INVITED REVIEW

THE MOLECULAR BIOLOGY OF HYPERSENSITIVITY TO PLANT PATHOGENIC BACTERIA

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SUMMARY

It has now been nearly 40 years since the first observations by Klement (Klement, 1963) that resistance to bacterial plant pathogens was correlated with a rapid necrotic response induced in artificially inoculated tissue. This response, subsequently termed the hypersensitive response (HR) (Klement *et al.*, 1964), was initially defined as a rapid necrosis observed within 24 h after inoculation of the tissue with a potentially pathogenic, but incompatible, bacterial strain. The response appeared to be part of a syndrome that resulted in localized plant cell death, bacterial localization and bacteriostasis. Two types of incompatible interactions have been identified that are associated with the elicitation of the HR (Klement, 1982). Inoculation of a pathogen of one plant species into another plant species elicited the HR associated with nonhost resistance. The HR could also be elicited when a virulent strain pathogenic to one plant species was introduced into resistant cultivars of that same plant species (race-specific resistance).

Although originally controversial, it is now clear that the defense responses associated with hypersensitivity are a primary line of defense to many pathogens, particularly biotrophic pathogens (Goodman and Novacky, 1994; Hutcheson, 1998; Dangl and Jones, 2001; Lam *et al.*, 2001). The response appears to involve protein-mediated recognition of pathogen-derived proteins to initiate several defense responses, including programmed cell death. Cells responding hypersensitively, in turn, signal adjacent cells to accumulate antimicrobial compounds and to initiate the hormonal signaling process associated with systemic acquired resistance. What is most remarkable is that the molecular events occurring during hypersensitivity appear to be very similar to the initiation of innate cellular immunity in mammals and insects (Hutcheson, 1998; Collmer *et al.*, 2000; Staskawicz *et al.*, 2001). Thus, hypersensitivity may represent an ancient form of cellular defense used by eukaryotes in general. For purposes of brevity and simplicity, the discussion below emphasizes the studies on the HR elicited by *Pseudomonas syringae* strains.

Key words: Tipe III secretion, *hrp* genes, *Pseudomonas syringae*, hypersensitive response, active oxygen.

EARLY PHYSIOLOGICAL STUDIES ON ELICITATION OF HYPERSENSITIVITY

Early studies on hypersensitivity made several significant observations regarding the mechanism for elicitation of the HR by plant pathogenic bacteria. Because the response was induced in response to 'incompatible' strains of the bacterium, it was logical to predict that the bacterium had to be producing some kind of stimulus, termed an elicitor, that resistant plants were responding to (Keen and Holiday, 1982). Cells of the resistant plant in turn must express a surveillance system that could detect these postulated elicitors. It could also be predicted that some source of variation had to exist in each system to explain the unique host ranges of pathogens and the resistance specificities of the plant hosts.

Initial studies used classic biochemical and immunological approaches to try to identify the postulated elicitor of the HR. Serogroups did not correlate well with host range (Young *et al.*, 1992), suggesting that the specificity of the interaction did not lie in an exposed surface feature of the bacterium, such as lipopolysaccharides or flagellar proteins. In nearly all cases, cell-free preparations derived from HR-eliciting bacteria failed to elicit the HR (Klement and Goodman, 1967; Lyon and Wood, 1976). These studies also indicated that the eliciting signal was not a conserved biochemical component of the cells. Living cells were required to elicit the response, thereby suggesting that perhaps some metabolic activity of the bacterial cells was required to produce the elicitor.

By using inhibitors of specific metabolic processes, such as antibiotics, it was established that de novo transcription and translation by the bacteria was needed to initiate the HR (Klement and Goodman, 1967; Sasser,

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1982). This apparent induced gene expression was limited to the initial stage of the interaction called the induction stage. For the HR elicited by *P. syringae* strains, this induction stage required 2-4 hours, depending upon the strain and environmental conditions. The induction stage was not fixed but could be altered by the culture conditions used to grow the bacteria. Growth of the bacteria in a minimal media lacking a broad spectrum amino acid source substantially reduced or abolished the induction stage for the HR (Hutcheson *et al.*, 1989; Yucel *et al.*, 1989). The requirement for an induction stage suggested that elicitor production was delayed until the requisite genes encoding the elicitor, or necessary for its phenotype, were expressed. Again attempts to define the biochemical nature of this delayed elicitor production by extracting the elicitor from inoculated tissue after the induction stage was completed or by flushing it into fresh tissue were unsuccessful (Klement, 1977).

Subsequent studies focused on the role of the living bacterial cell in the elicitation of the HR. These studies established that contact between live metabolically active bacterial cells and the plant cells was required to initiate the HR (Klement, 1977; Stall and Cook, 1979). Furthermore, a single bacterial cell was sufficient to elicit a response by a single plant cell (Turner and Novacky, 1974), suggesting that plant cells could respond to very low levels of the elicitor.

These early studies also demonstrated an active role for the plant cells in the HR. The HR was not simply induced necrosis in the plant cells caused by some toxic product produced by the bacterium. The HR also required *de novo* protein synthesis by the host cells (Keen *et al.*, 1981; Greenberg and Sussex, 1996; Mittler and Lam, 1996). Such phenomena had been described in animal cells as programmed cell death (Ghosh *et al.*, 1998). In summary these studies suggested that the HR was the result of a pathogen-derived elicitor, formed by *de novo* gene expression during an induction stage, initiating a genetic response in the plant cells that culminated in programmed cell death. Strains of distinct host ranges must either differ qualitatively or quantitatively in the production of the postulated elicitor(s). Plants in turn must differ in their ability to recognize and respond to these postulated elicitors.

GENETICS OF THE HYPERSENSITIVITY PHENOTYPE

It was clear from the preceding experiments that biochemical methods were unlikely to succeed in identifying the postulated elicitor(s) of the HR. By this time, however, transposons and broad host range cloning vectors had been developed that allowed the application of microbial genetics to the problem. These studies identified two gene systems essential to the elicitation of the HR by *P. syringae* strains and other plant pathogenic bacteria.

The simplest interactions to investigate genetically were the incompatible interactions observed during race specific resistance. Plant breeders had established that cultivar-specific resistance to several bacteria followed the predictions of Flor (Flor, 1971). A single dominant resistance gene conferred resistance to specific races of the bacterial pathogen. This in turn would predict that the races should carry single dominant genes conferring 'avirulence' (*avr* genes). To test this hypothesis, a genomic library from a race 6 strain of *P. syringae* pv. *glycinea* (virulent on a defined set of soybean cultivars) was cloned into a broad host range vector and transformed into a race 4 strain that was virulent on the indicator soybean cultivars tested (Staskawicz *et al.*, 1984). A clone was identified that reduced the virulence of the transformant to the same set of cultivars as those susceptible to race 6. In the now resistant cultivars, the transformant elicited the HR like the parent race 6 strain. A single *avr* gene, *avrA*, was identified within this clone that was responsible for the phenotype (Napoli and Staskawicz, 1987).

Subsequent studies showed that all bacteria capable of eliciting the HR carry *avr* genes. At least 50 distinct *avr* genes have now been identified in plant pathogenic bacteria (Dangl, 1994; Leach and White, 1996; Vivian and Gibbon, 1997). Many, if not all, *P. syringae* strains have since been found to carry multiple *avr* genes (see below). Preliminary analysis of the *P. syringae* DC3000 genome indicates that this strain carries alleles of 18 known *avr* genes and may carry as many as 43 additional genes that could exhibit Avr-like phenotypes (A. Collmer, personal communication).

