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Current Review

hrp Genes of Phytopathogenic Bacteria

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The plant-bacterial interaction. The interactions between bacterial pathogens and their plant hosts fall into two general categories: compatible, leading to intercellular bacterial growth and symptom development in the host, or incompatible, resulting in the absence of observable disease symptoms. Bacterial populations in compatible interactions increase dramatically within 48 hr of inoculation, and final cell numbers can increase 10^5-fold over initial inoculum levels. On nonhost plants, the incompatible interaction is often correlated with the elicitation of the hypersensitive reaction (HR) when bacteria are introduced into leaf tissue at high (greater than 10^6 colony-forming units per milliliter) inoculum levels (Klement 1963, 1982; Klement *et al.* 1964; Lelliott *et al.* 1966; Sequeira 1983). Below the threshold of 10^6 colony-forming units per milliliter, a macroscopic plant reaction is not normally seen in an incompatible interaction. Bacterial growth within the intercellular spaces of incompatible leaf tissue is limited, with the final populations either remaining unchanged or increasing only 10- to 100-fold within the first 48 hr. In contrast, the introduction of nonpathogenic bacteria, such as *Pseudomonas fluorescens* or *Escherichia coli*, into plant tissue at any inoculum level does not result in the appearance of the HR, and these bacteria do not multiply in plants.

Studies using metabolic inhibitors have suggested that active metabolism and *de novo* synthesis of macromolecules are required for induction of the HR on pepper (*Capsicum annuum* L.) by *Xanthomonas campestris* pv. *vesicatoria* (Meadows and Stall 1981). However, this requirement for *de novo* macromolecular synthesis may reflect the need to overcome media repression of bacterial genes necessary for plant interaction. With *P. syringae* pv. *glycinea*, Huynh *et al.* (1989) have shown that metabolic inhibitors such as rifampicin do not prevent the appearance of the HR on soybean (*Glycine max* (L.) Merr.) if the bacteria are grown on a defined minimal medium that does not repress plant-interaction genes. This work also shows that plant products are not essential for the expression of the *P. s.* pv. *glycinea* genes needed for the elicitation of the HR.

To better understand the biological mechanisms affecting the plant-pathogen interaction, researchers in many laboratories have undertaken the mutational analysis of bacterial plant pathogens. This work has led to the identification of genes, designated as *hrp* (*h*ypersensitive *r*eaction and *p*athogenicity), that are required for several plant-interaction phenotypes. As originally described for *P. s.* pv. *phaseolicola*, which is the causal agent of halo blight of bean (*Phaseolus vulgaris* L.), inactivation of *hrp* genes by transposon insertion has a pleiotropic effect: the mutant bacteria lose the ability to elicit the HR on nonhost plants, such as tobacco (*Nicotiana tabacum* L.), are severely attenuated in their ability to cause disease on their respective host plants, and are impaired in their ability to colonize plants (Lindgren *et al.* 1984, 1986). Since their initial discovery, *hrp* genes or *hrp*-like mutations have been described in numerous *P. syringae* pathovars and include the following: *P. s.* pv. *phaseolicola* (Anderson and Mills 1985; Deasey and Matthysse 1988; Somlyai *et al.* 1986), *P. s.* pv. *syringae* (Anderson and Mills 1985; Huang *et al.* 1988; Niepold *et al.* 1985), *P. s.* pv. *tomato* (Cuppels 1986), *P. s.* pv. *pisi* (Malik *et al.* 1987), *P. s.* pv. *tabaci* (Lindgren *et al.* 1988), and *P. s.* pv. *glycinea* (Huynh *et al.* 1989; Lindgren *et al.* 1988). Genes that appear to be *hrp*-like have also been described in taxonomically distinct plant pathogens such as *P. solanacearum* (Boucher *et al.* 1987; Huang *et al.* 1990b), *X. campestris* pathovars (Boucher *et al.* 1987; Daniels *et al.* 1988; Stall and Minsavage 1990), and *Erwinia amylovora* (Bauer and Beer 1987; Steinberger and Beer 1988). For the purpose of this review, we will concentrate on those bacteria from which *hrp* genes have been physically isolated and analyzed in some detail. While this approach is somewhat redundant, information clarifying the physical and functional relationships between the various *hrp* genes, which would make a review on the subject more succinct, is not yet in published form.

