Journal of Agriculture and Environmental Sciences
June 2014, Vol. 3, No. 2, pp. 265-285
ISSN: 2334-2404 (Print), 2334-2412 (Online)

Published by American Research Institute for Policy Development

Effectors-Role in Host-Pathogen Interaction

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Abstract

Effectors are pathogen secreted molecules that manipulate host cell structure and function thereby facilitating infection or triggering defense responses (Kamoun, 2006). These can be toxins or elicitors. This dual activity of effectors has been broadly reported in many plant-microbe pathosystems (Alfano and Collmer, 2004). The term effector is neutral and does not imply a negative or positive impact on the outcome of the disease interaction. Effectors are secreted from pathogens' secretion systems. So far four types of secretion systems (Types I-IV) have been identified. Among them, T3SS (Type III Secretion System) and T4SS (Type IV Secretion System) can cross bacterial cell walls and host eukaryotic cell membranes to deliver effectors into host cells directly without going through extracellular matrix. Those effectors can manipulate host cell functions once entering host cell (Leach, 2003). For a pathogen to survive and multiply, it produces effector molecules to obtain nutrients from its host plant and cultivate the right environment in which to establish infection. Phytopathogenic bacteria use a number of secretion pathways to deliver effector molecules, either into the intercellular spaces or even directly into the host cells. These pathways vary in their complexity for delivery of the effectors (Salmond and Reeves, 1994). The complexity of the pathways is based on the number of proteins involved in the assembly of a channel or pore formed between bacterial inner and outer membranes through which the effectors are transported from the cytosol to the outside of the bacterium.Two classes of effectors target distinct sites in the host plant *viz.* apoplastic effectors and cytoplasmic effectors. Apoplastic effectors are secreted into the plant extracellular space, whereby they target extracellular targets and surface receptors whereas cytoplasmic effectors are translocated inside the plant cell presumably through specialized structures like infection vesicles and haustoria that invaginate inside living host cells (Kamoun, 2006).Plant pathogenic bacteria and fungi have evolved the capacity to deliver effector proteins inside host cells through a diversity of mechanisms. Gram negative bacteria use specialized secretion systems such as T3SS to deliver proteins inside host cells (Block *et al.,* 2008). Biotrophic fungi have evolved haustoria for this purpose. Haustoria were initially thought to primarily function in nutrient uptake but more recently, evidence emerged that haustoria take part in secretion of particular classes of host-translocated fungal effectors (Whisson *et al.,* 2007). Oomycetes such as *Phytophthora infestans*, are also known to secrete apoplastic effectors in addition to host-translocated (cytoplasmic) effectors (Damasceno *et al.,* 2008). A recent study illustrates the concept that plant pathogenic fungi can evade host immunity by way of effectors that suppress R-gene mediated resistance e.g., the effector Avr1 of *Fusarium oxysporum* f.sp. *lycopersici* suppresses the resistance response conferred by the R genes I-2 and I-3 (Saskia *et al.,* 2009). However, various queries regarding effectors still remain unanswered e. g., Are effectors secreted at particular sites at the interface between microbe and plant? Are there waves of effector secretion? Do effectors have distinct functions etc. These are various other issues which need to be investigated in future.

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Introduction

As a complex and interesting relation between organisms in ecology and evolution, host-pathogen interaction is a basis of infectious diseases. Pathogens span a broad spectrum of biological species, including viruses, bacteria, fungi, protozoa, and multicellular parasites. In all these cases, a pathogen causing an infection usually exhibits an extensive interaction with the host during pathogenesis. The cross-talks between a host and a pathogen allow the pathogen to successfully invade the host organism, to breach its immune defense, as well as to replicate and persist within the organism. One of the most important and therefore widely studied groups of host-pathogen interactions is the interaction between pathogen protein (effectors) and host cells.

Effectors can be defined as parasite genes having phenotypic expression in host bodies and behavior (Dawkins, 1999). Effectors are pathogen secreted molecules that manipulate host cell structure and function thereby facilitating infection or triggering defense responses (Kamoun, 2006). These can be toxins or elicitors. This dual activity of effectors has been broadly reported in plant-microbe pathosystems (Alfano and Collmer, 2004).The term effector is neutral and does not imply a negative or positive impact on the outcome of the disease interaction. Effectors are secreted from pathogens' secretion systems. So far four types of secretion systems (Types I-IV) have been identified.

Among them, T3SS (Type III Secretion System) and T4SS (Type IV Secretion System) can cross bacterial cell walls and host eukaryotic cell membranes to deliver effectors into host cells directly without going through extracellular matrix. Those effectors can manipulate host cell functions once entering host cell (Leach, 2003).

Two classes of effectors target distinct sites in the host plant *viz.* apoplastic effectors and cytoplasmic effectors. Apoplastic effectors are secreted into the plant extracellular space, whereby they target extracellular targets and surface receptors whereas cytoplasmic effectors are translocated inside the plant cell presumably through specialized structures like infection vesicles and haustoria that invaginate inside living host cells (Kamoun, 2006).