As before, the mechanism by which the products of *avr* genes elicited the HR was not obvious. Most Avr products lacked significant similarity to other proteins of known function. Purified protein products were devoid of activity when infiltrated into tissue of a plant known to respond hypersensitively to strains expressing this *avr* gene (Staskawicz *et al.*, 1987). One explanation could be that *avr* genes encoded a product functioning in a biosynthetic pathway for the postulated elicitor. Strains expressing one *avr* gene, *avrD* isolated from a *P. syringae* pv. *tomato* strain (Kobayashi *et al.*, 1989), produced biochemically isolable elicitors by the enzymatic activity of AvrD (Keen *et al.*, 1990). These elicitors, acyl lactones of the syringolide family (Keen *et al.*, 1996), could cause a response in cultivars carrying the corresponding AvrD-linked *Rsg4* resistance gene. This excit-

ing result proved, however, to be of limited application. Other Avr products did not appear to function as part of biosynthetic pathways and no diffusible elicitors could be isolated from strains expressing other *avr* genes.

An alternative explanation for the cryptic activity of Avr products was that the phenotype of most *avr* genes was dependent upon other pathogenicity determinants of the bacterium. Transposon mutagenesis had identified a second class of genes associated with the elicitation of the HR (Anderson and Mills, 1985). Transposons inactivate a gene by physically inserting into the coding sequence. Several groups were able to isolate prototrophic transposon mutants of plant pathogenic bacteria that were incapable of eliciting the HR during previously incompatible interactions, and surprisingly, also turned out to be non-pathogenic as well (see references in Hutcheson *et al.*, 1989; Hutcheson, 1999). These mutations mostly clustered in a set of genes named *hrp* genes (for hypersensitive response and pathogenicity) (Lindgren *et al.*, 1986). All strains of a bacterial species were found to carry the homologous *hrp* gene cluster and phenotypically similar gene clusters were identified in nearly all Gram-negative bacteria capable of causing disease in plants. These include *P. syringae* (Lindgren *et al.*, 1988) (Huang *et al.*, 1995), *Erwinia* spp. (Bauer *et al.*, 1994; Bogdanove *et al.*, 1996), rhizosphere strains of *P. fluorescens* (Preston *et al.*, 2001), *Xanthomonas* spp. (Bonas *et al.*, 1991), *Ralstonia solanacearum* (Boucher *et al.*, 1992) and rhizobia (Viprey *et al.*, 1998; Gottfert *et al.*, 2001). The only exceptions thus far have been *Agrobacterium* ssp. and *Xylella fastidosa* (Lambais *et al.*, 2000).

The role of *hrp* genes in elicitation of the HR was initially equivocal. Hrp mutants also failed to multiply in the tissue making it difficult to resolve whether the HR minus phenotype of the mutants was due to the inactivation of some essential gene involved in the elicitation of the HR or was the indirect result of the inability to colonize that host which secondarily prevented some essential metabolic process from occurring. The role of *hrp* genes in the elicitation of the HR was firmly established when it was shown that the expression of the core *hrp* gene cluster from a *P. syringae* strain (Huang *et al.*, 1988), and later from an *E. amylovora* strain (Wei *et al.*, 1992), enabled previously nonpathogenic bacteria, such as *E. coli*, to elicit the HR. This gain of function could only have occurred if the *hrp* genes were essential to elicitation of the HR. Twenty seven *hrp* genes were identified in *P. syringae* strains that were necessary to produce the phenotype (Fig. 1) (Huang *et al.*, 1991; Heu and Hutcheson, 1993; Huang *et al.*, 1993; Lidell and Hutcheson, 1994; Xiao *et al.*, 1994; Huang *et al.*, 1995).

In these analyses, the *hrp* genes of *P. syringae* strains, *Erwinia* strains and *P. fluorescens* were found to be most closely related. These *hrp* clusters have since been classified as group I *hrp* clusters (Alfano and Collmer, 1997; Hutcheson, 1997). Within the bacteria carrying group I *hrp* clusters, 25 of 27 genes were at least partially conserved and the order of genes within operons retained. Two other differences were observed in group I *hrp* clusters: several operons have been relocated in *Erwinia* strains relative their counterparts in the *Pseudomonas* strains (Kim *et al.*, 1998); and the regulatory system was only partially conserved (see below) (Wei *et al.*, 2000). The *hrp* clusters of *Xanthomonas* strains and *R. solanacearum* were also closely related to one another but were substantially divergent from group I *hrp* clusters (VanGijsegem *et al.*, 1993; Bonas, 1994). This divergence extended not only to the translational similarities detected between partially conserved components, but also to the transcriptional organization of genes and to the regulatory mechanism. Because of the substantial differences, these *hrp* gene clusters were classified as group II *hrp* clusters (Alfano and Collmer, 1997; Hutcheson, 1997).

Several attempts were made to identify biochemical traits associated with expression of *hrp* genes. These studies initially failed to detect novel secreted proteins in culture filtrates, changes in LPS or other surface features that were correlated with expression of the *hrp* genes (Li *et al.*, 1992). In contrast to the *avr* genes, however, many *hrp* genes did have amino acid sequence homologs in the databases that could be used to generate hypotheses as to their function. The products of at least 9 *hrp* genes were found to share significant amino acid sequence similarity to the products of genes essential for pathogenicity of mammalian pathogens, such as *Yersinia pestis* and *Salmonella* serovars (see VanGijsegem *et al.*, 1993; Alfano and Collmer, 1996; Hutcheson, 1997; Hueck, 1998; Hutcheson, 1999). Up to this time, there had been little, if any, similarity in the mechanisms for pathogenesis used by bacteria pathogenic to plants or to mammals. These 9 products were the same proteins that were conserved between group I and II *hrp* clusters. These conserved genes were subsequently renamed hrc genes (hypersensitive response conserved) in the plant pathogenic bacteria (Bogdanove *et al.*, 1996). In the mammalian pathogens, these pathogenicity determinants were also clustered as observed in the plant pathogens (Rosqvist *et al.*, 1995). In addition to the amino acid sequence similarities detected, the order of conserved genes within the gene clusters was also partially conserved. To date, similar gene clusters have been identified in *Yersinia* spp. (Cornelis *et al.*, 1998), *Salmonella* spp. (Groisman *et al.*, 1999), *Shigella* spp.

