bacteria

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Gram-negative bacterial pathogens use common strategies to invade and colonize plant and animal hosts. In many species, pathogenicity depends on a highly conserved type-III protein secretion system that delivers effector proteins into the eukaryotic cell. Effector proteins modulate a variety of host cellular pathways, such as rearrangements of the cytoskeleton and defense responses. The specific set of effectors varies in different bacterial species, but recent studies have revealed structural and functional parallels between some effector proteins from plant and animal pathogenic bacteria. These findings suggest that bacterial pathogens target similar pathways in plant and animal host cells.

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Abbreviations

avr	avirulence
GAP	GTPase-activating protein
HR	hypersensitive response
Hrp	hypersensitive response and pathogenicity
IKKβ	I κ B kinase complex
NF-κB	nuclear factor κ B
NLS	nuclear localization signal
pv.	pathovar
R	resistance
TTS	type-III secretion
Yop	<i>Yersinia</i> outer protein

Introduction

Gram-negative pathogenic bacteria have evolved sophisticated strategies to exploit the attractive nutritional menu provided by plants and animals. The majority of bacterial pathogens are highly specialized for a limited number of eukaryotic host organisms. However, some bacterial strains, such as *Pseudomonas aeruginosa* PA14, are capable of infecting a wide range of diverse hosts that includes both plants and animals [1]. Screening of a mutagenized PA14 population allowed the identification of bacterial virulence determinants that are involved in

the interaction of PA14 with both *Arabidopsis* and mice [2]. The contribution of common proteins to bacterial virulence on plants and animals has also been revealed by studies of different bacterial taxa, as is described below [3].

Bacterial invasion and colonization of eukaryotic tissues involves a variety of extracellular factors, such as polysaccharides, adhesins, toxins and degradative enzymes. Furthermore, bacterial effector proteins are delivered into the host cell cytosol where they interfere with cellular responses to the pathogen's benefit. Interestingly, proteins that contribute to the host–pathogen interaction are often encoded by pathogenicity islands (PAIs), suggesting their acquisition by horizontal gene transfer [4]. Genetic mobility provides one explanation for the presence of conserved pathogenicity genes in different bacterial species. Broad conservation is well exemplified by type-III secretion (TTS) systems, which are key pathogenicity determinants and mediate the delivery of effector proteins into the host cell [5]. TTS systems have been intensively studied in bacterial model systems such as species (spp.) of the animal pathogens *Yersinia* (in which they were discovered), *Salmonella* and *Shigella*, and the plant pathogens *Erwinia* spp., *Pseudomonas syringae*, *Ralstonia solanacearum* and *Xanthomonas* spp. [6]. Despite the broad conservation of the core components of the secretion machinery, the number and sequences of the secreted proteins vary considerably. However, recent studies have unraveled sequence homologies among some type-III effector proteins from plant and animal pathogenic bacteria, suggesting that they exert similar functions in eukaryotic host cells.

In this review, we highlight some common themes in the molecular interactions between Gram-negative bacterial pathogens and their eukaryotic hosts. We focus particularly on the recently discovered structural and functional parallels between type-III effector proteins from plant and animal pathogenic bacteria.

Initial events at the host–pathogen interface

One of the first events in a host–pathogen interaction is the physical contact between the bacterium and the host cell. Bacterial attachment to the host cell surface is mediated by surface proteins, termed adhesins, that are assembled into pilus-like structures (fimbrial adhesins) or anchored in the outer membrane (afimbrial adhesins) [7]. In animal pathogenic bacteria, adhesins bind to specific host-cell receptors, thus allowing a tight contact between the pathogen and the host cell. In plant pathogenic bacteria, however, the role of adhesins in the interaction with the cell wall, a natural barrier that surrounds plant