Apoplastic Effectors

1. **Enzyme inhibitors:** Several plant-pathogenesis related (PR) proteins such as glucanases, chitinases and proteases are hydrolytic enzymes. Fungal and bacterial plant pathogens have evolved diverse mechanism for protection against the activities of these PR protein (Abramovitch and Martin, 2004 and Punja, 2004). Similar to these pathogens, plant pathogenic oomycetes, such as *Phytophthora,* have also evolved mechanisms to escape the enzymatic activity of PR proteins. Oomycetes contain little chitin in their cell wall and are therefore resistant to plant chitinases (Kamoun, 2003). *Phytophthora* also evolved active counter defense mechanisms by secreting inhibitory proteins that target host glucanases and proteases.
2. **Small cysteine-rich proteins:** Many *Avr* genes such as *Cladosporium fulvum Avr*2, *Avr*4, *Avr*9 and *Phytophthora* elicitins, encode small (<150 amino acids) secreted proteins with an even number of cysteine residues, which can induce defense responses when infiltrated into plant tissues (Vant Slot and Knogge, 2002). Several of these common structural features, most notably secretion and the disulfide bridges formed by the pairs of cysteines, are essential for defense induction and avirulence function (Luderer *et al.,* 2002). The disulfide bridges could enhance stability in the plant apoplast, which is rich in proteases (Joosten *et al.,* 1997).
3. **Nep1-like proteins:** These proteins are widely distributed in bacteria and fungi particularly in plant-associated species (Pemberton and Salmond, 2004). This necrosis- and ethylene-inducing protein (Nep1) was originally purified from culture filtrates of the fungus *Fusarium oxysporum* f.sp. *erythroxyli* (Bailey *et al.,* 1997).
   NLPs have subsequently been described in species as diverse as *Bacillus* (Takami and Horikoshi, 2000), *Erwinia* (Pemberton et al., 2005), *Verticillium, Phytophthora* (Fabritius *et al.,* 2002) and *Pythium*. Despite their diverse phylogenetic distribution, NLPs share a high degree of sequence similarity and several members of the family have the remarkable ability to induce cell death in as many as 20 dicotyledonous plants (Pemberton and Salmond, 2004). The wide phylogenetic conservation and broad spectrum activity of NLPs distinguish them from the majority of cell death elicitors and suggest that the necrosis inducing activity is functionally important.
4. **GP42 (PEP13) Transglutaminase:** GP42 is an abundant cell wall glycoprotein of *Phytophthora sojae* that triggers defense gene expression and synthesis of antimicrobial phytoalexins in parsley through binding to a plasma membrane receptor (Sacks *et al.,* 1995).

5. A 13-amino-acid peptide fragment (Pep 13) is necessary and sufficient for activation of defences in parsley and also triggers cell death in potato (Halim *et al.,* 2004). Biochemical analyses indicated that GP42 is a Ca^{2+} dependent transglutaminase (TGase) that is highly conserved in *Phytophthora* (Brunner *et al.,* 2002). The Pep 13 motif is important for activation of plant defences and TGase activity. This suggests that plants evolved receptors to recognize an essential epitope within the TGase proteins and that GP42 functions as a pathogen associated molecular pattern (PAMP) (Brunner *et al.,* 2002). However, it is not known whether *Phytophthora* GP42 TGases play an essential role in avirulence or fitness of the pathogen.

Cytoplasmic Effectors

1. **RXLR protein family:** Race specific resistance to *Phytophthora* spp. follows the gene for gene model, which implies that Avr genes from the pathogen are perceived directly or indirectly by matching resistance (R) genes from the plant (Hammond and Jones, 1997). Race specific Avr genes from oomycetes have been cloned only recently (Rehmany *et al.,* 2005). All four oomycetous Avr proteins (ATR1, ATR13, AVR3a and Avr1b) carry a signal peptide followed by a conserved motif (RXLR) that occurs in a large number of secreted oomycete proteins (Rehmany *et al.,* 2005). The RXLR motif is similar to a host targeting signal that is required for translocation of proteins from malaria parasites (*Plasmodium* spp.) into the cytoplasm of host cells (Hiller *et al.,* 2004), leading to the hypothesis that RXLR functions as a signal that mediates trafficking into host cells (Rehmany *et al.,* 2005). This finding raises the possibility that plant and animal eukaryotic pathogens share similar mechanisms for effector delivery into host cells.
2. **CRN protein family:** CRN1 and CRN2 were identified following an in planta functional expression screen of candidate screening proteins of *P. infestans* (Pex) based on a vector derived from Potato virus X (Torto *et al.,* 2003). Expression of both genes in *Nicotiana* spp. and in the host plant tomato results in a leaf crinkling and cell death phenotype accompanied by an induction of defense related genes. Torto *et al.* 2003 proposed that CRN1 and CRN2 function as effectors that perturb host cellular processes based on analogy to bacterial effectors, which typically cause macroscopic phenotypes such as cell death, chlorosis and tissue browning when expressed in host cells (Kjemtrup *et al.,* 2000). The two CRN genes are expressed in *P. infestans* during colonization of host plant tomato.