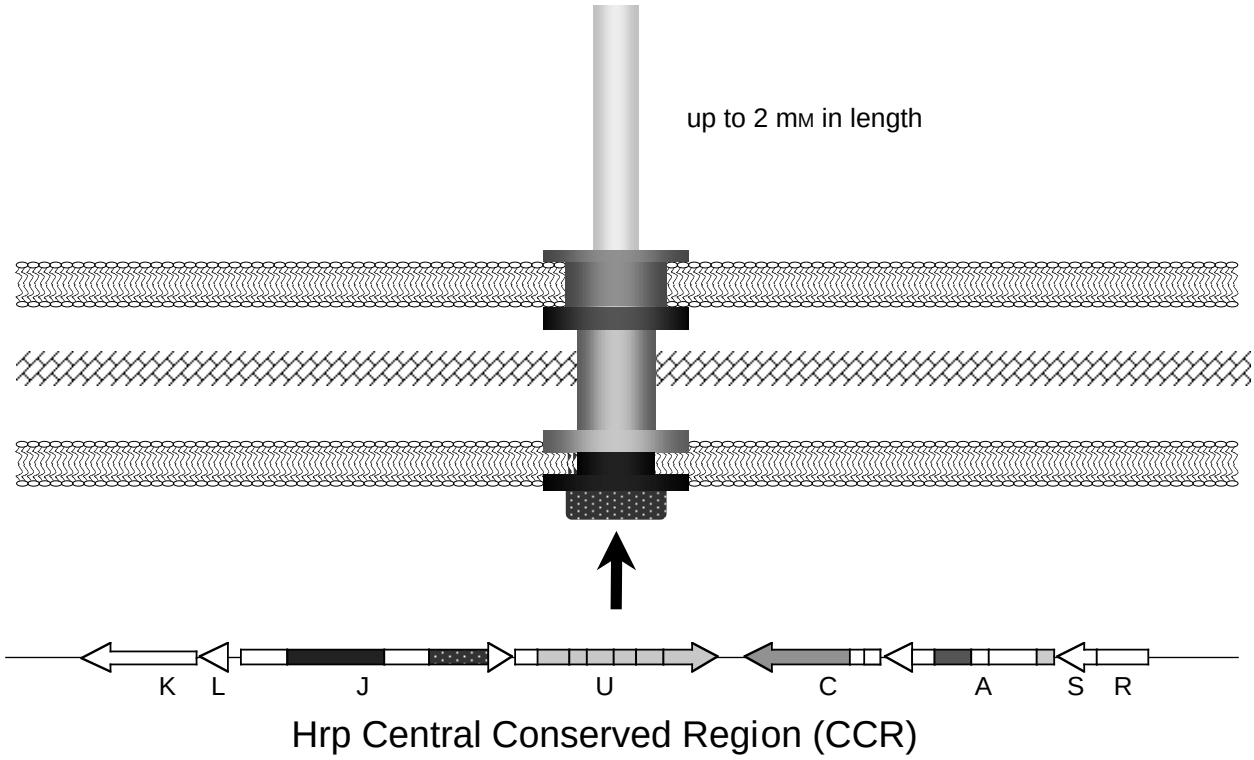

Fig. 1. The *P. syringae hrp* central conserved region and type III secretion system secreton. The operons of the *hrp* CCR are indicated by the labeled arrows. Genes are represented by the boxes. Except for hrpA encoding the hrp pilus structural protein (lightest gray), shaded boxes represent *hrc* genes whose products are conserved in all TTSS (Bodganove *et al.*, 1996). The deduced secreton structure is based upon the model of Blocker *et al.*, 2001. Gene products are predicted to be locatized as indicated by the shading of the gene products. hrpJ and hrpU operon products are associated with the inner membrane and have homologs in flagellar biosynthesis. hrpC and hrpA operons have products that associated with the outer membrane. The *hrp* CCR is shown on the cytoplasmic side of the inner membrane as drawn. The black arrow indicated the probable location of the secretion port.

(Parsot and Sansonetti, 1999), enteropathogenic and enterohemorhagic strains of *E. coli* (Nataro and Kaper, 1998), *P. aeruginosa* (Yahr *et al.*, 1997), *Chlamydia* spp. (Fields and Hackstadt, 2000), *Bordetella pertussis* (Yuk *et al.*, 2000) and *Burkholderia cepacia* (Parson *et al.*, 2001) that are essential for pathogenicity. Pathogenesis by these bacteria shares in common interactions with epithelial cells during early stages of pathogenesis (Galan and Collmer, 1999). During initial stages of pathogenesis, these bacteria adsorb to and elicit responses in the epithelial cells that form the surface of the gastrointestinal tract or the respiratory tract.

TYPE III PROTEIN SECRETION

The conservation of 9 core genes in these plant and mammalian pathogens suggested that the function of these gene clusters might be similar. In plant pathogenic bacteria, it was clear that *hrp* genes had to be involved in producing some form of an extracellular signal in order to elicit responses in host cells. Mammalian pathogens also elicited responses in the host cells during pathogenesis, suggestive of an extracellular function for these genes. For the mammalian pathogens, however, it was clear that the products of these genes were involved in the secretion of proteins. Virulent strains of *Yersinia* spp. (Straley *et al.*, 1993), and later *Salmonella* (Galan, 1996), *Shigella* (Menard *et al.*, 1996) and enteropathogenic and enterohemorhagic strains of *E. coli* (Jarvis *et al.*, 1995; Kaper *et al.*, 1999) secreted proteins into the growth medium that were required for virulence. Thirteen secreted proteins, called Yops (*Yersinia* outer proteins) could be detected, for example, in culture filtrates of *Yersinia* strains under the appropriate conditions (low Ca^{2+}, 37°C) (Straley *et al.*, 1993; Hueck, 1998). These proteins were only secreted from strains expressing the *Yersinia* homologs of the hrc genes. Because this pathway appeared to represent a distinct protein secretion pathway it was named type III protein secretion (Salmond and Reeves, 1993; VanGijsegem *et al.*, 1993). The genes conserved among the

various plant and mammalian pathogens encoded the conserved components of this type III secretion system (TTSS). Most TTSS also require proteins unique to that system. The function of these unique proteins has not yet been established.

It was also clear that there was something unusual about the TTSS. Even though strains of mammalian pathogens expressing the TTSS could secrete virulence proteins into the medium, the purified proteins, like Avr products, had minimal activity when simply added to the medium independently of the source bacteria (Hueck, 1998). Furthermore, many of the secreted proteins had domains, such as phosphatase or kinase domains, suggestive of activities within the cytosol of the host cells. Consistent with this observation, many of these effector proteins were shown to have activity once taken up or injected into cells. These activities included alterations to the cytoskeleton of the cells, insertion of membrane proteins, disrupted cell signaling cascades, or cytotoxicity, responses similar to the host cell responses observed during pathogenesis by the virulent bacterium. These proteins commonly cause morphological changes in cells, such as pedestal formation induced by EPEC and EHEC strains of *E. coli* (Kaper *et al.*, 1998). Secreted proteins that were necessary for pathogenicity were termed 'effector' proteins because they appeared to mechanistically function in pathogenesis. Their primary function for many mammalian pathogens is suppression of phagocytosis.

The most likely function for the TTSS appeared to be the polar translocation of effector proteins into host cells. Contact between the pathogen and host cells strongly enhanced expression and secretion of Yops, but most, if not all, of the newly secreted proteins were found to be internal to the host cells (Rosqvist *et al.*, 1994). Almost none were found in the medium, arguing that these proteins were being directly translocated into host cells. By using fusions constructed to the reporter protein, adenylate cyclase, which is only active in the cytoplasm of host cells, several Yop proteins were shown to be vectorally translocated into host cells (Sory and Cornelis, 1994; Sory *et al.*, 1995). Thus, consistent with the model, the effector proteins of *Yersinia* strains appeared to be 'injected' into host cells by the TTSS. Similar results were obtained for the other mammalian pathogens (see Hueck, 1998; Galan and Collmer, 1999).

A hint as to the mechanism by which bacteria could inject proteins into their host came from the other structural similarities detected to conserved components of the TTSS. In addition to the similarities identified between the components found in the various pathogenic bacteria, several of the conserved core genes also shared sequence similarity with gene products required for flagellar biosynthesis (Lidell and Hutcheson, 1994; VanGijsegem *et al.*, 1995) and another was related to a key protein required by filamentous phages for phage particle assembly on the outer membrane surface (Huang *et al.*, 1992; VanGijsegem *et al.*, 1993). In flagellar biosynthesis, a basal body is assembled in the inner membrane from the components that have homologs in the TTSS (Macnab, 1996; Chilcott and Hughes, 2000). Proteins for the rod, hook and flagellum are then sequentially loaded in an ATP-dependent process into a hollow channel within the basal body and added to the distal end of the growing structure. Flagella can thus be considered hollow tubes capable of translocating proteins. When flagella are sheared to remove their distal cap, the secretion of proteins into the medium via the flagellar apparatus can be detected (Hughes *et al.*, 1993). The process is highly specific and the signal associated with translocation of proteins into the flagellum was found to reside in the N-terminus of the translocated proteins (Kuwajima *et al.*, 1989; Chilcott and Hughes, 1998).

TTSS could thus be predicted to function similarly to flagellar biosynthesis. Proteins, carrying specific N terminal signals, were likely to be loaded into the central core of a basal body-like structure and translocated to the tip of a pilus/flagellum-like structure formed similarly to filamentous phage particle assembly. The predicted TTSS membrane 'needle' complex, described as a 'secreton', has been observed by electron microscopy in flagella-deficient mutants of *Salmonella* (Kubori *et al.*, 2000) and *Shigella* (Blocker *et al.*, 2001) strains. Component proteins were localized in the TTSS as predicted from their structural properties. We assume that a similar structure assembles in *P. syringae* and other plant pathogenic bacteria (Fig. 1) but this has not yet been observed directly. The basic model for how TTSS operates has been confirmed as described below.