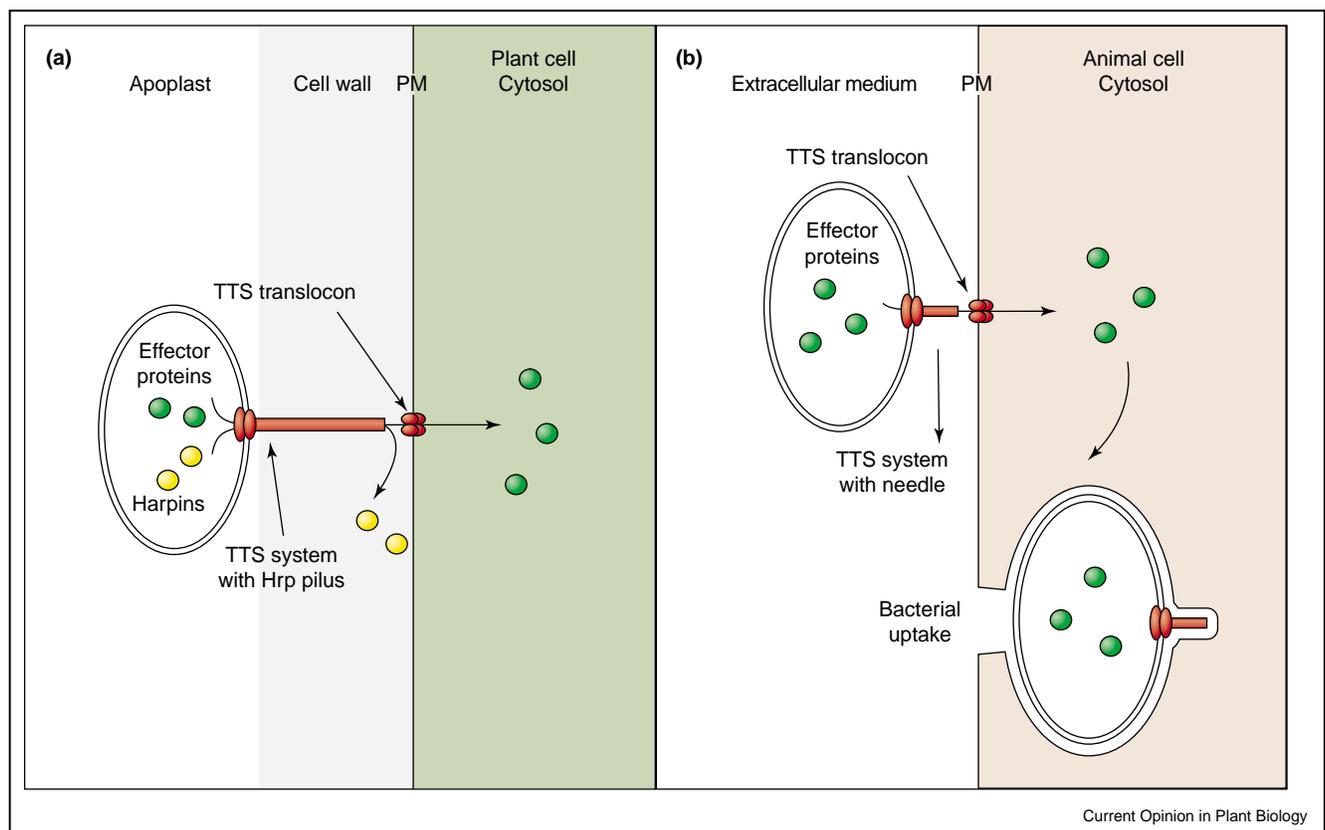
but not animal cells, is less clear. Two afimbrial adhesins have been characterized that show homology to adhesins from animal pathogenic bacteria. Both proteins, HecA from *Erwinia chrysanthemi* and XadA from *Xanthomonas oryzae* pv. *oryzae*, are involved in bacterial virulence [8,9]. Furthermore, HecA contributes to the bacterial competence in attaching to and aggregating on leaf surfaces [9]. DNA sequence analyses revealed the presence of *xadA* and *hecA* homologs in the genomes of the plant pathogens *Xylella fastidiosa*, *R. solanacearum*, *Xanthomonas axonopodis* pv. *citri* and pathovars of *Xanthomonas campestris* [10–13]. The broad conservation of adhesin-like proteins in plant and animal pathogens suggests that these proteins are commonly used to infect eukaryotic hosts.

TTS systems in plant and animal pathogenic bacteria

Once the bacteria are close to a host cell, they start to inject effector proteins into the cytosol of the eukaryotic

cell. The delivery of effector proteins is mediated by the TTS system, which spans both bacterial membranes and is associated with an extracellular appendage [6]. TTS systems are present not only in many Gram-negative pathogenic bacteria but also in some plant symbionts, such as *Rhizobium* spp., in which they presumably influence the host range. The structure and function of TTS systems have been extensively reviewed elsewhere [5,6,14] and will not be discussed in detail. It is worth noting, however, that major structural differences among the TTS systems of plant and animal pathogenic bacteria reside in the extracellular part of the secretion machinery. The TTS system of animal pathogens is associated with a needle, which is essential for the delivery of effector proteins into the host cell [15–17]. In plant pathogenic bacteria, the TTS system is connected to a pilus structure, which is up to 200 nm in length and can potentially cross the plant cell wall ([18]; Figure 1). The pilus serves as a conduit for secreted proteins [19,20].