The initial description of *hrp* genes. As mentioned above, the mutational analysis of *P. s.* pv. *phaseolicola* resulted in the identification and cloning of a gene cluster required for several plant-interaction phenotypes (Lindgren *et al.* 1986). This cluster was defined initially by seven linked Tn*5* insertions within mutants of *P. s.* pv. *phaseolicola* NPS3121. All of these mutations eliminated the ability of the bacterium to elicit the HR on nonhost plants, such as tobacco, but did not affect the ability of these mutants to grow on minimal media. Six of the mutant strains also lost lesion-forming ability on bean leaves and multiplied poorly *in planta*. The seventh mutation resulted in a strain that produced fewer lesions than wild-type on bean and had a 10-fold reduction of growth *in planta*. These seven Tn*5*-induced mutants could be restored to wild-type func-

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tion by a single cosmid clone. An eighth Tn*5* insertion that is not physically linked to this *hrp* gene cluster has also been described (Lindgren *et al.* 1986), and the *P. s.* pv. *phaseolicola* locus that contains this insertion has been found to be analogous to the *hrpM* gene of *P. s.* pv. *syringae* discussed below (Mills and Mukhopadhyay 1990; Mindrinos *et al.* 1990; Mukhopadhyay *et al.* 1988).

The *hrp* gene cluster of *P. s.* pv. *phaseolicola* has been analyzed in detail using a combination of subcloning and transposon mutagenesis (Lindgren *et al.* 1989; Mindrinos *et al.* 1990; Rahme *et al.* 1991). This work revealed that the *hrp* cluster of *P. s.* pv. *phaseolicola* spans a chromosomal region of approximately 22 kilobases (kb) and consists of nine complementation groups (*hrpL*, *hrpAB*, *hrpC*, *hrpD*, *hrpE*, *hrpF*, and *hrpSR*). Rahme *et al.* (1991) established the direction of transcription of all nine complementation groups and confirmed the earlier finding (Lindgren *et al.* 1989) that *hrp* genes are actively transcribed in plant leaves but only weakly transcribed in complex media. Mutations in all *hrp* loci, except *hrpC*, greatly reduced (10^5- to 10^7-fold) the ability of the bacteria to multiply in bean leaves. A *hrpC* mutant did not elicit the HR on tobacco but did grow, although reduced about 10^2-fold, and produced fewer lesions on bean leaves (Rahme *et al.* 1991).

The *hrp* cluster of *P. s.* pv. *phaseolicola* is physically and functionally conserved within two of the taxonomically related *P. syringae* pathovars, *P. s.* pv. *glycinea* and *P. s.* pv. *tabaci* (Huynh *et al.* 1989; Lindgren *et al.* 1988). A DNA segment from the *P. s.* pv. *phaseolicola hrp* cluster hybridized to genomic DNA isolated from *P. s.* pv. *glycinea*, *P. s.* pv. *tabaci*, and *P. s.* pv. *angulata*. Weak DNA hybridization was detected between the *hrp* cluster probe and the genomic DNA of *P. s.* pv. *tomato* (Lindgren *et al.* 1988). This homology appears to reflect a conservation of gene function within several pathovars. The exchange of a *hrpAB*::Tn*5* mutation via homologous recombination from *P. s.* pv. *phaseolicola* into either *P. s.* pv. *tabaci* or *P. s.* pv. *glycinea* resulted in the loss of pathogenicity on tobacco or soybean, the respective hosts of these pathovars, and the loss of elicitation of the HR on nonhost plants (Lindgren *et al.* 1988).

The *hrp* genes of other plant-pathogenic *P. syringae*. In *P. s.* pv. *syringae*, mutations in *hrp* genes were identified initially by screening mutants either for reduced virulence or for loss of the ability to induce the HR on nonhost plants. The first *P. s.* pv. *syringae hrp* mutants were found by assaying Tn*5* mutants of the bean pathogenic strain PS9020 on leaves of *P. vulgaris* cv. Red Mexican (Anderson and Mills 1985). The prototrophic mutant PS9021 was unable to induce disease symptoms on bean at an inoculum level 10^4-fold higher than was sufficient for production of symptoms by the wild-type strain. In addition, this mutant no longer elicited the HR on tobacco and had an altered, mucoid colony morphology. An 8.5-kb region of the cosmid clone pOSU3101 complemented all three mutant phenotypes (Niepold *et al.* 1985). Like the *P. s.* pv. *phaseolicola hrp* mutants, the *P. s.* pv. *syringae* mutant PS9021 was defective in the ability to multiply in bean leaves (Bertoni and Mills 1987).