Pathogenicity Functions of Effector genes from some Plant Pathogenic Bacteria

	Gene	Pathogenicity function
Erwinia amylovora	dspEF	Fire blight symptom expression in pear and apple.
Pseudomonas syringae pv. maculicola	avr RMP1	Water soaked symptom expression and bacterial multiplication.
P. syringae pv. phaseolicola	avrPphF	Water soaked symptom expression and bacterial multiplication in bean and soybean.
P. syringae pv. tomato	avrA, avrE	Symptom expression and bacterial multiplication.
	avrPto, avrRpt2	Aggressiveness and bacterial multiplication in tomato.
Xanthomonas axonopodis pv.citri	pthA	Intercellular growth and induction of cankers in citrus.
X. campestris pv.malvacearum.	avrb6, pthN	Water soaked symptom expression in cotton.
X. oryzae pv. oryzae	avrxa5	Lesion length and bacterial multiplication in rice.
	avrxa7	Aggressiveness, lesion length and bacterial multiplication in rice.

(Klaas *et al.,* 2002)

Pathogen Associated Molecular Patterns (PAMPS)

The key concept surrounding basal immune systems is that they recognize certain broadly conserved molecules associated with a wide range of pathogens. The term PAMP was developed by researchers of the mammalian innate immune system to describe this type of defense activating compound. The term MAMP (Microbe associated molecular pattern) is gaining favour because non-pathogenic microorganisms also possess PAMPs. Well developed examples of MAMPs that are detected by plants include bacterial flagellins, lipopolysaccharides or elongation factor-Tu, fungal chitin or oomycete Pep-13 or heptaglucosides (Zipfel and Felix, 2005). It is now accepted that plants contain two lines of defence.

The first line provides basal defence against all potential pathogens and is based on recognition of PAMPs by so-called PAMP-recognition receptors (PRRs) that activate PAMP-triggered immunity (PTI) and prevent further colonization of the host (Ioannis and Pierre, 2009).

One of best known PAMPs is chitin, a major structural component of fungal cell walls, for which two LysM-type of receptor-like kinases involved in its perception have been characterized in rice and *Arabidopsis*. Evidence is now accumulating that Avr genes encode effectors that suppress PTI, thus enabling a pathogen to infect its host plant and cause disease. Once the basal defence system of plants is overcome by pathogens, plants respond with the development of a more specialized recognition system based on effector perception by R proteins and subsequent activation of effector triggered immunity (ETI) that leads to rapid and acute defense responses in plants, the hallmark of which is the hypersensitive response (HR). This triggers a second wave of coevolutionary arms race between pathogens and plants, during which pathogens respond by mutating or losing effectors, or by developing novel effectors that can avoid or suppress ETI, whereas plants develop novel R proteins mediating recognition of novel effectors (Ioannis and Pierre, 2009). A related concept from both plant and animal research is that the genes for host MAMP receptors are relatively stable and heritable, allowing the capacity for early detection of microbial infections to be preserved and passed from generation to generation (Nurnberger *et al.,* 2004). A third concept is the perception that basal immunity has a relatively primitive and inferior immune capacity relative to adaptive capacity. This idea derives in part from the observation that basal defences are only partially effective at restricting pathogens. It also derives from the concept that basal defenses are relatively static, i.e., capable of evolving to recognize novel infection threats only over many generations, whereas plant disease resistance mediated by R genes is sometimes portrayed as the plant adaptive immune system.

For example, some R genes compose a more rapidly evolving component of the plant basal immune system than MAMP receptors, but they are not an adaptive immune system in that they do not regularly undergo useful diversification and selection in the somatic cells of individuals. Gene for gene hypothesis for disease resistance is economically important as it is used in numerous crops to confer highly effective disease resistance (Simmonds and Smartt, 1999). The gene for gene hypothesis states that for every dominant avirulence (Avr) gene in the pathogen there is a cognate resistance (R) gene in the host and the interaction between the products of these genes leads to activation of host defence responses such as the hypersensitive response (HR) that arrests the growth of biotrophic fungi.

Plants have many R genes and pathogens have many Avr genes. Disease resistance is observed if the product of any particular R gene has recognition specificity for a compound produced due to a particular pathogen Avr gene. The molecular cloning of first bacterial Avr gene was reported in 1984, the first fungal Avr gene in 1991 and the first oomycete Avr gene in 2004 (Ioannis and Pierre, 2009).

Difference between MAMP and an Avr Gene Product

Formally the latter are named avirulence genes because they cause avirulence in presence of R genes. In the absence of a cognate R gene, Avr genes often make a quantitative contribution to virulence yet are not essential for pathogen viability, although these are not defining features. Some Avr proteins can evolve substantially or may be entirely absent from certain strains of the pathogen, whereas MAMPs are defense elicitors that are evolutionary stable, forming a core component of the microorganism that cannot be sacrificed or even altered much without seriously impairing viability. The term MAMP is increasingly used in place of PAMP because it lends greater accuracy to our thinking. Many microorganisms carry these defense eliciting molecules yet are not pathogens or are not pathogens of many of the hosts that can detect their MAMPs (Ausubel, 2005). A plant normally grows in the presence of hundreds of microbial species including many non-pathogenic microorganisms that it would seemingly be counter productive to defend against. One can postulate that microorganisms must reach a critical mass in the plant interior before the basal immune system is strongly activated e.g., smaller or primary external/epiphytic microbial populations are usually less potent at inducing PR gene expression and other active defences.