Figure 1



Model describing the role of TTS systems in bacterial interactions with plants and animals. **(a)** The TTS system of plant pathogenic bacteria is associated with the Hrp pilus, which presumably spans the plant cell wall (200 nm thick; not drawn to scale) and serves as a conduit for secreted proteins. Among the secreted proteins are harpins (yellow) that presumably act at the plant cell surface and effector proteins (dark green). The translocation of effector proteins into the host cell cytosol is mediated by the putative TTS translocon, a bacterial protein complex in the host plasma membrane (PM) [59]. **(b)** The TTS system of animal pathogenic bacteria is associated with a needle structure that is significantly shorter than the Hrp pilus. The translocation of effector proteins into the host cell cytosol is mediated by a putative channel formed by the TTS translocon. Several animal pathogenic bacteria (e.g. species of *Salmonella* and *Shigella*) are able to induce their uptake into non-phagocytic cells [60].

Proteins that travel through the TTS systems include extracellular components of the apparatus as well as effector proteins. Harpin proteins represent another class of secreted proteins that are characteristic of plant pathogenic bacteria. They are heat-stable, glycine-rich proteins that lack cysteines and presumably act at the plant cell surface ([21]; Figure 1).

Crossing the borderline – bacterial type-III effector proteins

Type-III-mediated delivery into the host cell cytosol had initially been shown for *Yersinia* outer proteins (Yops) [5] and was only recently demonstrated for effector proteins from plant pathogenic bacteria. Here, evidence for protein translocation was provided by the use of reporter fusions and by direct visualization of effector proteins inside the infected plant cells using immunocytochemistry [22,23,24^{**},25^{*},26^{*}]. Because of the low secretion efficiency *in vitro*, effector proteins from plant pathogenic bacteria have mainly been identified genetically as the products of avirulence (*avr*) genes. Avr proteins induce specific defense responses in plants that express the corresponding resistance (*R*) genes [3]. Plant defense is often associated with the induction of the hypersensitive response (HR), a local programmed death of plant cells at the infection site [27], which can be easily scored. The fact that Avr proteins induce the HR when expressed *in planta* or when transfected into protoplasts strongly suggests that these effectors are translocated into the plant cell during the natural infection [28,29^{*}].

It should be noted that effector proteins act not only as avirulence factors. Effector proteins presumably provide a selective advantage for pathogens that infect host plants that do not contain a corresponding *R* gene [30]. However, knockout studies indicate that individual effectors often contribute little to bacterial virulence or are functionally redundant [31]. Plant pathogenic bacteria have presumably evolved multiple effectors that have similar functions in order to evade recognition by the plant's surveillance system. In fact, when compared to animal pathogens (e.g. six known effectors in *Yersinia* spp. [32]), the effector protein arsenal from plant pathogenic bacteria appears to be much larger. In *P. syringae* pv. *tomato* DC3000, for instance, at least 38 putative effector proteins are known to date (for review see [33,34]). The identification of candidate effectors in *P. syringae*, as well as in other plant pathogenic bacteria, has recently been fueled by bioinformatic approaches and by comparative analyses of genomic sequences [11,12].

Significant progress has been made in studying the biochemical functions and host cell targets of effector proteins from animal pathogenic bacteria. This is due in part to the fact that cultured eukaryotic cells, such as HeLa cells and macrophages, could be used for bacterial infection assays. Effector proteins have been shown to modulate a variety of

