COMMENTARY

Why do pathogens carry avirulence genes?

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In the gene-for-gene hypothesis, Flor [19] proposed the existence of avirulence alleles of virulence genes, and that the virulence genes actively conditioned cultivar-specific virulence on hosts. Yet cultivar-specific virulence genes have not been found, and many of the avirulence (avr) genes cloned to date (over 40) do not appear to condition pathogenicity in general, virulence or anything else of value to the microbe. There is strong indirect evidence that many, if not nearly all, Avr proteins are secreted from pathogenic bacteria, enter plant cells and signal a response phenotype, usually associated with a hypersensitive defense response (HR). Why the bother for avirulence?

Evidence is accumulating that most avr genes are, or once were, pathogenicity (pth) genes found in biotrophic pathogens that determine (d) host range, not in a cultivar-specific manner, but in a host species-specific manner. At least some of these genes appear to function for pathogenicity by encoding protein signals that are “injected” into plant cells by the hrp system, resulting in programmed host cell death, a characteristic normally associated with necrotrophs. A growing body of evidence indicates that most microbial genes conditioning pathogenicity, including the hrp, pth and avr genes, are present because of horizontal gene transfer, often involving movement of large gene clusters on “pathogenicity islands”.

Since horizontal transfer is a stochastic process, many avr genes are likely to be maladapted pth genes, following their horizontal transfer to strains in which their function may be either gratuitous or detrimental. The structure of some of these genes may allow rapid adaptive selection for pathogenic function.

INTRODUCTION

Plant breeders look for genes by which plants can defend themselves against microbial attack, and microbial pathologists look for genes in microbes that determine virulence. The breeding work resulted in a large number of studies documenting intraspecific variation in plant resistance, and the classical microbiology research resulted in more limited studies on intraspecific variation in microbial virulence. All classical genetic analyses were by necessity limited to intraspecific variation analysed by meiotic recombination. Until the advent of molecular genetics, a comprehensive and systematic approach for understanding why certain plant species and not others are hosts of certain pathogens, and how microbes become pathogens in the first place, was impossible. Is it due to a failure of resistance (pathogen recognition) or the acquisition of pathogenicity genes? Can saprophytes become pathogens quickly, and can pathogens change host range or become much more pathogenic quickly, or do these events require complex combinations of adaptations, requiring long evolutionary periods? Such questions are now being addressed experimentally and new paradigms are emerging to explain the origins of new disease epidemics and to better control plant disease.

CLASSICAL TERMINOLOGY, NEO-DARWINISM AND THE AVIRULENCE GENE DILEMMA

Naturally-occurring variation in degree of susceptibility or resistance in a particular host species to a particular pathogen has been shown to be controlled, in nearly all cases, by single resistance (R) genes. R genes trigger programmed plant defense responses, including cell death [21, 11, 23], most often resulting in the appearance of a plant HR. Naturally-occurring variation in the phenotype of microbial virulence on crop plants was also found to be determined by single genes. Harold Flor’s classic papers [19, 20] using flax rust (Melampsora lini) demonstrated that “pairs of factors” (specific avirulence and virulence alleles) in the pathogen determined virulence against each particular host (cultivar-specific) R gene, thus establishing...
compounds the conceptual dilemma, as being the active determinants of virulence and pathogenicity. Compounding the conceptual dilemma, Flor also demonstrated that avirulence/R gene interactions were phenotypically epistatic over any and all virulence/susceptibility gene interactions.

Since that time, many “pairs of factors” (still usually described as virulence genes) were discovered by classical genetic analyses, and the gene-for-gene hypothesis was demonstrated or suggested in many, primarily biotrophic, parasite/host systems [12]. Despite the fact that avirulence alleles were dominant and epistatic, Ellingboe’s idea that pathogens carried a number of genes where the active allele conditioned avirulence “superimposed upon a basic compatibility between host and parasite” [17], while the inactive allele allowed (but did not condition) the virulent state was not widely accepted until the first avirulence genes were cloned in the 1980s. The primary objections raised were based on neo-Darwinian selection arguments: there could not be selection for avirulence, therefore the genes must function for virulence. For example, Person & Mayo write: “Although avirulence usually segregates as a dominant character, microevolutionary history suggests that in its primary function an A gene associates not with avirulence but with virulence…” [36]. In the only and classic textbook on the subject, Day’s “Genetics of Host-Parasite Interactions”, avirulence as a phenotype is explicitly compared to microbial auxotrophy and to sensitivity to a poison [12]. In this widely accepted view, the avirulent pathogen must be lacking something a virulent pathogen has, exactly analogous to lacking an appropriate allele to grow on minimal medium or to break down a poison.