Further, tissue specificity was suggested by a recent study in which stomata closure was discovered as a plant defense against bacterial infections (Melotto *et al.,* 2006). Purified MAMPs triggered stomata closure and bacteria did as well, but only when they swarmed around the stomatal opening. Apparently, a threshold level of MAMP must be present before the response is activated.

Maintenance of avr (Virulence) Genes by the Pathogens

Many effectors were first identified on the basis of their avirulence activity. These were appropriately called Avr genes since their R gene mediated activity induces defenses that prevent virulence (Lucas, 1998). However, it was widely assumed that effectors must contribute in some way to pathogen fitness, e.g., by contributing to virulence on a susceptible host. Today the virulence role of many effectors is well established. Effector genes were first isolated as avirulence genes by screening bacterial genomic libraries for genes that convert virulent bacteria to avirulence (Staskawicz, 1984). A powerful clue to effector biology emerged when the first R genes were cloned and found to encode cytoplasmically localized proteins. The hypersensitive response (HR) is a robust defense response frequently associated with R gene mediated resistance and includes the death of plant cells local to the site of infection (Heath, 2000).

The hrp mutations disrupted the ability of phytopathogenic bacteria to cause the hypersensitive response on the resistant hosts and pathogenesis on susceptible hosts, providing evidence that avirulence and virulence activities of effectors are fundamentally related. Two major breakthroughs led to an appreciation that bacterial effectors are active inside the cells of the host-effector proteins expressed directly inside host cells frequently possessed avirulence activity similar to that observed when they are expressed by the pathogen (Alfano and Collmer, 2004) and some of the proteins encoded by hrp genes form a pilus capable of secreting bacterially encoded proteins into the extracellular matrix (Jin and He, 2001). The hrp pilus is now called the type-three secretion system (TTSS) and is known to be central to the virulence of numerous bacterial pathogens of plants and animals. Together, these results led to the hypothesis and subsequent confirmation that type III effector proteins, as they are now called, can be delivered via the TTSS from the bacteria into the cytosol of plant cells where they contribute to virulence (Casper *et al.,* 2002).

Recognition of Effectors by R Proteins

MAMPs can be recognized by direct interaction with a defense receptor. Similarly, it was widely hypothesized that cytosolic R proteins would serve as receptors that directly interact with intracellular effectors. Indeed, it appears true in number of cases (Dodds *et al.,* 2006). However, in many cases, direct interaction between effector and R protein does not explain effector detection.

An alternative model came with the formulation of the "guard hypothesis" which postulated that R proteins recognize effectors indirectly (Vander Biezen and Jones, 1998). It was proposed that effectors target host proteins other than R proteins and that perturbation of those host targets is the trigger that leads to R protein activation. The *Pseudomonas syringae* effector protein AvrPphB provides a straight forward example. This protease cleaves a host protein kinase and the cognate R protein (RPS5) detects such cleavage (Ade *et al.,* 2007). Thus, these types of R proteins guard the target of effectors and induce defence responses when those targets are perturbed (Rooney *et al.,* 2005). So perception of pathogen effectors by R proteins occurs in one of the two ways- either directly, analogous to recognition of MAMPs by MAMP-receptors or indirectly via their perturbation of guarded host targets. The two types of recognition have important ramifications with respect to the durability of resistance conferred by a particular R gene.

How do Effectors avoid Recognition by R Proteins

The evolution of effectors is influenced by how they are perceived by R proteins. An effector contributes to virulence only if recognition by R proteins is avoided. Mutation to avoid recognition is a viable option for effectors that are directly recognized. Changes in effector protein sequence can potentially disrupt the physical interaction with R protein. If the effector can maintain its activity in the context of such mutations, it will escape recognition while maintaining virulence function. There is interesting evidence concerning proteins from virus, fungus and oomycete plant pathogens that apparently have evolved to escape host detection (Liu *et al.,* 2005). However, for effectors that are recognized indirectly it may be much more rare to evolve forms that escape recognition while maintaining virulence activity. The effector would generally have to stop perturbing the host target to avoid detection but the virulence contribution of such effectors will usually be dependent on perturbing the host target.

The effector may attack more than one different host targets while continuing to impact other targets. But a trend seems to be emerging that directly recognized effectors often undergo diversification while indirectly recognized effectors are either present or deleted (Mackey and McFall, 2006).