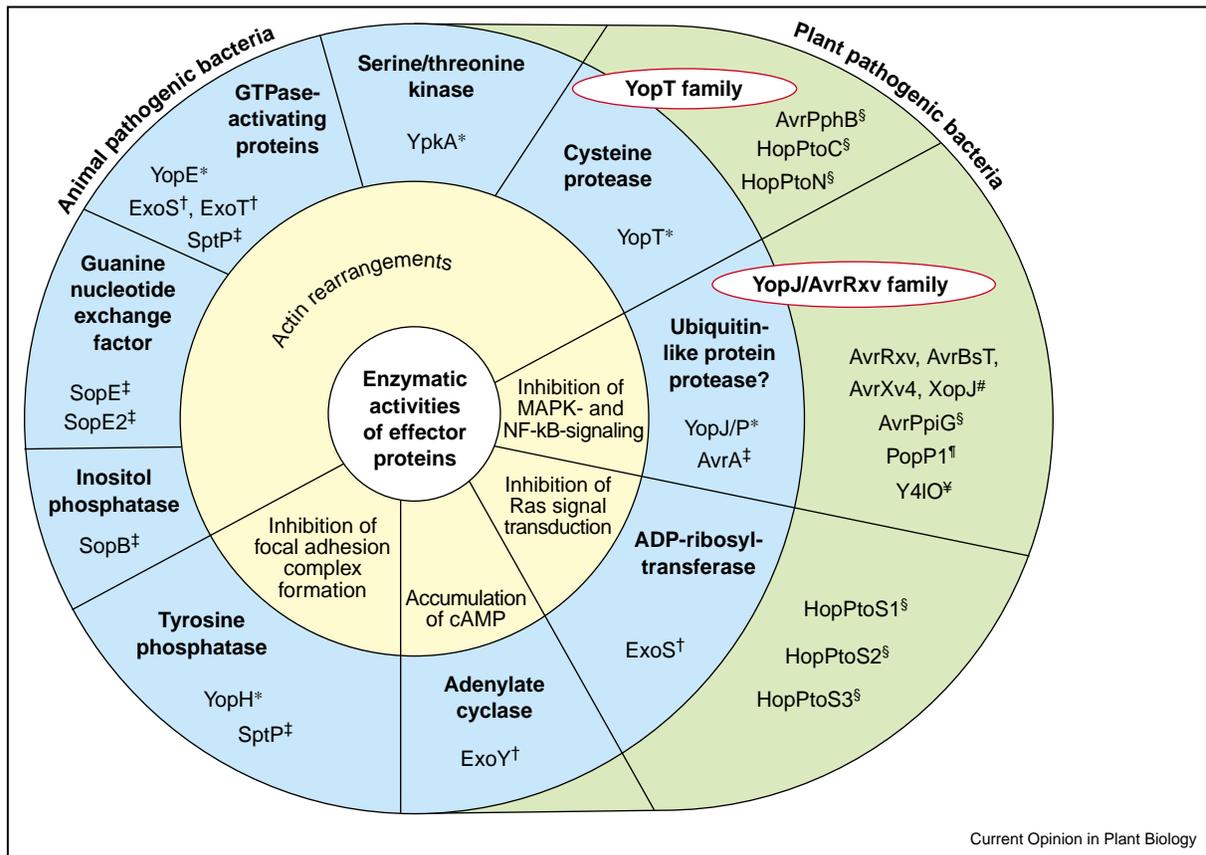
cellular activities, such as the control of host cell survival, immune response, actin rearrangement and vesicle trafficking [5]. The similarity of some effector proteins (e.g. YopH, SptP, YpkA and SopB; see Figure 2) to eukaryotic enzymes such as phosphatases and kinases indicates that mimicry of host proteins is one important strategy for interference with eukaryotic pathways [35,36]. A summary of the known enzymatic activities of effector proteins and their influence on the host cell is given in Figure 2. Some examples are presented in more detail below.

It has been difficult to deduce possible functions for effector proteins from plant pathogenic bacteria as the respective mutant strains often do not display phenotypic effects and most effectors are not homologous to proteins with a known function [30]. Host target proteins that were identified by interactor screens provided the first indications of how effectors modulate host cellular processes. The recent observation that the effector proteins AvrRpm1, AvrB and AvrRpt2 from *P. syringae* interact with the *Arabidopsis* protein RIN4, a component of the basal plant defense, supports the hypothesis that type-III effectors interfere with host defense responses [37^{**},38^{*},39^{*}]. Furthermore, these findings demonstrate that a given host protein can be targeted by several distinct effectors. In addition to virulence targets, interactor studies will uncover host proteins that are recruited by effectors to help them reach their final destination in the host cell. One notable example is pepper importin α , which mediates nucleocytoplasmic trafficking of *X. campestris* pv. *vesicatoria* AvrBs3, an effector protein that presumably acts as a modulator of the host's transcriptome [40,41^{*}].