The cloning of the first avirulence gene by Staskawicz et al. [60] from a readily cultured bacterial pathogen (Pseudomonas syringae), overcame the objections caused by the difficulty of accepting the idea that microbial pathogens carry genes that primarily and simply function to limit their virulence. One of the hallmarks of that paper was the deliberate search not only for an avirulence gene, but also for a virulence gene sensu Flor, a gene that actively conditioned virulence against a specific resistant host cultivar. Cultivar-specific virulence genes were not found. In fact, not even a DNA fragment hybridizing to the avr gene was found in other P. syringae strains in that study that might have indicated a corresponding “allele” in the species. Since then, many avirulence (avr, following bacterial nomenclature) genes (over 40) have been cloned from a variety of different bacterial plant pathogens [20, 42, 76] with similar results. Furthermore, many gene

disruption experiments of bacterial avr genes in several labs failed to reveal any effect on growth or pathogenicity of the microbial strain on (or off) hosts, other than the expected loss of R gene-specific avirulence. That is, the majority of bacterial avr genes appear to be dispensable. Although only a few fungal avirulence genes have been cloned to date [38], similar data emerges. With many microbial avr genes, there is no evidence for alleles or analogues that condition anything of value to the pathogen. Such avr genes appear to be not only dispensable, but also gratuitous. At the very least, cultivar-specific virulence in many gene-for-gene interactions is determined by the mere absence of avr genes; virulence alleles are not necessary for cultivar-specific virulence (Fig. 1).

It became clear that use of the term “virulence gene” to refer to the hypothetical alternate allele of cultivar-specific avr genes was a misnomer; in R gene for avr gene interactions, a “virulence” allele often does not exist, and if it does, does not condition the cultivar-specific state it describes. Unfortunately, there is considerable confusion in the literature brought about by use of the term “virulence” as synonymous with “pathogenicity”. For example, in their review of the terms virulence and pathogenicity, Shaner et al. [36] recount that three well-known scientists gave three different definitions of the term “virulence” at a 1989 international congress. With the exception of the virulence (vir) genes of Agrobacterium tumefaciens, formal use of the term “virulence” as synonymous with “pathogenicity” is incorrect. The vir genes of A. tumefaciens control pathogenicity and host range, but in other plant pathogenic microbes, these
would be called pathogenicity genes, since these vir genes are not cultivar-specific. The term “pathogenicity” is generally used with reference to genes that function to condition that state, and this terminology will be used throughout this paper. The term “virulence” will only be used to refer to cultivar-specific situations, keeping in mind that there are no known “vir” genes by this definition.

WHY ARE THERE SO MANY avr GENES?

Some avr genes are pth genes

There appear to be several valid explanations for the enigma of why pathogens carry avr genes, and the explanations are not mutually exclusive. First, some avr genes have selective value in terms of pathogenicity [22, 38, 42]. That is, they function pleiotropically to condition pathogenicity on all hosts tested (usually with increased pathogen growth in planta) in the absence of a cognate (“recognized” in a formal, gene-for-gene sense) R gene. It is important to emphasize that the pathogenicity, as opposed to the avirulence, encoded by these genes, appears from limited data to include entire host genicity, as opposed to the avirulence, encoded by these R genes. It would be called pathogenicity genes, since these genes are not cultivar-specific. The term “pathogenicity” is generally used with reference to genes that function to condition that state, and this terminology will be used throughout this paper. The term “virulence” will only be used to refer to cultivar-specific situations, keeping in mind that there are no known “vir” genes by this definition.

Another explanation for the enigma is that many avr genes could occur simply because of coincidental linkage with another factor on the same plasmid that has selective value, for example copper resistance. Copper sprays are widely used in some areas of the country, and copper resistance has been found linked to avr genes on an X. campestris pv. vesicatoria self-mobilizing plasmid recovered from strains in Florida [59].