Transposon mutagenesis further delimited the *hrp* region that restored mutant PS9021 to a length of 3.9 kb (Mills and Niepold 1987), while sequence analysis revealed two open reading frames, ORF 1 and ORF 2, as determined by an assay for promoter activity (Mukhopadhyay *et al.* 1988). The predicted sizes of the two polypeptides, 40 and 83 kDa, respectively, were in agreement with polypeptides expressed in *E. coli* maxicells (Mills *et al.* 1985). An *E. coli* consensus promoter sequence was present upstream of and overlapping with ORF 1, while a sequence containing the features of a transcriptional terminator, and previously shown to inhibit expression of *galK* (Mills *et al.* 1985), was found downstream of ORF 2. The gene for ORF 2 was designated *hrpM*. It remains uncertain whether ORF 1 constitutes a separate *hrp* locus, whether it positively regulates *hrpM*, or whether the insertions into ORF 1 are polar on *hrpM*, because a separate promoter was not found upstream of ORF 2. No homology to any known protein was detected in a search of the GenBank and EMBL data bases. Sequences homologous to the region surrounding the original Tn*5* insertion in PS9021 are present in *P. s.* pv. *phaseolicola* (Niepold *et al.* 1985), and it has now been established that *hrpM* is conserved both physically and functionally within *P. s.* pv. *phaseolicola* (Mills and Mukhopadhyay 1990; Mindrinos *et al.* 1990).

More recently, *hrp* genes in *P. s.* pv. *syringae* were identified by a second group of researchers. Six mutants of *P. s.* pv. *syringae* strain 61 were isolated following screening of 1,600 Tn*5* mutants for failure to induce the HR on tobacco (Baker *et al.* 1987). All six mutants showed reduced virulence and were defective in their ability to grow in bean leaf tissue and, thus, are considered to be *hrp* mutants (Atkinson and Baker 1987). A cosmid clone, pHIR11, which contains a 36-kb chromosomal insert, restored the ability of these mutants to incite the HR on the nonhost, tobacco (Huang *et al.* 1988). The ability of this clone to complement the defect in virulence has not been reported. Derivatives of pHIR11 containing transposon insertions that destroy the complementing activity were used to construct chromosomal marker exchange mutants of two strains of *P. s.* pv. *syringae*. As expected, the resulting mutants did not elicit the HR on tobacco. One of the mutants no longer produced disease symptoms on its host plant, tomato (*Lycopersicon esculentum* Mill.); the phenotype of the other marker exchange mutant on its host plant, bean, was not reported. These data suggest that the complementing clone pHIR11 contains one or more *hrp* genes. Further, mutagenesis of pHIR11 with Tn*phoA* revealed 11 complementation groups, two of which appear to produce either transmembrane or exported proteins (Huang *et al.* 1990a).

The pHIR11 cosmid is unique among the cloned *hrp* gene clusters isolated from *P. syringae* in that it enables the nonpathogens *P. fluorescens* and *E. coli* to produce a full or a weak HR on tobacco, respectively; responses of the strains on tomato are much less pronounced. Similarly, strains of *P. s.* pv. *tabaci* and *P. s.* pv. *syringae* carrying pHIR11 are unable to cause disease symptoms on their respective hosts. It has not yet been determined whether these phenomena resulted from increased *hrp* gene copy number or from the presence of an avirulence (*avr*) gene or genes on the cosmid (Huang *et al.* 1988; Hutcheson

et al. 1989). It has been reported recently that an *avr* gene in *P. s.* pv. *tomato* is located within a *hrp* cluster (Lorang *et al.* 1990). In addition, introducing the *avrD* gene of *P. s.* pv. *tomato* into *E. coli* conferred on this bacterium the ability to incite the HR, but only on soybean cultivars that are incompatible with *P. s.* pv. *glycinea* races carrying *avrD* (Keen *et al.* 1990).