The host cell cytoskeleton as an effector protein target

The host cell cytoskeleton is a major virulence target of effector proteins from animal pathogenic bacteria (see Figure 2). YopE, YpkA and YopT from *Yersinia* spp., for instance, directly influence the activity of Rho GTPases [42], which are key regulators of the actin cytoskeleton. Rho GTPases act as molecular switches that are active when bound to GTP and inactive when bound to GDP [43]. YopE is a GTPase-activating protein (GAP) that directly regulates the activity of Rho GTPases [42]. Homologs of YopE have been identified in *Salmonella typhimurium* (SptP) and *P. aeruginosa* (ExoS) (Figure 2). In all three proteins, the GAP activity domains contain an 'arginine finger' that is also involved in the catalytic activity of mammalian Rho GAPs [44]. This is an intriguing example of convergent evolution and shows that YopE and its homologs mimic eukaryotic enzymes. Another case of host mimicry has been reported for YpkA (YopO in *Yersinia enterocolitica*), which contains a domain with sequence similarity to eukaryotic serine/threonine kinases [45]. YpkA binds to actin and to Rho GTPases, and presumably phosphorylates proteins that are involved in actin regulation [42].

Figure 2



Enzymatic activities of bacterial effector proteins. Known enzymatic activities of effector proteins from animal pathogenic bacteria (blue circle) and their major effects on infected host cells (yellow circle). Homologous effector proteins that have been identified in plant pathogenic bacteria are indicated in the green area. An enzymatic activity has not yet been demonstrated for members of the YopJ/AvrRxv family or for effector proteins from plant pathogenic bacteria. *Proteins from *Yersinia* spp. (see text for details). YopH dephosphorylates components of focal adhesions, thereby inhibiting their complex formation [32]. †Proteins from *P. aeruginosa*. ExoS is a bifunctional effector protein with an amino-terminal GTPase-activating protein domain and a carboxy-terminal ADP-ribosyltransferase activity [61]. The ExoS homolog ExoT is a GTPase-activating protein but does not exhibit ADP-ribosyltransferase activity *in vivo* [62]. ExoY is a calmodulin-independent adenylate cyclase [63]. ‡Proteins from *Salmonella* spp. [64]. SopB is an indirect activator of the Rho GTPase Cdc42 and induces actin rearrangements. SopE and SopE2 directly activate Rho GTPases. This effect is reversed by the amino-terminal GTPase-activating activity of SptP. The function of the SptP phosphatase activity is unclear. One potential substrate is the intermediate filament protein vimentin. §Proteins from pathovars of *P. syringae* [33]. #YopJ/AvrRxv homologs from *X. campestris* pv. *vesicatoria* [14]. ¶YopJ/AvrRxv homolog from *R. solanacearum* [65]. ¥YopJ/AvrRxv homolog from *Rhizobium* NGR345.

Both YopE and YpkA induce the disruption of actin stress fibers in HeLa cells [42]. A similar cytological effect has been observed for the cysteine protease YopT, which cleaves Rho GTPases near the carboxyl terminus, leading to their release from the plasma membrane [46]. Interestingly, the plant pathogenic bacterium *P. syringae* pv. *tomato* DC3000 expresses a homolog of YopT, AvrPphB, which triggers the HR in resistant *Arabidopsis* plants ([46]; Figure 2). In AvrPphB, the invariant amino-acid residues that are essential for YopT cytotoxicity are required for autocatalytical processing of an AvrPphB precursor to the mature protein, as well as for the induction of HR in resistant plants, indicating that AvrPphB acts as a protease. It remains to be investigated whether AvrPphB targets Rho GTPases.

To date, effector proteins from plant pathogenic bacteria have not been shown to modulate the host cytoskeleton. It is interesting to note, however, that AvrBs3 from *X. campestris* pv. *vesicatoria* induces hypertrophy symptoms (i.e. an enlargement of mesophyll cells) in susceptible plants [41]. The expansion of plant cells involves multiple processes that probably include changes in microtubules and actin filaments [47].