The most controversial feature of TTSS is the mechanism by which effector proteins are directed to the secreton. The initial studies employing fusions to adenylate cyclase showed that the secretion signal resided in the amino terminal 15 amino acids of several Yops (Sory *et al.*, 1995). This region, however, was not conserved in any obvious feature between the various Yops. Mutagenesis did not indicate a requirement for any specific amino acid within this region for secretion (Anderson and Schneewind, 1997). Even a frameshift mutant, producing a completely altered amino acid sequence for the region, appeared to be secreted. This latter result was interpreted to indicate that the secretion signal most likely resided in the mRNA (Anderson and Schneewind, 1997; Anderson *et al.*, 1999). Subsequent

studies, however, have not supported this conclusion as the experimental system used to identify the mRNA signal has been recently challenged (Lloyd *et al.*, 2001). Thus the mechanism by which protein are directed to the TTSS remains elusive. What has been confirmed is that the secretion signal is located at the 5' end of the coding sequence for the effector protein, irrespective of the bacterium and TTSS.

SECRETION OF PROTEINS BY THE *P. SYRINGAE* HRP-ENCODED TTSS

The function of the *hrp* gene products could be deduced by analogy. Since the apparent TTSS of *P. syringae* strains and other plant pathogenic bacteria required structurally related proteins to their counterparts of mammalian pathogens, it must mechanistically be similar. Pathogenesis by *P. syringae* strains could be predicted to involve the translocation of effector proteins into the cytoplasm of host cells by a needle-like TTSS structure (Fig. 2).

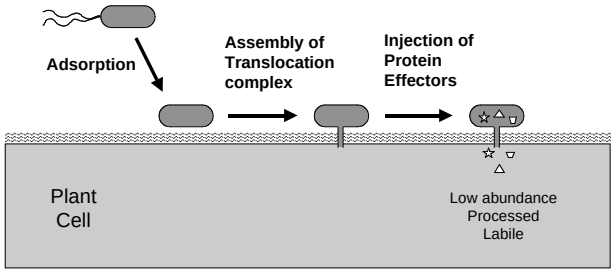

Fig. 2. Pathogenesis by bacteria utilizing TTSS. After introduction into plant tissue, bacterial cells adsorb to the surface of plant cell walls. Little is known about this process. Within the first 2-3 h of the interaction, expression of *hrp/hrc* genes is induced and a TTSS assembles. Bacteria are then thought translocate effector proteins into the cytoplasm of plant cells. Strains are predicted to differ qualitatively, and possibly quantitatively, in the effector proteins translocated into host cells by the TTSS. Effector proteins appear to be translocated at a low level (difficult to detect) and can be processed by host systems as described in Fig. 3. Translocated proteins appear to have a short half-life once recognized by resistence gene-associated defense systems (Dangl and Jones, 2001).

The first protein shown to be secreted by a *P. syringae* TTSS was HrpZ (He *et al.*, 1993), also known as harpin$_{Pss}$. It is a 34.5 kDa, glycine-rich, cysteine-deficient, heat stable protein found in culture filtrates of some *P. syringae* strains induced to express *hrp* genes. Secretion of HrpZ required the *hrp*-encoded TTSS (Alfano *et al.*, 1996). At high concentrations, HrpZ could elicit a necrotic response in host tissue, but genetic analyses indicated that the HrpZ was not the primary elicitor of the HR (Alfano *et al.*, 1996), and in fact, was fully dispensable for the phenotype (Pirhonen *et al.*, 1996). Mutants carrying nearly complete deletions of *hrpZ* retained an HR positive phenotype. Recent studies suggest that HrpZ can open cation-conductive pores in membranes (Lee *et al.*, 2001) (see below). Release of K^+ from cells is known to be an early host response elicited by *P. syringae* strains (Atkinson and Baker, 1987; Baker *et al.*, 1987). The altered membrane physiology caused by the activity of HrpZ (Novacky *et al.*, 1994) could facilitate the release of nutrients from the cell, and thus, would be essential for pathogenesis but not for elicitation of the HR.

A second protein secreted by the *hrp*-encoded TTSS is HrpA. HrpA is a 11 kDa protein that is the structural protein of TTSS-associated pilus in *P. syringae* strains (Roine *et al.*, 1997; Romantschuk *et al.*, 2001). HrpA appears to be one of the most variable proteins produced by the *hrp* gene cluster (Deng *et al.*, 1998). Amino acid sequence identity between strains of *P. syringae* can be as low as 28%. This pilus was shown to be associated with type III secretion (Wei *et al.*, 2000). Most significantly, studies on the *P. syringae* TTSS-associated pilus provided direct experimental evidence for the postulated mechanism for type III protein secretion. In an elegant set of experiments, newly synthesized proteins were shown to accumulate at and near the tip of the TTSS-associated pilus in electron micrographs as predicted by the flagellar biosynthesis model described above (Brown *et al.*, 2001; Jin *et al.*, 2001). Thus the Hrp pilus is likely to be a hollow tube involved in the secretion of proteins via the *hrp*-encoded TTSS as predicted.

One other protein has been demonstrated to be secreted by the native *hrp*-encoded TTSS of *P. syringae*. HrpW is a 43 kDa protein with two distinct domains: the amino terminal domain is HrpZ-like (glycine-rich, cysteine-deficient) whereas the C-terminal domain shows sequence similarity to pectate lyases (Charkowski *et al.*, 1998). The purified protein did not exhibit any detectable enzymic activity but could elicit a necrotic response in tissue at high concentrations. Mutants carrying nonpolar mutations in this gene retained the ability to elicit the HR.

Other plant pathogenic bacteria expressing TTSS can also secrete proteins. The first protein shown to be secreted by any TTSS associated with a plant pathogenic bacterium was Harpin secreted by *E. amylovora* strains (Wei *et al.*, 1992). This TTSS has also been shown to secrete DspE (Bogdanove *et al.*, 1998). Much later it was shown that the cloned *E. amylovora hrp*-

encoded TTSS expressed in *E. coli* secreted several *P. syringae* effector proteins (Ham *et al.*, 1998). The TTSS encoded by the group II *hrp* cluster of *R. solanacearum* forms a pilus of the pilin protein HrpY and has been shown to secrete several Pop proteins (Gueneron *et al.*, 2000; VanGijsegem *et al.*, 2000).

ROLE OF TTSS IN AVR PHENOTYPES

The *hrp*-encoded TTSS, thus, was required for elicitation of the HR, could secrete proteins, and could be predicted to translocate effector proteins into the cytoplasm of host cells, but the preceding analyses had not identified the primary elicitor of the HR. In the case of the *P. syringae* strains, there were hints that at least some of the translocated effector proteins eliciting the HR were products of *avr* genes. First, the phenotype of Avr products was lost in *hrp* mutants in which the TTSS was inactive (Lindgren *et al.*, 1989). Second, resistance gene products mediating responses to *P. syringae* strains, when cloned and characterized, were apparent cytoplasmic proteins (see below). If these proteins were to function as receptors for specific Avr products as predicted, a mechanism would be needed to translocate Avr products into plant cells. Finally, several groups noted that *avr* genes were co-expressed with *hrp* genes in *P. syringae* strains via the same regulatory mechanism (Huynh *et al.*, 1989; Innes *et al.*, 1993; Shen and Keen, 1993; Xiao and Hutcheson, 1994).

By using the plasmid-borne TTSS isolated from *P. syringae*, phenotypic expression of *avr* genes was linked to the secretion activities of the *hrp*-encoded TTSS (Pirhonen *et al.*, 1996). *E. coli* transformants carrying any of seven *P. syringae avr* genes could produce an R gene-dependent HR only when the *hrp*-encoded TTSS was co-expressed with a specific *avr* gene. This gain of function was consistent with the translocation of the Avr products into plant cells by the *hrp*-encoded TTSS.