***hrp* genes that are also present in a bacterial wilt pathogen.** *P. solanacearum* is the causal agent of bacterial wilt of many plants, including members of the family Solanaceae such as tobacco and tomato. While many strains of *P. solanacearum* have a relatively broad host range, for example causing wilt on both tobacco and tomato, some strains are relatively restricted in their host range and may wilt tomato but elicit an HR on tobacco.

Strains with a Hrp$^-$ phenotype have been isolated in either one of two ways: using acridine orange (AO) treatment (Boucher *et al.* 1986, 1988b; Message *et al.* 1978) or transposon mutagenesis (Boucher *et al.* 1986, 1985). AO-resistant mutants of the tomato pathogenic strain GMI1000 usually differ from the wild type in several characteristics, including loss of pathogenicity on tomato, loss of ability to induce the HR on tobacco, methionine auxotrophy, production of large amounts of brown pigment (Boucher *et al.* 1986; Message *et al.* 1978), and variant lipopolysaccharide and extracellular polysaccharide structures (Drigues *et al.* 1985). Such mutants are difficult to characterize because of the large (>85 kb) deletion induced by AO treatment (Boucher *et al.* 1986). *P. solanacearum* contains a megaplasmid (Boucher *et al.* 1988a; Rosenberg *et al.* 1982), and since AO is often used to cure plasmids (Hirota 1960), it is possible that treatment of cells with this agent could have prompted deletion or loss of the megaplasmid and thus resulted in the pleiotropic phenotype.

Analysis of deletion derivatives of the megaplasmid found in AO-resistant mutants has led to the identification of a DNA region that is involved in the HR and virulence and which has been further studied by transposon mutagenesis. Boucher and colleagues screened 8,250 Tn*5*-induced mutants of *P. solanacearum* GMI1000 on axenically grown tomato seedlings and identified 13 prototrophic avirulent mutants (Boucher *et al.* 1986, 1985). Several of these were also affected in their ability to incite the HR on tobacco and were designated as *hrp* mutants. Most of the Tn*5*-generated avirulent mutants were weak invaders of young tomato plant roots and stems as compared to the wild-type strain and produced no wilt symptoms 1 month after inoculation, whereas wild-type bacteria caused complete wilting in 8 days (Trigalet and Demery 1986). This lack of invasiveness is probably the primary reason why these mutants were detected using a wilting assay on tomato seedlings. Southern hybridization analysis showed that the nine Tn*5* insertions which gave a Hrp$^-$ phenotype were located within the >85-kb region deleted from the megaplasmid following AO treatment (Boucher *et al.* 1986). A cosmid clone, pVir2, containing a 25-kb chromosomal insert, restored pathogenicity and the HR to eight of the nine Tn*5* mutants that mapped to the deleted region. Mutagenesis of pVir2 with Tn*5-lac* further localized the *hrp* gene cluster to a 17.5-kb region at the left end of the insert in pVir2 and extending out of the cloned region (Boucher *et al.* 1987). A second clone, pAFE8 (Arlat *et al.* 1990), extended the size of the *hrp* cluster to at least 22 kb. Following mutagenesis of pVir2 with the *lac*-reporter transposon Tn*5*-B20 (Keller *et al.* 1988), assays for β-galactosidase indicated the presence of a minimum of nine transcriptional units, all of which were expressed on minimal medium. In addition, β-galactosidase activity from three of the transcriptional units increased threefold to fivefold in the presence of tomato root exudate or tobacco cell culture filtrate (Arlat *et al.* 1990). No information is currently available on the function of these genes, and the inability to produce merodiploids is preventing complementation analysis of this region (Arlat *et al.* 1990; Boucher *et al.* 1987).

A second region of the *P. solanacearum* genome containing *hrp* genes has been identified in strain K60 (Huang *et al.* 1990b). Cosmid pTS34 contains this second *hrp* region, and this clone does not share homology with pVir2 (Huang *et al.* 1990b). The 7-kb insert of clone pTS34 was mutagenized with Tn*5-lac*, and two possible transcription units have been identified. In β-galactosidase assays, the activity of both of these transcription units was increased threefold to sixfold by coculturing with potato callus tissue (Huang *et al.* 1990b).