Effector proteins interfere with the host's surveillance system

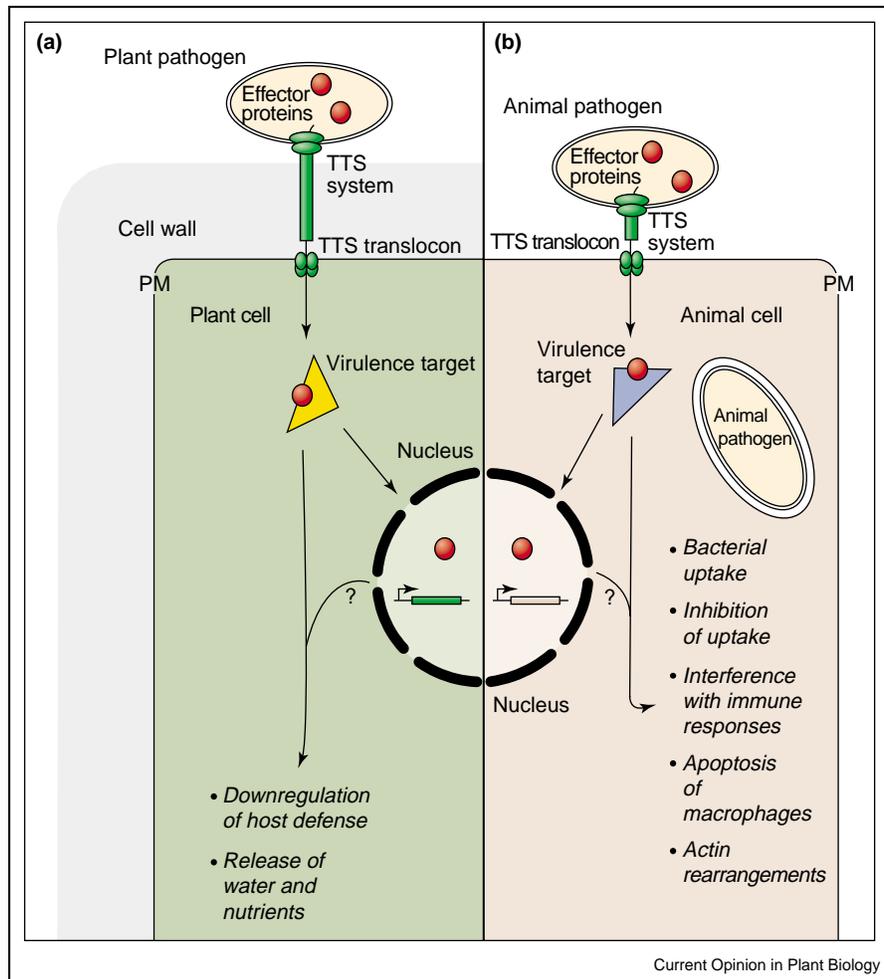
One major capability of bacterial effector proteins appears to be the suppression of host defense responses. This has been well studied for YopJ from *Yersinia pestis* (YopP in *Yersinia pseudotuberculosis* and *Y. enterocolitica*), which

belongs to the YopJ/AvrRxv family of effectors (Figure 2). YopJ inhibits cytokine production by the host cell and induces apoptosis in macrophages [48]. This global effect is caused by the ability of YopJ to downregulate multiple mitogen-activated protein kinases and to block the activation of the transcription factor NF- κ B. Activation of NF- κ B requires its release from its cytosolic inhibitor (I κ B), which is degraded upon phosphorylation by the I κ B kinase complex (IKK β). YopJ binds to and thus inhibits IKK β , resulting in the cytosolic capture of NF- κ B.

Intriguingly, the prediction of secondary structures revealed a similarity between YopJ and adenovirus protease (AVP), a cysteine protease that resembles the yeast ubiquitin-like protein protease 1. The catalytic residues

of AVP are conserved in YopJ and are essential for the virulence function of YopJ. However, a proteolytic activity of YopJ has yet to be demonstrated [49]. Homologs of YopJ have been identified in *Salmonella* spp. (AvrA) as well as in several plant pathogenic bacteria (e.g. AvrRxv, AvrBsT, AvrXv4 and XopJ from *X. campestris* pv. *vesicatoria*) ([48,50]; Figure 2). The putative catalytic residues are strictly conserved in all YopJ-like proteins [48], indicating that they function as proteases. In AvrBsT, mutation of these amino acids abolishes the ability to induce the HR in resistant host plants [49], suggesting that the corresponding R protein recognizes the products of the AvrBsT protease [51]. It is not yet clear whether all members of the YopJ/AvrRxv family target the same host cellular pathways. AvrA from *Salmonella* spp., for instance,