Direct demonstration of the translocation of Avr products into plant cells has proved difficult. In most cases, the secretion of Avr products into the medium or plant cells was below the sensitivity of the assays employed. Fusions to reporter proteins, such as adenylate cyclase, ß-glucuronidase (GUS), green fluorescent protein (GFP) or eukaryotic transcriptional factor domains, impeded secretion (Lidell, 1998; Mudgett and Staskawicz, 1999) (Wood, Lidell, Katagiri and Hutcheson, unpublished results). Epitope-tagged proteins were secreted at too low of a level to produce a signal in host cells in the early studies. What initially succeeded was to bypass the translocation issue altogether and ask a more direct question: where does an Avr product produce a phenotype in planta? These experiments produced results consistent with the hypothesis: *avr* genes transiently expressed in the cytoplasm of plant cells from any of several eukaryotic expression systems could elicit the HR in cells expressing the corresponding resistance gene independently of bacterial TTSS (Fig. 3) (Gopalan *et al.*, 1996; Leister *et al.*, 1996; Van den Ackerveken *et al.*, 1996; Lidell, 1998).

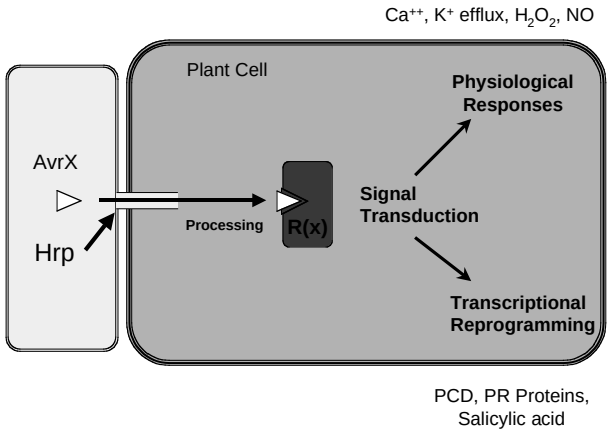

Fig. 3. Translocation of Avr products into plant cells and resistance gene-associated defense responses. Avr products are translocated by the hrp-encoded TTSS into the cytoplasm of the host cell. Some effector proteins appear to interact directly with resistance gene-associated sensors. Others, such as avrRpt2, undergo proteolytic processes prior to the interaction with its cognate sensor system. Some, *e.g.* AvrRpm1, undergo myristoylation at their amino termini and associate with the plasma membrane. AvrBs3 family members, associate with Importin and are compartmentalized into the nucleus. Mechanisms by which resistance genes associated sensor systems function are discussed in the text. The interaction of an Avr effector and its cognate sensor system initiates a signal transduction cascade involving protein kinases and Ca^{++} influx. Physiological responses observed include K^+ efflux, oxidative bursts and nitric oxide production. Transcriptional reprogramming leads to programmed cell death and production of salicylic acid and other signal molecules.

More recent studies have confirmed that the *hrp*-encoded TTSS can translocate an Avr product into plant cells. AvrRpt2 was shown to be secreted into culture filtrates at low levels from *P. syringae* DC3000 as the full length polypeptide (Mudgett and Staskawicz, 1999). In plant tissue, AvrRpt2 transiently expressed from an introduced construct was processed by proteolytic cleavage of the amino terminal 71 amino acids. The same result was observed when bacteria expressing the *hrp*-encoded TTSS and *avrRpt2* were inoculated into plant tissue. Since processing of AvrRpt2 occurred in the plant

cells, the protein had to be translocated as postulated. The avirulence activity of AvrRpt2 was found to reside in the carboxy terminal 135 amino acids. Thus AvrRpt2 consists of dispensable amino terminal secretion domain and a carboxy terminal effector domain (Guttman and Greenberg, 2001). Another group noted that several Avr products have myristyolation motifs at their amino termini that in eukaryotic proteins are used to direct the attachment of a short chain fatty acid (Nimchuk *et al.*, 2000). The myristyolated proteins then associate with membranes. The myristyolation motif of AvrRpm1 was important for its activity and AvrRpm1 was shown to be myristyolated in planta. Thus AvrRpm1 must also be translocated into plant cells. Nuclear localization signals have been identified in the AvrBs3-family proteins secreted by *Xanthomonas* strains that are essential for their activity and mediate localization to the nucleus of host cells (Yang and Gabriel, 1995; Van den Ackerveken *et al.*, 1996). Transcriptional activator domains necessary for function have also been identified in AvrBs3 (Szurek *et al.*, 2001).

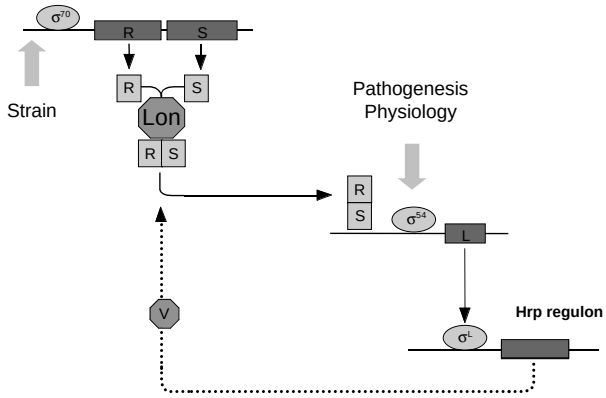

Fig. 4. The regulatory system controlling expression of the *P. syringae hrp* regulon. Sigma factors are indicated by the shaded ovals and known positive transcriptional factors by the square boxes. Negative acting regulatory determinants are shown by the octagons. The block arrows represent probable sites where the indicated effect occurs. The region upstream of the hrpR promoter is poorly conserved between strains (Hutcheson *et al.*, 2001). Since this area can include regulatory sites, it is possible that hrpR promoters are differentially regulated in different strains. Lon protease functions as a negative regulator of HrpR/HrpS activity (Bretz *et al.*, submitted). Environmental regulation of the hrp regulon occurs primarily at the level of the hrpL promoter.

ROLE OF THE INDUCTION STAGE

The involvement of a TTSS in the elicitation of the HR was consistent with the initial physiological studies. Bacteria produced elicitors in the form of effector proteins. Effector proteins were inactive in the elicitation of the HR unless translocated into the cytoplasm of host cells by the TTSS. Living bacteria would be required for TTSS to be active and the translocation process would require the bacteria to be in contact with the responding cell. Assembly of the *hrp*-encoded TTSS and the time required for production and translocation of effector proteins could explain the induction stage.

Several groups were able to show that transcription of *hrp* and *avr* genes in *P. syringae* strains was induced during the period corresponding to the induction stage. Expression of group I *hrp* genes and the genes for effector proteins (*avr*, *hrm*, *hop*) was found to be low during exponential growth in most rich media but enhanced expression could be detected beginning 1-2 h after inoculation of plant tissue (Huynh *et al.*, 1989; Rahme *et al.*, 1992; Wei *et al.*, 1992; Xiao *et al.*, 1992). Expression of *hrp* genes could also be induced in culture by incubation of the bacteria in acidic minimal salts media that lacked broad spectrum amino acid sources, such as casein hydrolysates (Xiao *et al.*, 1992). These media are thought mimic the conditions in planta.

In *P. syringae* strains, a complex regulatory system was identified that controls the environmental regulation of the *hrp* regulon (Fig. 4) but its role in modulating expression of *hrp* genes during pathogenesis has not been fully elucidated. This regulatory system involves several unusual regulatory proteins. The *hrpRS* operon encodes HrpR and HrpS which are both atypical enhancer binding proteins that lack a key modulatory domain found in most other members of the protein family (Grimm and Panopoulos, 1989; Xiao *et al.*, 1994). In the absence of this modulatory domain, these proteins are constitutively active as are HrpR and HrpS (Xiao *et al.*, 1994). HrpR and HrpS were subsequently shown to physically interact to activate the σ^{54}-dependent *hrpL* promoter (Hutcheson *et al.*, 2001). HrpL in turn is a constitutively active alternative sigma factor necessary for expression of the rest of the *hrp* regulon (Hutcheson *et al.*, 1996; Hendrickson *et al.*, 2000) and was a founding member of a new family of sigma factors distinguished by their small size and role in controlling extracellular activities (Xiao *et al.*, 1994; Xiao and Hutcheson, 1994; Wei and Beer, 1995). A HrpL-dependent promoter sequence has been identified that is present in all known *hrp* regulon promoters and is associated with initiation of transcription (Xiao and Hutcheson, 1994).