Other stochastic events may account for the presence of dispensable avr genes. Redundancy through duplication is especially indicated for members of the avrBs3/pthA gene family. Sixty-two bp terminal inverted repeats mark the boundaries of homology among all members of this gene family, and the terminal 38 bp of these inverted repeats are highly similar to the 38 bp consensus terminal sequence of the Tn3 family of transposons [14]. It is therefore possible that these genes can, or once could, transpose. A different stochastic event might be loss of host range on a particular wild plant species due to mutation and adaptation to new plant species, leaving behind functional but unnecessary genes that once assisted in conditioning pathogenicity on the original plant species. Evidence for nonfunctional relics of avr genes in both Pseudomonas and Xanthomonas have been reported, for example, [39, 76]. Of course, experiments designed to reveal potential pathogenicity function(s) of avr genes are conducted on known hosts of the pathogen in which the genes currently reside.

Recent advances in our analyses of microbial genomes has revealed that they are much more fluid than previously thought, and horizontal gene transfer between species is thought to occur to a greater extent than generally assumed [57]. In fact, one of the primary surprises to come as a result of recent extensive genomic sequencing analyses is the discovery that both prokaryotic and eukaryotic genomes are chimeras, with large blocks of genes from a variety of sources, sometimes even mixtures from prokaryotic and eukaryotic sources [11]. It seems that horizontal transfer of clusters of genes conferring adaptive phenotypes in bacteria is the rule, not the exception [33]. Horizontal transfer of gene clusters is not limited to prokaryotes: horizontal transfer of an entire supernumerary chromosome has been demonstrated in Colletotrichum [30]; supernumerary chromosomes are known to carry genes that condition growth and/or pathogenesis in some filamentous plant pathogenic fungi [16, 67].
Evidence for the horizontal transfer of many _avr_ genes is indicated by their function (avirulence), structural features, % GC content, residency on plasmids, lack of conservation within pathogenic groups and the fact that so many appear to be dispensable. However, as several authors have noted, the fact that nearly all bacterial _avr_ genes require the specific action of the _hrp_ genes (discussed below) in order to express the avirulence phenotype, leads back to the conclusion that most of these genes must have originally been elaborated for pathogenic purposes.

Even the _hrp_ system itself seems to be acquired by horizontal gene transfer. The function of most _hrp_ genes is to provide for a specialized protein secretion system called type III [31, 68]. This type III secretion system is a generally conserved mechanism found in both plant and animal pathogens for the export, secretion and often delivery of specific proteinaceous effector molecules (virulence or pathogenicity factors) directly and/or indirectly into host cells in a contact-dependent manner [24, 31, 46]. The _Salmonella typhimurium_ secretion system appears to form a hollow, needle-like structure [46], that functions as a toxic protein injection system [38]. The _hrp_ pili may form channels through which macromolecules travel to reach the plant cell cytoplasm [45]. Interestingly, widely diverse bacteria probably acquire the entire export/injection system on a "pathogenicity island" in a single horizontal gene transfer event [46]. The evidence for this is that the genes encoding these protein secretion systems in various animal and plant pathogens are all clustered and the codon usage and % GC content of the genes encoding these type III systems are typically different from that of the rest of the genome.

Pathogenicity islands are not limited to phytopathogenic bacteria. A pathogenicity island consisting of at least six _avr_ genes and differing in codon usage and % GC content from that of other chromosomal genes in the fungal pea pathogen, _Nectria haematococca_, has been found on a dispensable, supernumerary chromosome [37]. Interestingly, the genes of this cluster appear to be involved in both offense and defense: some function to condition pathogenicity by killing host cells, while others function to degrade pisatin, a phytoalexin elicited in response to the fungal attack [27]. Also found on the same dispensable chromosome a gene allowing utilization of homoserine as a sole carbon and nitrogen source [54]. Homoserine is one of the nutrients found in relative abundance in pea root exudates and also released from dying pea cells. As mentioned earlier, at least one fungal supernumerary chromosome is known to be capable of horizontal transfer [30]; the large number of genes related to pathogenicity found on the _N. haematococca_ chromosome indicates that many, if not all of the tools required for pathogenicity and host range might be transferred to a nonpathogenic saprophyte in a single event. Evolution from saprophyte to pathogen might not always involve a long and complex series of incremental improvements in pathogenicity [32] as is commonly assumed, but rather could be achieved in a single horizontal transfer events (acquisition of a pathogenicity island or two) followed by a "clean up" operation, involving mutational shedding of maladapted _avr_ genes that are on the island.