The *hrp* gene cluster from pVir2 showed structural homology by Southern hybridization analysis with all 53 *P. solanacearum* isolates tested and with eight *X. campestris* pathovars, but not with *E. carotovora* subsp. *carotovora*, *E. c.* subsp. *atroseptica*, *P. s.* pv. *syringae*, *P. s.* pv. *phaseolicola*, *Rhodococcus fascians*, or two other closely related bacteria, *Alcaligenes eutrophus* and *P. cepacia* (Boucher *et al.* 1988a, 1987). Other researchers have used plasmids containing *hrp* genes from several strains of *P. solanacearum* to probe Southern blots of DNA from widely distributed *P. solanacearum* isolates. Homology to the *hrp* gene probes was detected in all 150 isolates tested (Barlow *et al.* 1990; Cook *et al.* 1989).

***hrp* genes within the Enterobacteriaceae.** *E. amylovora*, an enteric bacterium closely related to *Escherichia coli*, causes fire blight of pome fruits such as apple and pear and elicits the HR on tobacco. Mutational analysis of *E. amylovora* led to the identification of a *hrp* gene cluster that spans approximately 40 kb of the chromosome (Bauer and Beer 1987; Beer *et al.*, in press; Beer *et al.* 1989; Laby *et al.* 1989; Steinberger and Beer 1988). Eighteen distinct transposon-induced *hrp* mutants have been described. All of these mutant strains and two naturally occurring *hrp* mutants were restored by a single cosmid, designated as pCPP430, that contains a 45-kb chromosomal insert (Beer *et al.*, in press; Beer *et al.* 1989; Laby and Beer 1990; Laby *et al.* 1989). As with pHIR11 described above, pCPP430 imparted the ability to elicit the HR on tobacco and other plants to *E. coli* and all other members of the Enterobacteriaceae tested (Beer *et al.*, in press; Wei and Beer 1990). *E. amylovora hrp* genes and corresponding wild-type clones have also been described independently by other researchers (Barney *et al.* 1990).

A significant aspect of the analysis of the *E. amylovora hrp* cluster is the conservation of both homology and gene function among phytopathogenic bacteria and other

members of the Enterobacteriaceae that are not plant pathogens. The use of low-stringency hybridization conditions revealed hybridization between the chromosomal insert of pCPP430 and genomic DNA of *E. c.* subsp. *carotovora*, *E. chrysanthemi*, *E. lupinicola*, *E. mallotivora*, *E. nigrifluens*, *E. rubrifaciens*, *E. salicis*, and *E. stewartii*. Under the same low-stringency conditions, hybridization was seen between the chromosomal inserts within pCPP430 and pHIR11, which contain the *hrp* clusters from *E. amylovora* and *P. syringae*, respectively (Laby and Beer 1990). The cosmid pES1044, which was isolated from an *E. stewartii* genomic library (Coplin *et al.* 1986), shared homology with pCPP430 and was able to restore the Hrp^+ phenotype to several of the Tn*5*-generated *E. amylovora hrp* mutants. This conservation of function was reflected by significant hybridization between portions of pES1044 and pCPP430 (Beer *et al.*, in press; Beer *et al.* 1990). Finally, the predicted protein product of one of the *E. amylovora hrp* genes was found to be related to the *hrpS* gene product of *P. s.* pv. *phaseolicola* (Sneath *et al.* 1990).

It appears that *Escherichia coli* contains genes which complement some of the *E. amylovora hrp* mutants (Beer *et al.*, in press; Wei and Beer 1990). Two cosmids, designated as pCPP440 and pCPP450, partially overlap pCPP430 and do not contain some of the *hrp* genes at one end of the *E. amylovora hrp* cluster. However, strains of *E. coli* containing either pCPP440 or pCPP450 elicited the HR on tobacco. Transposon insertions in a 2.9-kb *Hin*dIII fragment present within pCPP430, but not contained in pCPP440 or pCPP450, were introduced by marker exchange mutagenesis into the chromosome of *E. coli*, suggesting a high degree of homology. Mutant *E. coli* transconjugants from this exchange mutagenesis that contained either pCPP440 or pCPP450 did not elicit the HR. This ability was restored when a subclone containing the 2.9-kb *Hin*dIII fragment of pCPP430 was introduced *in trans*. These results strongly suggest that *E. coli* genes can functionally complement a portion of the *E. amylovora hrp* cluster.