Mechanisms to Deliver Bacterial Effectors to Plant Cells

For a pathogen to survive and multiply, it produces effector molecules to obtain nutrients from its host plant and cultivate the right environment in which to establish infection. Phytopathogenic bacteria use a number of secretion pathways to deliver effector molecules, either into the intercellular spaces or even directly into the host cells. These pathways vary in their complexity for delivery of the effectors (Salmond and Reeves, 1994). The complexity of the pathways is based on the number of proteins involved in the assembly of a channel or pore formed between bacteria inner and outer membranes and through which the effectors are transported from the cytosol to the outside of the bacterium.

There are four basic types of secretion pathways. Type I and II pathways secrete proteins to the host intercellular spaces, whereas type III and IV pathways can deliver proteins or nucleic acids directly into host cell (Fig.1).

Type I pathway: This is structurally the simplest. It allows direct secretion of effectors from the bacteria cytosol to the external environment. Examples of plant pathogen effectors secreted via the type I pathway are proteases and lipases from the soft rot pathogen *Erwinia chrysanthemi* (Palacios *et al.,* 2001).

Type II pathway: It is composed of more complex secretion structure and two steps are required for secretion of an effector-1) transport to the periplasm and 2) secretion across the outer membrane. Transport into periplasm requires an N-terminal signal sequence and during transfer to the periplasm, the protein is processed by a signal peptidase. The intermediate location in the periplasm allows proper folding of the effector before it is secreted (Chapon et al., 2001). Pathogen effectors involved in cell wall degradation such as pectate lyase, polygalacturonase and cellulase from *Erwinia* and *Xanthomonas* species, are secreted by the type II pathway.

Type III pathway: It is also known as TTSS for type III secretion system. TTSS has been widely studied because it is present in disease causing bacteria of plants and is generally not found in their non-pathogenic counterparts.

Type III secretion pathways use complex structures similar to flagella structures (Blocker *et al.,* 2003) to interact with the eukaryotic host cells and deliver their effectors. The genes encoding the TTSS are called the hrp (hypersensitive response and pathogenicity) genes in phytopathogenic bacteria.

The hrp genes are usually arranged in clusters and are located in pathogenicity islands (PAIs), which are discrete regions that vary in G+C content from the overall genome and are flanked by insertion sequences, bacteriophage genes and transposable elements (Galan and Collmer, 1999). These features suggest that PAIs originated from other species and that they were acquired by horizontal gene transfer. The newly acquired genetic material may confer new pathogenic and fitness traits to bacteria (Hacker *et al.,* 2003). The hrp genes encode proteins that either regulate synthesis or assembly of the TTSS, are structural components of TTSS, or are extracellular proteins (e.g., harpins) secreted by the TTSS (Galan and Collmer, 1999). The hallmark characteristic of the TTSS structure, a needle-like protruding structure with a channel along which proteins travel, is its resemblance to bacterial flagella (Blocker *et al.,* 2003) both at the structural and functional level. Like the type I pathway, secretion of effector proteins via the TTSS is a one-step process with no intermediates in the periplasm. Some effectors require small acidic proteins called chaperones to stabilize the effector protein or aid in its secretion through TTSS (Page and Persot, 2002). Unlike type IV secretion, TTSS effector proteins can be delivered either directly into the plant cell or into the extracellular spaces (Jin and He, 2001).

The TTSS effectors described so far are structurally very diverse, suggesting bacteria may target multiple host functions to cause disease. Most of these effectors were detected by using screens for avirulence functions. Now, more and more candidate TTSS effectors are being discovered through bioinformatic analysis of bacterial genome sequences (Guttman *et al.,* 2002). These screens make use of conserved sequences, such as conserved regulatory domains or the signal sequence, to target these proteins through the TTSS that reside in the extreme N-terminus of the protein and are rich in polar amino acids (Guttman *et al.,* 2002).

Type IV pathway: This pathway is best known from studies on *Agrobacterium tumefaciens.* This pathway is the only secretion pathway described to translocate both proteins and nucleic acids. For example, VirD2/T-DNA nucleoprotein complex is delivered through the type IV pilus from *A. tumefaciens* directly into the plant cell (Citovsky et al., 1994).

Type IV secretion pathways use complex structures similar to conjugation structures (Blocker *et al.,* 2003) to interact with the eukaryotic host cells and deliver their effectors.