**WHAT DO _avr_ GENES DO BIOCHEMICALLY?**

The capacity of _avr_ genes to trigger programmed cell death (PCD) and defense responses, plus their dependence on the _hrp_ secretion system, indicates that most _avr_ genes encode plant cell signal proteins that are perceived by receptors and trigger signal transduction pathways [6, 11, 59, 75]. The hypothesis that _avr_ genes either are, or once were, _pth_ genes indicates that pathogenicity function is also by way of plant cell signal proteins that are perceived by receptors and trigger signal transduction pathways. The fact that the defense responses triggered by _avr_ genes appear to be programmed responses strongly indicates that, if they also have a pathogenic purpose, it must be to trigger (a) programmed event(s) in the plant cell other than defense. Since _avr_ genes are found primarily in those pathogens thought to be biotrophic, their purpose must be related to the pathogenicity lifestyle unique to biotrophs.

**WHAT IS REQUIRED FOR PATHOGENICITY?**

Once it is realized that _avr/pth_ genes encode signals affecting plant cell programs, it is obvious to ask what kinds of programs might enhance a biotrophic pathogenic lifestyle. This involves a consideration of two related questions: What adaptations are required on the part of microbes to become biotrophic pathogens, and what is required by a biotroph to attack a specific plant species, i.e. to make the species a host? Biotrophic pathogens, whether of plants or animals, appear able to cause changes in host cell functions that increase both the pathogen’s ability to reproduce and to survive. Ability to reproduce involves deriving nutrition from plants, considered in the next section, and ability to survive involves ability to move to new infection sites, considered in the following section.

**Ability to derive nutrition**

The essential difference between parasites and saprophytes is that parasites derive nutrition from a living plant at its expense. The essential difference between pathogens and parasites is that pathogens cause overt disease symptoms. In many cases, the disease symptoms appear to directly favour the pathogen, at least in terms of pathogen dispersal. Plant pathogens have traditionally been roughly divided into two groups: those which are necrotrophic
and those which are biotrophic. The distinction is thought to be useful in determining the primary means by which nutrition is derived. For example, necrotrophs are easy to culture, produce abundant degradative enzymes and toxins, tend to have limited host cell contact, hardly stimulate host protein synthesis, and cause early necrosis, seemingly in advance of colonization. By contrast, biotrophs are often difficult to culture, produce few degrading enzymes or toxins that are important in pathogenicity, have extensive contact with living host cells, greatly stimulate host protein synthesis and cause necrosis only after extensive colonization [50].

Most pathogenic bacterial biotrophs kill plant cells and thereby derive major nutritional benefits. An exception is *Agrobacterium*, which “engineers” its hosts to produce novel compounds that only it can catabolize [36]. Note that *A. tumefaciens* does not utilize type III secretion as a primary means of pathogenicity and that there are no *avr* genes in *A. tumefaciens*. Another possible exception is *Rhizobium*, which can be pathogenic under high nitrogen conditions, but which normally confers an advantage to its hosts, and is more properly considered as a mutualistic parasite. As a general rule, the essential difference between necrotrophs and biotrophs that kill host cells is that necrotrophs kill at a distance, while pathogenic biotrophs kill after intimate cellular contact. Both types of pathogen derive nutritional benefits from dead or dying cells. Interestingly, it is the contact-dependent *hrp* system that provides the ability for biotrophs to kill both host and nonhost cells.

Of all phytopathogenic bacteria that require *hrp* genes, members of the genus *Xanthomonas* are by far the most biotrophic. Unlike *Pseudomonas*, *Ralstonia* and *Erwinia*, members of the genus *Xanthomonas* are all plant-associated, nearly all growth is endophytic, and each strain is highly host-specific. In fact, among prokaryotic plant pathogens, *Xanthomonas* exhibits by far the highest known level of plant specialization, with over 120 pathovars. The second most biotrophic group is *P. syringae*, with over 40 pathovars and with strains typically exhibiting less host specificity than a typical xanthomonad. Is there a significant difference in the nature of the effector molecules secreted by *Xanthomonas* and *P. syringae* strains that reflects a more biotrophic lifestyle?