***hrp* gene function.** While there has been substantial progress in the molecular analysis of *hrp* genes, little is known concerning the biological role that the gene products of these loci play in the plant-pathogen interaction. Perhaps the best defined *hrp* gene is the *hrpS* portion of the *hrpSR* transcription unit of *P. s.* pv. *phaseolicola*. The deduced protein product of *hrpS* shares homology with known two-component procaryotic regulatory proteins (Grimm and Panopoulos 1989; Ronson *et al.* 1987). In support of a regulatory role, a functional *hrpS* gene is required for the transcription of reporter fusions within *hrpAB*, *hrpC*, and *hrpD* (Mindrinos *et al.* 1990) as well as within *hrpE* and *hrpF* (Rahme *et al.* 1991). It has been shown previously that some *hrp* mutations inhibit the transcriptional induction or function of other genes involved in the plant-pathogen interaction, namely avirulence genes in *P. s.* pv. *glycinea* (Huynh *et al.* 1989; Lindgren *et al.* 1988). Thus, evidence is accumulating that *hrpS* is a positive regulator of bacterial genes which are required for several aspects of the plant-pathogen interaction.

The biological function of the remaining *hrp* genes remains unclear. As mentioned above, the *hrp* loci of *P. s.* pv. *phaseolicola* were repressed in complex media but expressed at a significant level *in planta*. However, these genes were transcribed in defined minimal medium, and this expression was significantly reduced by raising the osmolyte concentration (Mindrinos *et al.* 1990). In a similar manner, transcription from eight *E. amylovora hrp* genes, as determined by expression of β-glucuronidase (GUS) reporter fusions, was low in complete medium (Beer *et al.*, in press). Growth in minimal medium increased the expression of the GUS fusions. The addition of $(NH_4)_2SO_4$ to 50 mM reduced GUS expression to levels equivalent to those seen in complex media. An effect of pH on expression was also apparent; GUS activity was 10- to 50-fold greater at pH 5.5 than at pH 7 in low-phosphate medium (Beer *et al.*, in press). It is not known at this time how the response of *hrp* genes to culture conditions *in vitro* correlates with their function *in planta*.

Recently, *hrp* mutants of *P. solanacearum* and *P. s.* pv. *tabaci* were used to analyze the induction of pathogenesis-related proteins and some of the plant stress response genes. Expression of several plant proteins, including phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and chalcone isomerase (CHI), enzymes in the phytoalexin biosynthetic pathway, and the pathogenesis-related proteins chitinase and β-1,3-glucanase have been correlated with the elicitation of the HR and plant resistance to pathogen attack (see Lamb *et al.* 1989 for review). While it is beyond the scope of this review to discuss the biological role of these plant proteins, *hrp* mutants have been used to analyze their induction in two systems. Vacuum infiltration of detached leaves of tobacco with cultures of *P. solanacearum* strains GMI1000 (incompatible), K60 (compatible), or GMI1178 (an AO-induced *hrp* deletion mutant of GMI1000) led to the accumulation of chitinase and β-1,3-glucanase transcripts and proteins 18 hr after inoculation (Godiard *et al.* 1990). Accumulation of these transcripts and proteins was also observed when the detached tobacco leaves were infiltrated with water. A different result was seen when whole bean plants were vacuum-infiltrated with *P. s.* pv. *phaseolicola* (compatible), *P. s.* pv. *tabaci* (incompatible), or with a *hrp* mutant of *P. s.* pv. *tabaci* (Jakobek and Lindgren 1990). As was seen in detached tobacco leaves, there was no significant difference in the ability of either the wild-type strain or *hrp* mutants of the incompatible bacterium *P. s.* pv. *tabaci* to induce PAL-, CHS-, CHI-, or chitinase-specific transcripts. However, infiltration of living bean plants with either the compatible pathogen *P. s.* pv. *phaseolicola* or water did not induce these transcripts. These transcripts were also induced by inoculation of the nonpathogenic plant bacterium *P. fluorescens* or heat-killed *P. s.* pv. *tabaci*. While the contrasting effects of the inoculation of compatible bacteria can be explained by differences in the host-pathogen systems or the inoculation of attached versus detached leaves, both of these experiments indicate that *hrp* mutants which do not elicit the HR are still able to induce plant genes that have been correlated with disease resistance.