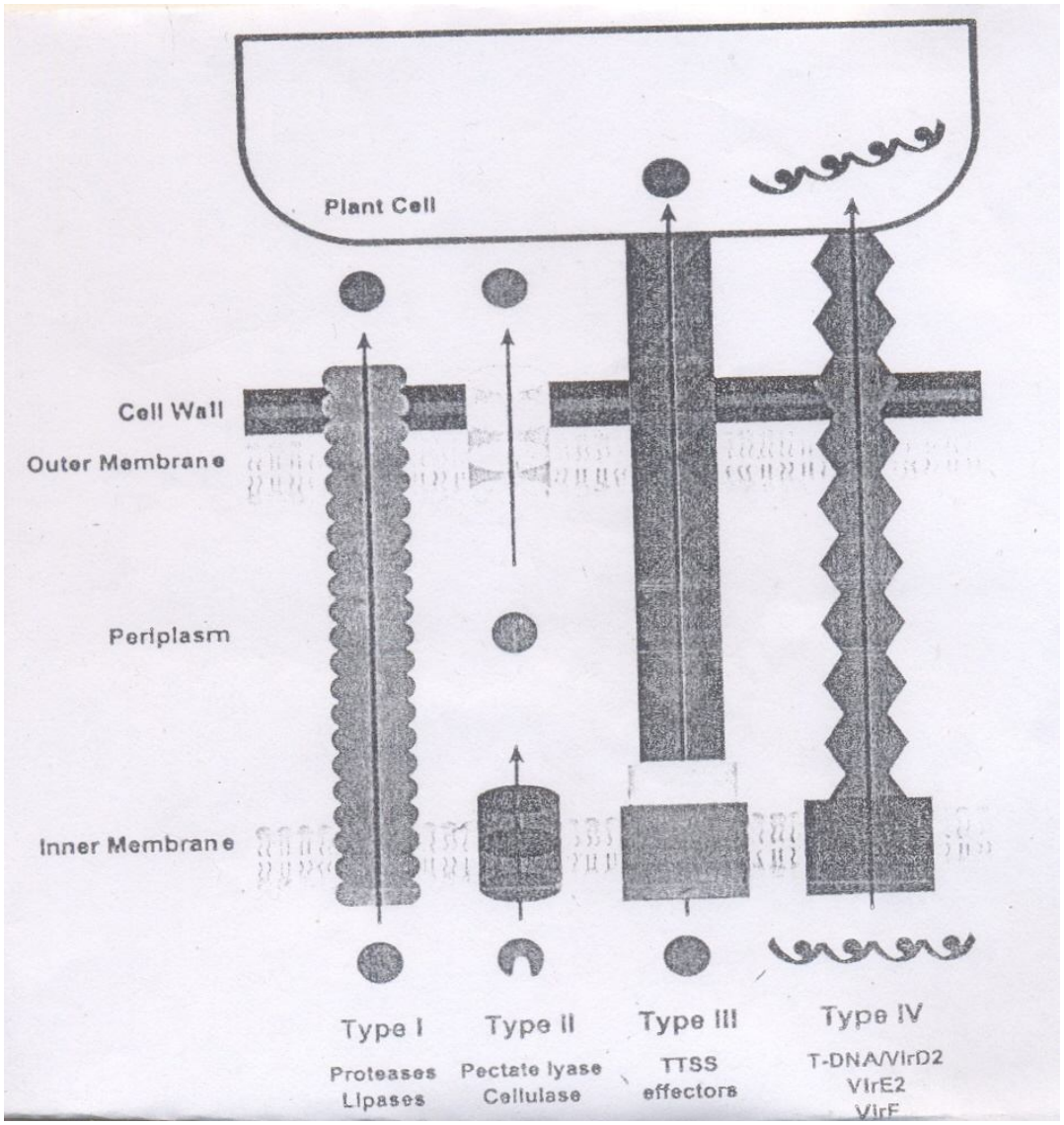

Fig.1: Roles of TTSS Effectors in Pathogenicity and Resistance

Although the mechanisms are not clear, TTSS effector proteins are predicted to play following roles:

1) Stimulate an increase in pH and nutrient content of the plant apoplast, making the apoplastic fluids more hospitable for bacterial multiplication (Atkinson and Baker, 1987).
2) Activate the host defence responses through recognition by a corresponding host R protein (avirulence function) (Bonas and Lahaye, 2002).

3) Inhibit the activation of host defence responses that are signaled by other TTSS avirulence effectors (Ritter and Dangl, 1996).
4) Inhibit basal plant resistance mechanisms (Hauck *et al.,* 2003).

Most of the over 40 known bacterial TTSS effectors were originally identified by their avirulence function in gene for gene interactions (Bonas and Lahaye, 2002) and as a consequence, most research has focused on understanding how thes proteins interact with plant proteins to activate defense responses (Martin *et al.,* 2003). Such plant defence responses are characterized by many cellular and molecular events, including the formation of active oxygen species, defense gene induction, and in many cases, a rapid, localized cell death called the hypersensitive response (HR). Activation of the HR, which is a genetically controlled and regulated process similar to programmed cell death (Gilchrist, 1998), is frequently used in studies to indicate activation of defense responses by effectors.

Some TTSS effectors modify plant target proteins inside the plant cell to stimulate either the activation of defense (Fig2 A and B) or the induction of disease (Fig2 C). Interactions involving TTSS effectors from *Pseudomonas syringae* and their target protein from *Arabidopsis thaliana* provide excellent examples of these possibilities. *P. syringae* TTSS effectors AvrPphB and AvrRpt2 are cysteine proteases whose proteolytic activity is essential for elicitation of the HR (Axtell and Staskawicz, 2003). HR elicitation by AvrRpt2 in *Arabidopsis* plants containing the corresponding resistance gene product, RPS2, requires cleavage of a plant membrane-bound target protein called RIN4. RIN4 is complexed with the RPS2 resistance protein and cleavage of RIN4 by AvrRpt2 protease results in the release of RIN4 from the complex (protein degradation in Fig2 A). Change in the overall structure of the complex is thought to be detected by RPS2, and thus RPS2-mediated resistance response is signaled. Interestingly, two other bacterial effectors from *P. syringae*, AvrRpm1 AvrB, which are not proteases, also use RIN4 as a plant target, but they signal resistance through another R protein, RPM1 (Mackey *et al.,* 2002) and by a different protein modification mechanism.