The simplest est model for regulation of the cluster by these regulatory proteins invokes induced expression of the *hrpRS* promoter during pathogenesis, but this promoter has been observed to be constitutively active

in several strains (Hutcheson *et al.*, 2001). Instead activity of HrpR and HrpS appears to be controlled by degradative turnover mediated by the regulatory protease LonB (Bretz *et al.*, submitted), and by HrpV, a product of the HrpC operon (Preston *et al.*, 1998), that binds to and modulates the activity of HrpS (Sussan *et al.*, unpublished results). LonB activity presumably sets the threshold for the level of *hrpRS* expression necessary to activate *hrpL* expression. Induction of *hrp* regulon expression during pathogenesis appears to occur at the level of the *hrpL* promoter (Hutcheson, 1997). The activity of LonB, HrpR, HrpS and/or HrpV under these conditions is currently being investigated. The involvement of σ^{54} in the expression of *hrpL* provides a pathway by which physiological conditions, such as amino acid starvation, can influence expression of the *hrp* regulon. The activity of σ^{54}-dependent promoters can be influenced by physiological conditions such as amino acid starvation during stringent responses (Sze and Shingler, 1999; Carmona *et al.*, 2000).

The regulation of group I *hrp* clusters in *Erwinia* strains and rhizosphere strains of *P. fluorescens* also utilize a HrpL homolog to control expression of the *hrp* regulon (Wei and Beer, 1995; Preston *et al.*, 2001). HrpS together with another regulatory protein, HrpX have been reported to control the expression of the *hrpL* promoter in *E. amylovora* strains (Wei *et al.*, 2000). A distinct regulatory mechanism linked to the activities of the HrpX (HrpB) and HrpG controls the expression of group II *hrp* cluster (Genin *et al.*, 1992; Wengelnik and Bonas, 1996; Brito *et al.*, 1999). There is strong evidence in the case for at least one group II *hrp* cluster that contact with plant cells mediates expression of the *hrp* regulon in these bacteria (Brito *et al.*, 1999; Alden *et al.*, 2000). Models for contact-dependent regulation of *hrp* genes are described in Fig. 5.

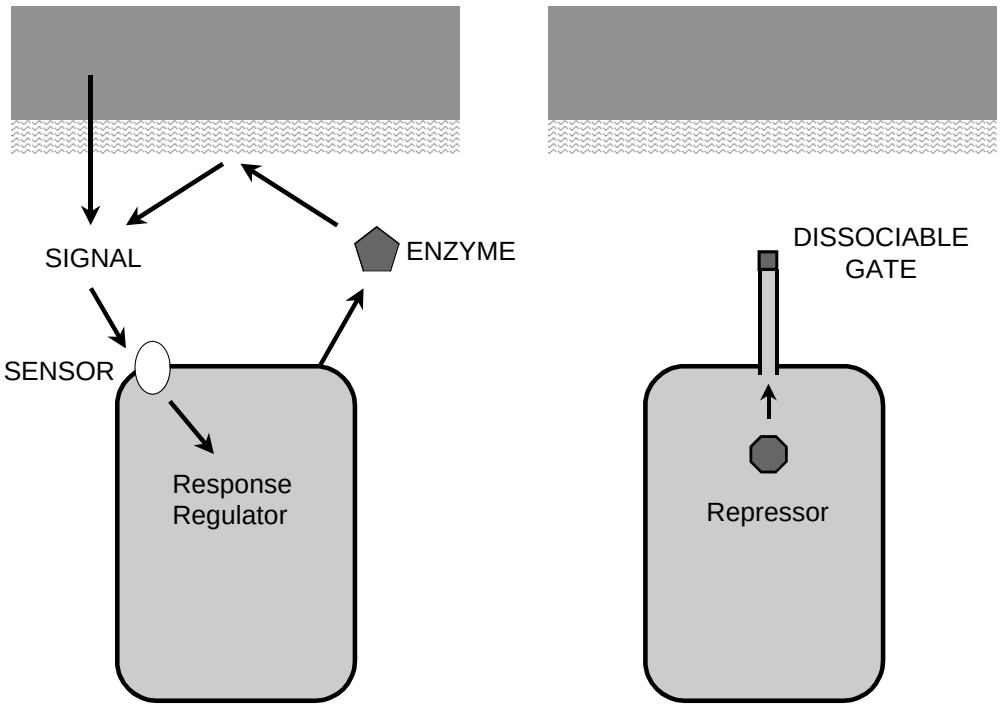

Fig. 5. Hypothetical models for contact-dependent regulation of *hrp* expression. On the left is the sensor model in which a signal molecule produced by the plant cells or released by an enzymic activity of the bacterium binds to a bacterial sensor protein to activate expression of the TTSS encoding genes. Such sensor proteins are common in bacteria and usually consist of a sensor domain linked to a protein kinase domain. The binding of the signal molecule activates the kinase activity which then phosphorylates one or more response regulators. The phosphorylated response regulators activate transcription of target genes such as the hrp genes. A modification of this scheme appears to regulate expression of hrp genes in Ralstonia solanacearum (Aldon *et al.*, 2000). An alternative model is the dissociable gate model based upon the mechanism for regulation of flagellar biosynthesis. In this model shown on the right, the TTSS is present at a low basal level in the pathogen but is gated to prevent untimely secretion of effector proteins. In proximity to host cells or by altering of the culture conditions, the gate protein complex dissociates from the hrp pilus opening the secretion channel. This would allow proteins to now be secreted by the TTSS. Transcriptional repressor proteins analogous to the TTSS-secreted FlgM (Chilcott and Hughes, 2000) could now be secreted to activate expression of the hrp regulon.

PERCEPTION OF TRANSLOCATED EFFECTORS

For plants to be able to recognize and respond to translocated elicitor proteins, they in turn, must have a mechanism to recognized these foreign proteins that have been introduced into their cells. Initial studies focused on race-specific resistance in which specific resistance genes were postulated to mediate the recognition of specific Avr products (Gabriel and Rolfe, 1990). Most resistance genes have been identified through map-based cloning. Genetic markers and recombination frequencies are used to map the gene of interest to a specific region of a chromosome (Leyser and Chang, 1996). Sequence markers are then used to refine location of the gene within this region through sequence polymorphisms generated by recombination. Eventually, a specific region within a yeast and/or bacterial artificial chromosome carrying this portion of the chromosome is identified and sequenced. The first resistance gene cloned by this method was the tomato *Pto* gene (Martin *et al.*, 1993), mediating hypersensitivity to *P. syringae* strains expressing AvrPto. Structural analysis indicated that Pto was a Ser-Thr protein kinase and yeast two-hybrid analyses (Bartel *et al.*, 1994) showed that AvrPto and Pto could physically interact as the model predicted (Tang *et al.*, 1996).

Through strategies similar to that described above, five classes of R genes have now been described in plants: 1) Pto-like Ser-thr kinases (STK); 2) LRR-NBS-CC proteins; 3) LRR-NBS-TIR proteins [these carry a Tol /Interleukin 1 Receptor (TIR) domain]; 4) extracellular, membrane-anchored LRR proteins; and 5) extracellular, LRR – transmembrane domain – cytoplasmic STK (Bent, 1996; Hammond-Kosack and Jones, 1997; Ellis *et al.*, 2000; Dangl and Jones, 2001). Since the characterized class 1 resistance gene products also require a LRR-NBS protein (Prf) for activity (Salmeron *et al.*, 1996), the common feature for these resistance genes is the involvement either directly or indirectly of LRR domains. LRR domains have been shown to be involved in pattern recognition during protein-protein interactions in several systems (Jones and Jones, 1996; Kajava, 1998). The NBS-TIR/CC domains are most likely involved in the interface with cellular signal transduction systems.