Summary. It is clear that *hrp* genes play a central role in the ability of plant pathogenic bacteria to interact with the plant. They are conserved with respect to homology

and function within *P. syringae* pathovars, *P. solanacearum*, *Erwinia* species, and, apparently, *E. coli*. The most perplexing aspect of *hrp* gene analysis is the pleiotropic nature of the mutations. It is difficult to separate the attenuation of growth *in planta* from the loss of the elicitation of the HR and pathogenicity. Are the phenotypic effects of a *hrp* mutation due to the inability of the mutant bacterium to grow normally within plant tissue, or do *hrp* gene products participate more directly in the pathogenic response? It is important not to limit the analysis of these loci to their effect on pathogenicity and the elicitation of the HR. The finding that some *hrp* genes are structurally and functionally conserved within nonplant-associated bacteria such as *E. coli* strongly suggests that they act at a basic metabolic level. This idea is supported by the induction of *hrp* genes by varying nutrient conditions in bacterial cultures, independent of the plant environment (Beer *et al.*, in press; Lindgren *et al.* 1989; Mindrinos *et al.* 1990; Rahme *et al.* 1991). In fact, recent experiments suggest that the requirement of *hrp* genes for the transcription of the *avrB* gene of *P. s.* pv. *glycinea*, a gene that is also regulated by nutrient conditions, can be explained by assigning to *hrp* genes the role of integrating information about carbon source availability (Huynh *et al.* 1989). Based on their phenotype and the presence of functional *hrp* genes in *E. coli*, we would expect functionally similar loci to be present within other nonpathogenic bacteria such as *P. fluorescens*.

The analysis of *hrp* loci and their function would be enhanced considerably by a focus of research efforts on one bacterial system. Based on currently published information, we found it difficult, if not impossible, to ascertain the physical or functional relationship between the various *hrp* mutations in the numerous genetic backgrounds that have been studied. Although some information concerning conservation of DNA homology between *hrp* genes from several bacterial species is now becoming available, it is unclear whether this DNA similarity will be reflected in the conservation of function of the various gene products. *Erwinia amylovora*, due to the conservation of this bacterium's *hrp* genes with those of *E. coli*, provides the greatest potential for the determination of *hrp* gene function through direct genetic comparison with the *E. coli* genome. Mapping of the location of the *E. coli hrp* genes, by traditional methods or through homology to the *E. coli* ordered clone bank (Kohara *et al.* 1987), will establish the relationship of *hrp* genes to known *E. coli* genetic loci and may lead to the determination of function. Once the functions of the *E. coli* and *E. amylovora hrp* genes are established, this information can be extrapolated to bacteria such as *P. syringae* and *P. solanacearum* that are not as genetically well-studied.

In conclusion, it can be said that a significant amount of information has been gathered concerning the molecular nature of *hrp* loci, but we are only just beginning to see a hint of the biological role of their gene products. It is hoped that future research efforts will focus on the biological role of *hrp* gene products in the plant-pathogen interaction, perhaps by using *E. amylovora* as a model system.

NOTE ADDED IN PROOF

A *hrp* gene cluster has recently been identified and cloned from *X. c.* pv. *vesicatoria* (U. Bonas, R. Schulte, S. Fenselau, G. V. Minsavage, B. J. Staskawicz, and R. E. Stall 1991. Isolation of a gene cluster from *Xanthomonas campestris* pv. *vesicatoria* that determines pathogenicity and the hypersensitive response on pepper and tomato. Molecular Plant-Microbe Interactions 4:81-88). This *hrp* region is approximately 25 kb in length and contains at least six complementation groups. Homology to this region is present in several pathovars of *X. campestris*, and the homology is high enough to permit genetic exchange of *hrp* mutations among some pathovars.

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