It is clear that one of the most significant classes of effect or molecule delivered by type III secretion is the harpins. Harpins are glycine rich, cysteine-lacking proteins that are secreted in culture media under appropriate growth conditions. Externally applied harpins from *E. amylovora*, *E. chrysanthemi*, *R. solanacearum* and *P. syringae* have the capacity to elicit a rapid K⁺ efflux and H⁺ influx exchange reaction (XLR) that results in colonization of the apoplast, sucrose efflux and also PCD [17]. Harpins are therefore toxin-like. Harpins are clearly important pathogenicity factors for *E. amylovora* (fire blight) [71] and *E. chrysanthemi* (soft rot) [6], and are also found in phytopathogenic ralstonias and pseudomonads [1]. Their role in the latter two genera is unclear [1, 2]. The fact that harpins cause alkalization of the apoplast, sucrose efflux and death of host cells indicates a primary role in nutrition acquisition. Although individual and double harpin gene knockout mutations in *P. syringae* did not affect pathogenicity, *P. syringae* appears to produce multiple harpins [9], which might compensate the loss of one or more (but not all) of the genes. Do even the more biotrophic pathogens, such as *P. syringae*, have to kill cells for nutrition? The surprising answer seems to be “yes”; growth of *P. syringae* pv. tomato in tomato plants is significantly inhibited in plants in which PCD is inhibited [25, 26].

It is possible that acquisition of nutrients from living cells by *P. syringae* is a two stage process: one stage that can involve harpins and a later stage that must involve PCD. Some nutrition can be derived from plant cells by way of harpins without killing plant cells via an exchange reaction (XR) that results in sucrose leakage from the plant cell. *P. syringae* induces an XR and increased efflux of sucrose from host cells within 2–4 h after inoculation [4]. This XR exchange precedes onset of *P. syringae* population growth [2], which strongly indicates that it is a required step for growth.

An XR reaction (and resulting nutrient efflux) is also induced by *Xanthomonas*, and possibly by way of (a) completely different mechanism(s). No harpins have been reported to date from *Xanthomonas*, and by contrast with induction in a matter of hours by *Pseudomonas* and *Erwinia*, the XR reaction induced by *Xanthomonas campestris* pv. malvacearum (bacterial blight of cotton) does not occur until 1–2 days after inoculation [52]. Since *Xanthomonas* is well into logarithmic population growth by this time [69], the XR does not appear to precede the onset of population growth. The *Xanthomonas* XR may be induced by ammonium ion accumulation in the intercellular space of the apoplast [65]. Ammonium ions do not kill green plant cells grown in light, because they are rapidly recycled to amino acids by way of the GS-GOGAT pathway. It is reasonable to assume that the nutrient release induced by the XR contributes to endophytic growth of *Xanthomonas*. However, as with *P. syringae*, it is unclear if the XR is essential for early stage growth of *Xanthomonas*. There are also at least two other potential mechanisms that may cause nutrient efflux at an early stage of infection: extracellular proteases [13] and additional effectors secreted by the *hrp* system.

It seems likely that there are effector molecules secreted by the *Xanthomonas* *hrp* system that are yet not discovered. *X. campestris* pv. malvacearum is found close to host cell walls as early as 12 h after inoculation [3] and in contact with host cell walls by 24 h after inoculation [49]. Thus, there is the potential for *hrp* system involvement in
pathogenesis even before the XR. Not all xanthomonads kill host cells [45], however, the xanthomonads that cause the major disease problems (cankers, blights and rots) do kill host cells. For example, cotton blight causes vesiculation between the plasmalemma and cell wall by 48 h after inoculation, and major degenerative changes resulting in cell death occur over the next 3 days [3]. Rapid host cell death per se does not inhibit X. campestris pv. malvacearum growth in cotton, since plants held in continuous darkness became necrotic, while permitting logarithmic bacterial growth for 5 days [45].

The ability of a pathogen to produce an externally applied, toxic compound that results in cell death is a characteristic of necrotrophs: they kill in advance of colonization in order to obtain nutrition. If P. syringae strains do not use harpins to kill plant cells and Xanthomonas strains do not even have harpins, what do they use? Necrotrophs do not carry gene-for-gene avr genes, whereas biotrophs do. Unlike harpins, the protein products of avr genes fail to elicit any symptoms at all when artificially introduced or infiltrated as purified or crude extracts into the spongy mesophyll of plants. Could some avr genes, functioning pleiotropically in a pathogenic role, be injected into plant cells and signal susceptible hosts to give up nutrients by causing PCD?