With the completion of the *Arabidopsis thaliana* genome (Lin and Al, 1999; Consortium *et al.*, 2000; Institute *et al.*, 2000; Mayer and Al, 2000; Theologis *et al.*, 2000) a more complete picture of the presence and deployment of R-products can be deduced. Approximately 50 class 1, 90 class 2, 60 class 3, 30 class 4, and as many as 174 class 5 R genes have been identified in the *Arabidopsis* genome based upon domain structure analysis (Dangl and Jones, 2001). Plants most likely carry a wide array of resistance genes with different specificies and affinities for effector molecules. In addition to translocated effector proteins, plants can also respond to bacterially produced syringolides (Keen *et al.*, 1996) and flagellin (Gomez-Gomez *et al.*, 2001).

The mechanism by which most R products recognize translocated Avr products to initiate a response has not been established. Presumably recognition involves the LRR domain (Jones and Jones, 1996). Specificity of class 4 R products has been linked to the LRR domain (Parniske *et al.*, 1997). Compensatory mutations to mutations in an Avr product have mapped to the LRR domain of the R product in another case (Anderson *et al.*, 1997). Point mutations altering the specificity of R gene mediated recognition also map to the LRR (Bent *et al.*, 1994; Mindrinos *et al.*, 1994; Leister *et al.*, 1996). Unfortunately, the direct interaction between bacterial Avr products and their corresponding R products has only been demonstrated for the class 1 Pto-AvrPto interaction (Tang *et al.*, 1996). Apparent complexes between a class 2 R product and AvrRpt2 have been detected in vivo (Leister and Katagiri, 2000). *Magnaporthe grisea avrPita* has been shown by two methods to physically interact with a class 2 R product as the model predicts (Jia *et al.*, 2000).

Most likely, R products function similarly to their counterparts involved in the initiation of innate cellular immunity in animals (Dangl and Jones, 2001). This process can also culminate in programmed cell death, called apoptosis (Navarre and Zychlinsky, 2000), and can involve homologs to Avr products (Galan, 1998). In animals, the Tol-Interleukin 1 receptor carries 'LRR-like', transmembrane domain, and TIR domains that functions in the initiation of apoptosis (Imler and Hoffman, 2000; Mackey and Rosen, 2000; Zhang and Ghosh, 2001). Binding of the elicitor causes the recruitment of a second receptor to the complex to form a dimer. This dimer then activates a kinase cascade that involves a STK (IRAK), MAP (Mitogen Activated Protein) kinases and Ca^{2+} influx. Eventually, a transcription factor (NFκB) is released from phosphorylated IκB and activated by translocation into the nucleus to initiate the transcriptional reprogramming required for programmed cell death. Cysteine proteases, called Caspases, are expressed to regulate subsequent steps of apoptosis (Green, 2000).

A similar process may occur in plants (Lam *et al.*, 2001). MAP kinases have been implicated in the signal transduction cascade leading to the initiation of the HR (Ligternik *et al.*, 1997; Daniel *et al.*, 1999; Fyre *et al.*, 2001). Interestingly, one of the class 3 R products also requires a STK for activity (Swiderski and Innes, 2001).

Ca^{2+} influx has been associated with elicitation of the HR (Atkinson *et al.*, 1990; Levine *et al.*, 1996; Grant *et al.*, 2000). Proteins related to IκB have been identified in plants and shown to function in elicitation of defense responses, but have not yet been demonstrated to directly function in the initiation of programmed cell death [*e.g.* NPR1; (Century *et al.*, 1997; Ryals, 1997)]. Leucine zipper (bZIP) proteins, that might function analogously to NFκB, have been implicated in transcriptional reprogramming associated with programmed cell death (Zhang *et al.*, 1999; Depres *et al.*, 2000). Caspase inhibitors block development of the HR in plants (Lam and Del Pozo, 2000), probably by their activity on meta-caspases (Lam *et al.*, 2001).

The mechanism for elicitation of nonhost resistance has not yet been elucidated but can be predicted to occur by a mechanism similar to race-specific resistance. In these interactions the resistant plant is proposed to express multiple R products that can independently initiate a response to any of several Avr products. Thus nonhost resistance would be expected to be polygenic and redundant. It should also be pointed out that plants can also responded to flagellin, and possibly LPS. It may be that the combination of recognition events mediates nonhost resistance. In addition, some effector proteins may also function to suppress defense responses during compatible interactions (see below). Strains lacking this suppressor function would also be incompatible.

MECHANISM FOR DISEASE RESISTANCE

The most obvious manifestation of the HR is the cell death observed. Assays for hypersensitivity traditionally score for tissue collapse and necrosis after infiltration with inocula sufficient to cause 50% or more of the cells to respond. At lower inocula concentrations tissue necrosis is not observed but individual cells can be seen to respond and die (Turner and Novacky, 1974). For obligate biotrophic pathogens, such as rusts, powdery mildews and viruses, the death of cells could function as a resistance mechanism by limiting the flow of nutrients or access to cellular functions necessary for replication. The death of plant cells, however, may be insufficient to restrict or limit the growth of bacterial pathogens. Most bacterial pathogens are not obligate biotrophs. *P. syringae* strains, for example, can grow asymptomatically on shoot surfaces as epiphytes (Hirano and Upper, 2000). As such they do not appear to directly parasitize mesophyllic tissue yet are still able to grow externally to the cuticle. Extracellular fluids contain sugars and reduced nitrogen sources that could support growth of bacteria once introduced into the tissue (Xiao *et al.*, 1992). Capillary action would keep walls of individual cells wetted with extracellular fluids unless modified to reduce their hydrophilicity.

To induce stasis or cell death for bacterial pathogens, other plant defense mechanisms are likely to be required. One such mechanism could be the oxidative bursts detected during the HR. Primary responding cells undergoing programmed cell death also produce active oxygen in the form of hydrogen peroxide and superoxide (Baker and Orlandi, 1995; Doke, 1997). Production of superoxide was first observed during an incompatible interaction between *Phytophthora infestans* and a resistant potato cultivar (Doke, 1983). Subsequently oxidative bursts were observed during the HR to incompatible bacterial strains during nonhost and race-specific resistance (Adam *et al.*, 1989; Orlandi *et al.*, 1992; Baker and Orlandi, 1995). Two phases of the oxidative response have been identified. The first occurs within a few minutes of inoculation of the tissue and is nonspecifically elicited by biotic and abiotic stimuli. The second is a sustained oxidative burst beginning 2-3 h later during incompatible interactions. The second phase oxidative burst is associated with lipid peroxidation and altered membrane permeability (Baker *et al.*, 1987; Keppler and Baker, 1989; Croft *et al.*, 1990). Localized active oxygen production adjacent to attached bacteria has been detected during incompatible interactions (Bestwick *et al.*, 1997). The oxidative bursts observed have been shown to be the consequence of but not the initiator of the HR (Glazener *et al.*, 1996). A calcium-dependent NADPH oxidase has been implicated in the oxidative burst (Keller *et al.*, 1998).

Whether the oxidative bursts produced during an HR are sufficient to be antimicrobial *in vivo* has not been established. Oxidative bursts are important in macrophage-mediated cellular resistance in mammals (Janeway *et al.*, 2001). In plants, oxidative bursts could promote cell wall modification (Bradley *et al.*, 1992; Brisson *et al.*, 1994), such as lignification and cross-linking of structural proteins, altered membrane permeability (Keppler *et al.*, 1988), secondary metabolite biosynthesis (Levine *et al.*, 1994; Tenhaken *et al.*, 1995) and cytotoxicity (Wu *et al.*, 1995). To be pathogenic, bacterial strains would need to produce anti-oxidants. *P. syringae* strains contain high levels of catalase (Klotz and Hutcheson, 1992) which could potentially function to protect the bacterium from high concentrations of hydrogen peroxide, but their effectiveness when hydrogen peroxide concentrations are below the K_m for the enzyme (Baker and Orlandi, 1995) has not been established. Catalase inhibitors have little effect on the levels of hydrogen peroxide detected in tissue (Bestwick *et al.*, 1997).