Figure 3



Proposed virulence functions of type-III effector proteins from plant and animal pathogenic bacteria. The TTS system of plant and animal pathogenic bacteria delivers effector proteins into the host cell cytosol where they interfere with specific host target proteins. **(a)** Some effector proteins from plant pathogenic bacteria presumably localize to the plant cell nucleus and modulate host gene expression, as has been shown for AvrBs3 (see text for details). The molecular activities of effector proteins inside the plant cell lead to a suppression of host defense responses. Furthermore, effector proteins probably cause the release of water and nutrients into the extracellular medium. **(b)** In animal host cells, effector proteins trigger a variety of cellular responses. Nuclear localization and modulation of host gene expression has been demonstrated for the effector protein YopM (see text for details). PM, plasma membrane.

blocks the NF- κ B pathway downstream of IKK β activation [52,53].

Besides YopJ-like proteins from animal pathogenic bacteria, several effector proteins from plant pathogenic bacteria also suppress host defense responses [54]. Recently, AvrPtoB from *P. syringae* [55*] was shown to act as a general inhibitor of programmed cell death [56**]. However, the molecular mechanisms underlying the effector-protein-triggered suppression of plant defense remain to be elucidated.

Modulation of host gene expression by effector proteins

Conceivably, the interference of effector proteins with eukaryotic signaling pathways leads to alterations in the host's transcriptome. Modulation of host gene expression has indeed been demonstrated for the YopJ homolog YopP from *Y. enterocolitica* by microarray analysis of infected macrophages [57]. Besides these indirect effects, some effector proteins presumably target the host transcription machinery directly and thus regulate the expression of host genes to their own benefit (Figure 3). This appears to be the case for the effector protein YopM from *Y. enterocolitica*, which localizes to nuclei of infected host cells [32]. YopM is a leucine-rich repeat (LRR)-containing protein that modulates the expression of host genes that are involved in the control of cell growth and the cell cycle [57]. LRRs are typically involved in protein-protein interactions [58], and so one could speculate that YopM binds to components of the host's transcription machinery. It has not yet been demonstrated, however, that the nuclear localization of YopM is indeed required for its regulatory activity.

Nuclear localization and modulation of host gene expression has also been shown for the effector protein AvrBs3 from *X. campestris* pv. *vesicatoria* [26*,41*]. AvrBs3 belongs to a family of highly homologous effector proteins that contain a central region of nearly identical 34-amino-acid repeats, as well as carboxy-terminal nuclear localization signals (NLSs) and an acidic activation domain (AAD) [50]. NLSs and AAD, typical features of eukaryotic transcription factors, are essential for the nuclear localization of AvrBs3 and the modulation of host gene expression, respectively [26*,41*]. Several plant genes that are induced by AvrBs3 show homology to auxin-induced and expansin-like genes that are presumably involved in cell enlargement. These findings provide a first link to the AvrBs3-induced phenotype in susceptible mesophyll cells.

Conclusions

In the past decade, it has become apparent that many plant and animal pathogenic bacteria share common infection strategies. Recent studies have revealed that distinct type-III effectors from plant and animal pathogenic bacteria appear to employ similar strategies to

interfere with the host cellular machinery. Furthermore, certain effector proteins share significant homology at the amino-acid level. These findings indicate that the molecular crosstalk between host and pathogen is determined by a set of common effector proteins that target similar pathways in different hosts. Comparative sequence analyses of whole bacterial genomes indicate, however, that bacterial pathogens also express unique effectors. These proteins have presumably evolved for specific interactions with distinct host organisms and might play a role in determining the host range.

The identification of effector proteins from plant pathogenic bacteria has been a major challenge. Recently, sensitive genetic screens and computational analyses have been developed to unravel the complete panoply of effector proteins [33,34]. The elucidation of the functions of effector proteins in the host plant cell remains another demanding task. First clues have been obtained by interactor screens and through the analysis of host gene expression. The use of cultured host cells, which has been instrumental in the rapid functional characterization of effector proteins from animal pathogens, is not well established for infection studies with plant pathogens. On the other hand, however, the relative ease of working with the intact plants allows the identification of virulence factors by large-scale screening of mutagenized bacterial populations. Furthermore, recent progress in gene silencing in plants, and the availability of mutant lines from model plants such as *Arabidopsis*, will facilitate the identification of host proteins that are involved in host-pathogen interactions. The characterization of molecular events that underlie effector protein functions inside the host cell will not only advance our knowledge on bacterial pathogenicity and essential cellular processes but also help us to develop new strategies for disease control.

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