Several Xanthomonas and P. syringae avr genes elicit an HR when expressed inside plant cells carrying specific R genes [13, 16, 28, 53, 91, 94, 96]. More importantly, both avrB from P. syringae pv. glycinea and avrRpt2 from P. syringae pv. tomato cause cell death and necrosis when expressed in plants lacking cognate R genes, and suggesting a possible role for these genes in pathogenicity [28, 47]. A role for a known pathogenicity determinant, phlA from X. citri, in programmed killing of host cells were recently established. Expression of phlA in citrus cells was sufficient to cause symptoms diagnostic of the disease caused by the pathogen: division, enlargement and death of host cells [16]. Since PthA is not a toxin and does not appear to be an enzyme, the cell death that is signalled must be programmed. Furthermore, the protein signal is specific for citrus.

As mentioned earlier, pthA is a member of the largest group of avr genes cloned and sequenced to date: the Xanthomonas avrBs3/phlA gene family [22, 42]. Members of the gene family, all of which are gene-for-gene avr genes, are either known or thought to be required for pathogenicity of xanthomonads causing cotton blight, Asian citrus canker, false citrus canker, Mexican lime cancerosis, common bean blight and rice blight [22]. Among other symptoms, the primary disease symptom elicited in common by those members appears to be PCD. Such PCD would likely have to occur without the oxidative burst and phytoalexin induction. Evidence to support this idea has been found using X. campestris pv. malvacearum, causing cotton blight. Cotton phytoalexins have been convincingly shown to be sufficient for the resistance observed in cotton against X. campestris pv. malvacearum [52]. However, on susceptible cotton, host cell death occurs without the production of phytoalexins [49]. This host cell death on susceptible cotton is caused by multiple members of the avrBs3/phlA gene family in X. campestris pv. malvacearum [46]. As discussed above, phlA appears to cause PCD, and it is likely that other members of the gene family that function for pathogenicity also cause PCD. In fact, there is strong evidence that the oxidative burst and initiation of the HR are independent of PCD in a number of systems [21, 35, 31].

### Ability to move to new infection sites

Since there is a common export mechanism for PCD signals shared by animal and plant bacterial pathogens, how do they cause such diverse diseases? Since most of the type III secretion system genes are involved in the export machinery per se, the answer may again lie in the effector molecules. The results of disease phenotype screens with several xanthomonads yielded only a relatively few genes that determine specific disease phenotypes [21, 25]. Examples are phlA of X. citri (hyperplastic cankers) and avrb6 and avrb7 (water soaking and blight) of X. campestris pv. malvacearum. These are all bona fide, gene-for-gene avr genes.

The PthA protein signals citrus plant cells to divide. In a natural infection, enough plant cells are affected by X. citri to result in tissue hyperplasia, eventually resulting in rupturing the leaf epidermis and allowing egress of the canker pathogen to the leaf surface. This is important because the pathogen is spread by wind-blown rain. A similar story emerges from studies of some other avr genes, affecting other pathogenicity phenotypes (reviewed in [22, 42]). In a well studied case, avrb6 and other members of the avrBs3/phlA gene family found in X. campestris pv. malvacearum cause water soaked, necrotic lesions on cotton, a phenotype which results in 1600 times more bacteria available on the leaf surface than if the genes were not present [76]. Once again, since this pathogen is also primarily spread by wind-blown rain, availability of infection units in quantity on the leaf surface would have epidemiological significance. Therefore the host–specific disease phenotype may serve an epidemiological purpose independently of multiplication within the host, to enable more efficient movement to uninoculated hosts.