AvrB and AvrRpm1 TTSS effectors induce phosphorylation of RIN4. The phosphorylation of RIN4 is detected by RPM1-mediated resistance response (protein modification in Fig2 A).The above studies show that the TTSS effector modification of a plant target protein is required for avirulence function.

The requirement for modification by proteolytic cleavage or phosphorylation in the pathogenicity functions has not been determined, but it is speculated for AvrRpt2, again through interaction with the target RIN4 (Axtell and Staskawicz, 2003). Mackey *et al.* (2003) suggested that the normal function of RIN4 may be to activate basal defense in plants. Cleavage of RIN4 by effector AvrRpt2 proteases in the absence of any R proteins is predicted to suppress these basal defence responses and therefore result in enhanced susceptibility. Indeed, in absence of RPS2, cleavage of RIN4 by AvrRpt2 leads to more pathogen growth in tissues (Mackey *et al.,* 2003). Thus, RIN4 is the virulence target of AvrRpt2 and its proposed function in the plant cell is to regulate a basal level of defence. RIN4 may also function in plant development because inactivation of RIN4 resulted in a dwarf phenotype in *Arabidopsis* (Mackey *et al.,* 2003). RIN4 is likely a critical intermediary protein in plant pathogen interactions in *Arabidopsis* because several TTSS effector proteins, including AvrRpt2, AvrRpm1 and AvrB, all interact with and modify this protein.

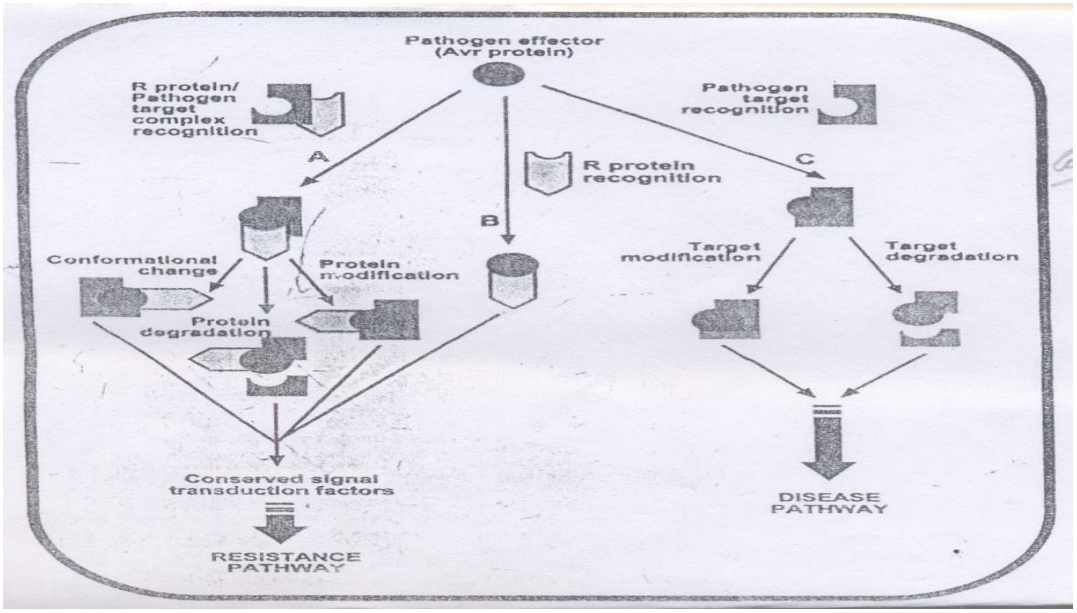

Fig. 2: Emerging Concepts in Effector Biology

1) **Many effectors are delivered into host cells:** Plant pathogenic bacteria and fungi have evolved the capacity to deliver effector proteins inside host cells through a diversity of mechanisms. Gram negative bacteria use specialized secretion systems such as TTSS to deliver proteins inside host cells (Block *et al.,* 2008).