Also observed during some primary responses are alterations in cell walls adjacent to the attached bacteria. Papillae formation has been observed at these sites as cell wall thickening (Bestwick *et al.*, 1995). Presumably this response impedes the flow of nutrients from the parasitized host cell.

Additional contributing factors to disease resistance during the HR are the secondary responses observed in adjacent cells. Increased phenylpropanoid metabolism needed for lignification of cell walls and phytoalexin biosynthesis has been observed during many host-pathogen interactions (see Dixon, 2001). Many of the alterations in defense-associated gene expression detected in tissue responding hypersensitively occur after the primary responding cells have died. As an example, if cells are inoculated with constructs to express a reporter gene, such as GUS, and an HR-eliciting effector protein, minimal expression of a GUS reporter gene can be detected in tissue (Leister *et al.*, 1996). Therefore, much of the altered gene expression detected in plants during incompatible interactions must be secondary responses. Rings of responding cells have been observed in planta that accumulate phytoalexins for example (Graham and Graham, 1991). In this manner, cells that may not be immediately affected by virulence factors released by the pathogen, such as toxins, could mount a defense response to effectively isolate the pathogen from the rest of the tissue as observed in inflammatory responses in mammals (Abbas *et al.*, 1998; Janeway *et al.*, 2001).

Like the inflammatory responses observed in mammalians (Abbas *et al.*, 1998; Janeway *et al.*, 2001), the primary responding cell appears to release messenger molecules that in turn elicit responses in adjacent cells (Fig. 6). Three classes of molecules have been implicated as messenger molecules during the HR. Hydrogen peroxide and nitric oxide are well known signaling molecules during macrophage-mediated defense responses to bacteria (Abbas *et al.*, 1998; Janeway *et al.*, 2001) and have been implicated in intercellular signaling in plants (Levine *et al.*, 1994; Delledonne *et al.*, 1998; Wendehenne *et al.*, 2001). An oxidative burst on its own was insufficient to elicit many facets of the HR (Glazener *et al.*, 1996; Delledonne *et al.*, 1998) but was a necessary component. Working with active oxygen is nitric oxide. Nitric oxide scavengers and biosynthesis inhibitors abolished the HR elicited by a *P. syringae* strain expressing *avrRpm1* (Delledonne *et al.*, 1998). Salicylic acid or a close relative has also been implicated in intercellular signaling. Salicylic acid was originally identified as a possible mediator of systemically acquired resistance (Delaney *et al.*, 1994; Ryals *et al.*, 1996). Consistent with at least a partial role in signaling,

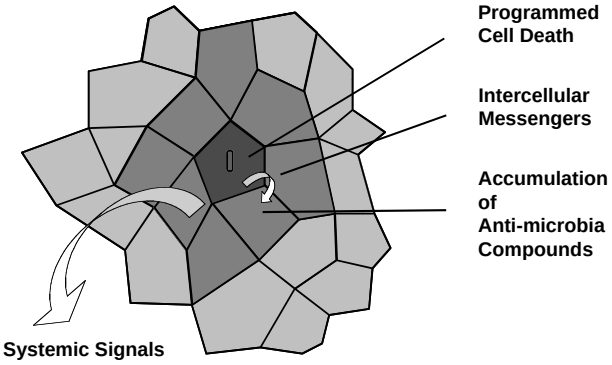

Fig. 6. Tissue responses during incompatible interactions. The involvement of a TTSS in pathogenesis restricts the interaction of an invading bacterial pathogen to the host cell to which it has adsorbed. The TTSS appears to only deliver effector protins to this cell. During incompatible interactions this host cell undergoes programmed cell death within 6 h. The oxidative burst occurring concurrently with PCD produces hydrogen peroxide and nitic oxide that can stimulate surrounding host cells to response like observed in mammalian inflammatory responses. These surrounding cells accumulate antimicrobial compounds (Graham and Graham, 1991) that could function to isolate the pathogen in the tissue. This complex of primary and secondary responding cells in turn appears to release hormonal signals, probably in the form of salicylic acid, to initiate systemic acquired resistance throughout the plant.

plants unable to accumulate salicylic acid show reduced defense responses and enhanced susceptiblity to disease (Delaney *et al.*, 1994; Ryals *et al.*, 1996). Mutants in the SA-dependent signal transduction pathway were more susceptible to disease (Cao *et al.*, 1994; Delaney *et al.*, 1995). Since no single signal molecule mediates all aspects of the apparent secondary defense responses, it may be that all three function together to elicit secondary responses.

CONCLUDING REMARKS

What emerges from the preceding discussions is that the *hrp*-encoded TTSS plays a central role in the interaction of *P. syringae* and other plant pathogenic bacteria with their plant hosts, irrespective of the outcome of the interaction. Bacteria expressing the *hrp*-encoded TTSS are able to grow and multiply within tissue of susceptible plants whereas saprophytic bacteria lacking a TTSS do not. Thus, the primary function for the effector proteins translocated by the *hrp*-encoded TTSS must be to facilitate parasitism of the host plant. There are two basic mechanisms by which this could occur.

The secreted effector proteins could alter the physiology of the host cells by disrupting membrane function, for example, as seen with HrpZ. Leakage of nutrients has been observed early in compatible interactions between virulent *P. syringae* strains and susceptible hosts (Atkinson and Baker, 1987). The products of *avr* genes are also implicated in this process. Mutants in specific *avr* genes can exhibit reduced virulence on their normally susceptible hosts (Ritter and Dangl, 1995; Chen *et al.*, 2000; Shan *et al.*, 2000). A second function for these proteins could be the suppression of host defenses. Underlying the HR must be other defense mechanisms that do not culminate in programmed cell death (Klement *et al.*, 1999). The nature of these defense response is currently under study. Acquisition of TTSS and effectors appears to have occurred by horizontal transfer. The *hrp* gene of cluster of *P. syringae* strains is part of a pathogenicity island (Hutcheson, 1999; Alfano *et al.*, 2000). Many of the *avr* genes are associated with mobile genetic elements (Kim *et al.*, 1998).

Resistant plants in turn have acquired the ability to recognize and respond to translocated effector proteins. This process need only involve any one of a multitude of resistance gene products. These resistance gene products can be classified into classes based upon their structural features but not as the eliciting pathogen. At least 4 of the 5 classes of resistance gene products mediate responses to bacterial effectors. The same classes of resistance gene products also mediate responses to fungi, viruses and nematodes (Hammond-Kosack and Jones, 1997; Hutcheson, 1998). Other pathogens are likely or are known to be producing effector proteins that elicit defense responses. Thus the molecular events described above are broadly applicable to many host-pathogen interactions.

Finally pathogenesis by bacteria and the process by which hosts defend themselves against pathogens shows remarkable similarities irrespective of the host. Pathogenesis by many bacterial pathogens of plants and animals involves a TTSS and the translocation of effector proteins into host cells. Surprisingly, both groups of pathogens secrete a homolog to YopJ that is essential for pathogenesis and functions as an Avr product in plant pathogens (Leach and White, 1996; Hardt and Galan, 1997). It remains to be determined what the biochemical functions are for many of the effector proteins secreted by plant pathogenic bacteria. The recognition of effector proteins mediating cell death appears to occur by similar mechanisms and the initiation of programmed cell death involves related signal transduction cascades. The programmed cell death that results from the aforementioned recognition process may involve similar processes. Even the intercellular signaling mechanisms functioning to limit the spread of the pathogen in tissue involves similar signaling molecules. It should come as no surprise that many immunologists have now become quite interested in disease resistance in plants as we have in immunology.

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