The ability to elicit the division of differentiated plant cells, the ability to water soak cotton and the ability to elicit PCD are radically different phenotypes. Yet all three phenotypes are determined by genes which are 97% identical in DNA sequence (pthA, avrb6 and avrBs3) [74]. The dozen or more 102 bp direct tandem repeats found in the middle of these genes are known to facilitate intragenic recombination, which generates mutant variants of these...
genes with altered specificity and with altered pathogenicity phenotypes [74]. Reshuffling of repetitive tandem repeats has become well recognized as one of several mechanisms used by organisms to achieve genetic adaptation [57]. If one of these avr/pt genes moves horizontally into a new bacterial pathogenic strain to which they were not previously adapted, the most likely result would be to confer either cultivar-specific avirulence or no phenotype at all. Like avrBb3 in X. campestris pv. vescicatoria, the gene would be both dispensable and gratuitous. However, intragenic or intergenic recombination might generate a variant avr/pt gene with selective value in the pathogen, one that enhanced pathogenicity. Such a change would require that the Avr/Pth protein interact not with a resistance factor in the host, but rather with a “susceptibility” factor. The fact that nearly identical genes from the avrBb3/ptA gene family can cause radically different diseases on plants in different families indicates that this has already happened several times and will likely happen again.

THE CONVERSE OF R GENES: COGNATE PLANT SUSCEPTIBILITY GENES?

There is strong evidence that avr genes encode protein signals that are delivered into the plant cell in order to cause disease. Some are “intercepted” by R genes and result in an HR, while others are nonfunctional because of various stochastic processes, but the original purpose appears to be for pathogenicity. These conclusions are based primarily on: (1) the hrp dependency of all avr genes; (2) the discovery of functional nuclear localization signal (NLS) sequences on some of the genes [21, 73]; (3) the ability of many tested avr genes to elicit an HR when expressed inside resistant plant cells and (4) the ability of pthA to elicit all symptoms diagnostic of citrus canker disease when expressed inside citrus cells [16]. Such protein signals must interact with plant receptors. A direct physical binding of the avirulence protein AvrPto from P. syringae with the tomato resistance protein Pto has been demonstrated [61]. The specificity of the avrBb3/ptA gene family has been shown to reside in the leucine rich repeats (LRRs) of the protein products [34, 72], and the specificity of 13 alleles of the flax rust resistance gene L was recently demonstrated to reside in the LRRs of the protein products [18]. LRRs are thought to be involved in direct protein-protein interactions. The fact that pthA and avrb6 elicit pathogenic phenotypes specific for the entire range of citrus and cotton hosts, respectively, provides evidence that the receptors in citrus and cotton must be conserved among respective citrus and cotton hosts, and that they are specific cognate receptors.

The idea that avr/pt genes encode signal proteins that bind and activate plant receptors may be generally true for all biotrophic pathogens that utilize type III secretion as a primary means of pathogenicity. If so, it might not be the race-specific R genes and an active defense response (so useful in breeding resistant cultivars) that are generally responsible for limiting the host range of biotrophic plant pathogens, as has often been suggested. In fact, several labs have attempted to find evidence for this by mutational analysis of avr genes, including pthA, but with negative results, reviewed by [22, 42]). Rather, it might be a failure of the pathogenicity signal to bind an appropriate cognate plant response receptor (to condition disease) that would limit host range. The host range of biotrophic plant pathogenic bacteria would in part be determined by possession of a type III protein injection system and appropriate protein effectors capable of binding receptors that trigger host cell death and/or other plant responses useful to the pathogen, such as cell division.

CONCLUSIONS

Plant cells are killed by both necrotrophic and biotrophic pathogens, most likely primarily for nutritional purposes, but also for dissemination. The bacterial hrp genes, which encode a contact-dependent (Type III) secretion system, appear to deliver signal proteins directly to plant cells to help condition pathogenicity, and involving host cell death. Secreted harpins are utilized by the more necrotrophic bacterial plant pathogens to kill cells, while the more biotrophic pathogens appear to utilize protein signals encoded by pth genes to kill cells. Some pathogens use both. The limited number of microbial pathogenicity signals that have been identified implies a limited number of plant receptors that are responsive to such signals. These receptors appear to determine host range and specific susceptibility to biotrophs.

By contrast with pth genes, a relatively large number of microbial avr genes have been identified, and the majority appear to be dispensable or gratuitous. Many avr genes, and even the entire hrp system, appear to have transferred horizontally among gram negative bacteria. The avr genes that are dispensable require the same contact-dependent secretion system as pth genes, and the hypothesis is advanced that most avr genes: (a) are, or once were, host species-specific pth genes; (b) encode protein signal molecules that must bind plant response receptors in or on the plant cell, and (c) often possess a physical structure that enhances recombination and genetic adaptability. Some of these may be able to evolve new host specificities and pathogenicity phenotypes.

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REFERENCES