2) Biotrophic fungi have evolved haustoria for this purpose. Haustoria were initially thought to primarily function in nutrient uptake but more recently, evidence emerged that haustoria take part in secretion of particular classes of host-translocated fungal effectors (Whisson *et al.,* 2007).
3) **Other effectors act in the apoplast:** Some effectors act in the extracellular space at the plant-microbe interface, where they interfere with apoplastic plant defences (Kamoun, 2006). Examples include the secreted protein effectors of the tomata fungal pathogen *Cladosporium fulvum*. This fungus is an extracellular parasite of tomato that grows exclusively in the apoplast and does not form haustoria or haustoria-like structures (Rivas and Thomas, 2005). All known *C. fulvum* effectors such as Avr2, Avr9, Avr4 and ECP2, are small cysteine-rich proteins that are thought to function exclusively in the apoplast (Thoma *et al.,* 2005). Oomycetes such as *Phytophthora infestans*, are also known to secrete apoplastic effectors in addition to host-translocated (cytoplasmic) effectors (Damasceno *et al.,* 2008). One common activity ascribed to many apoplastic effectors of *C. fulvum* and other fungal pathogens is their ability to inhibit and protect against plant hydrolytic enzymes such as proteases, glucanases and chitinases.
4) **One effector and many host targets:** Plant pathogen effectors frequently have more than one host target. *Pseudomonas syringae* AvrRpt2 is a TTSS effector with proteolytic activity against atleast five *Arabidopsis* proteins (Takemoto and Jones, 2005).
5) **Many effectors suppress plant immunity:** Recent work shows that effectors have highly adapted virulence functions. They perturb specific host targets in order to disrupt specific host processes-often host defenses. The most information to date about the virulence activity of pathogen encoded effectors has come from studies type III effectors from bacterial pathogens. Suppression of plant innate immunity has emerged as the primary function of effectors, particularly of TTSS effectors of plant pathogenic bacteria (Block *et al.,* 2008). Several TTSS effectors contribute to virulence by suppressing basal defenses. Other TTSS effectors suppress hypersensitive cell death elicited by various Avr proteins.

TTSS effectors probably interfere with host immunity via a diversity of mechanisms but the effectors studied so far are known to target three plant processes that are key to innate immunity namely, protein turn-over, RNA homeostasis and phosphorylation pathways (Block *et al.,* 2008).

A recent study illustrates the concept that plant pathogenic fungi can evade host immunity by evolving effectors that suppress R-gene mediated resistance e.g., the effector Avr1 of *Fusarium oxysporum* f.sp. *lycopersici* suppresses the resistance response conferred by the R genes I-2 and I-3 (Saskia *et al.,* 2009).

6) **Some effectors alter plant behavior and development:** Some effectors alter host plant behavior and morphology. One elegant example is coronatine which was shown to trigger stomatal reopening in *Arabidopsis* and thereby facilitate bacterial entry inside the plant apoplast (Melotto *et al.,* 2006). *Xanthomonas* effectors of AvrBs3 family of transcriptional activators are known to induce cellular division and enlargement in susceptible host plants. Many other plant associated organisms are known to alter morphology of their host plant, resulting in malformations that either create a protective niche or enhance dispersal. Classic examples include rhizobial nodules, galls induced by *Agrobacterium* spp. and other bacteria and witches broom and other developmental alterations caused by several pathogens such as phytoplasmas (Hogenhout *et al.,* 2008).
7) **Molecular mimicry by effectors:** Although effectors are encoded by pathogen genes, they function in a plant cellular environment and therefore could have been selected to mimic plant molecules. Strikingly, many effectors produce analogs and mimics of plant hormones. One example is coronatine, a toxin secreted by several pathovars of *Pseudomonas syringae* that is a structural and functional mimic of the plant hormone jasmonoyl-isoleucine. Coronatine has many effects that enhance bacterial colonization of plants. These include impacting phytohormone pathways such as jamming the induction of the salicylic acid-mediated resistance response and increasing the opening of plant stomatas. Other classic examples of phytohormone mimicry in plant pathogens include auxins and cytotoxins produced by various bacteria including *Agrobacterium* and gibberellins produced by several fungi such as *Gibberella fujikoroi* which causes the foolish seedling disease of rice.

Conclusion

Identifying effectors and exploring their molecular mechanisms not only are critical to understanding the disease mechanisms but also provide theoretical foundations for infectious disease diagnosis, prognosis and treatment.

Understanding the relative contribution of effectors to fitness is also useful information for predicting how durable a given resistance gene might be, or for designing plant genotypes that contain optimally effective combinations of resistance genes.

Comprehensive knowledge of the structure and function of pathogen effectors and the perturbations they cause in plants is a precondition for understanding the molecular basis of pathogenesis and disease. Identification of effectors that make a major virulence contribution may allow identification of the R genes to utilize. Directly recognized effector proteins might be used to screen for improved R genes that recognize effector domains that can tolerate little or no change. The understanding that effectors often attack host targets may allow placements of those host targets and their guardian R proteins into heterologous plant species, thereby converting adapted pathogens into non-host pathogens. Effectors can allow identification of host processes perturbed to promote disease, possibly allowing modification of those targets toward insensitivity. A new paradigm for disease activation has emerged in which plants recognize microbe-associated molecular patterns (MAMPs) and thereby activate local defenses, pathogens express effectors that suppress basal defenses, some plants express R proteins that directly or indirectly recognize effectors and activate strong defenses, and some pathogens modify or eliminate the effectors that the host can recognize so that the pathogen regains atleast some virulence on hosts that express these R proteins. R proteins may recognize pathogen effectors by direct physical interaction, or they may recognize them indirectly by sensing the host proteins upon which effectors have acted.

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