Genetics of Plant-Pathogen Interactions Specifying Plant Disease Resistance

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Understanding the mechanistic basis of why a certain pathogen causes disease in one host plant and not in another has long intrigued and motivated plant pathologists. Plants, in nature, are generally resistant to most pathogens. The ability of a pathogen to cause disease in a host plant is usually the exception, not the rule. This is because plants have an innate ability to recognize potential invading pathogens and to mount successful defenses. In a converse manner, successful pathogens cause disease because they are able to evade recognition or suppress host defense mechanisms or both.

Since the early days of the 20th century, classical breeding for disease resistance in plants has been a major method for controlling plant diseases. However, it was not until the 1940s when H. H. Flor published his seminal work on the genetics of the interaction between flax and its obligate rust pathogen, *Malamspora lini*, that we gained a substantial understanding of the genetic interactions that control disease resistance in plants (10). Flor’s work was novel, insightful, and under-appreciated at the time as he concurrently studied the inheritance of resistance in the host and virulence in the pathogen. This work resulted in formulation of the gene-for-gene hypothesis. In its most simple form, the gene-for-gene hypothesis states that plants contain single dominant resistance (R) genes that specifically recognize pathogens that contain complementary avirulence genes. Avirulence genes can be defined as genes in the pathogen that encode a protein product that is conditionally recognized directly or indirectly only by those plants that contain the complementary R gene. Specific recognition results in the induction of defense gene expression and the inhibition of pathogen growth. However, if the plant host does not contain the R gene, the pathogen is still capable of causing disease on that plant even though it still contains the avirulence gene. It was the work of H. H. Flor that set the stage for the subsequent molecular cloning of pathogen avirulence genes and plant R genes.

MOLECULAR CLONING AND CHARACTERIZATION OF PATHOGEN AVIRULENCE GENES

As a graduate student at U.C. Berkeley in the late 1970s, I became interested in studying why certain races of *Pseudomonas syringae pv. phaseolicola (Psp)* cause disease in certain cultivars of bean, but induced a hypersensitive resistance response in other cultivars. At the time it was known that a single dominant R gene was present in a certain cultivar of bean that specifically controlled resistance to certain races of *Psp*, but that others races of the pathogen could cause disease in that cultivar. A review article written by Al Ellingboe on the genetics of host-parasite interactions greatly influenced my thinking at the time (2). After many discussions concerning this article with my major advisor Nick Panopoulos we predicted that it should be possible to chemically mutagenize an avirulent race of *Psp* to cause disease in the previously resistant bean variety. We were right.

The molecular cloning of the first pathogen avirulence gene occurred several years later in the early 1980s while I was a research scientist at one of the first plant biotechnology companies, International Plant Research Institute (IPRI) (15). A seminar by Fred Ausubel on the construction of a *Rhizobium* cosmid library and the cloning of *Rhizobium* genes (*nod* genes) by genetic complementation inspired the development of a strategy to clone a bacterial avirulence gene. We hypothesized that we could clone a bacterial avirulence gene based on the fact that in previous genetic studies with fungal pathogens, Flor had shown that avirulence genes were genetically dominant. In collaboration with Noel Keen we set out to clone an avirulence gene by constructing the genomic library of a race 6 strain of *Pseudomonas syringae pv. glycinea (Psg)* in a wide host range cosmid cloning vector, pLARFI. We reasoned that we could test the idea whether avirulence genes could be detected in bacteria by conjugating a cosmid genomic library of *Psg* race 6 into a *Psg* race 5 strain. Because the two races have the reciprocal phenotype on two different cultivars of soybean, we could test whether virulence or avirulence was dominant by inoculating the exconjugants on both cultivars and scoring the phenotype (Table 1).
Our results, published in 1984, revealed that a *P. syringae* race 5 strain containing the avrulence gene *avrA* from race 6 was now recognized by the Harosoy cultivar and induced a hypersensitive response. We never detected any exconjugants that converted *P. syringae* race 5 strains from an HR to a susceptible reaction on the cultivar Norchief confirming the dominant nature of the *avrA* gene. Although we cloned and sequenced a pathogen avirulence gene, we did not gain much insight into how this gene could trigger disease resistance in plants that contained a complementary R gene. The sequencing of *avrA* and the predicted protein sequence did not show homology to any proteins in any database and attempts to isolate a factor from bacterial supernatants were unsuccessful. A clue of how avirulence genes induced a resistance response in host plants had to await the cloning and characterization of the Hrp genes (see below) in phytopathogenic bacteria several years later.

**HRP GENES IN PHYTOPATHOGENIC BACTERIA ENCODE A SPECIALIZED TYPE III SECRETION APPARATUS TO DELIVER AVR (EFFECTOR) PROTEINS TO PLANT CELLS**

Early genetic studies in the 1980s carried out in the group of Nick Panopoulos resulted in the identification of bacterial genes controlling the ability of phytopathogenic bacteria to elicit a plant infection (9). These genes were termed “Hrp” genes, as bacteria containing these mutations concomitantly lost the ability to induce an HR on resistant plants and to cause disease in susceptible plants. Hrp genes were subsequently identified and characterized in several phytopathogenic genera, including *Pseudomonas* (Collmer group), *Xanthomonas* (Bonas group), *Erwinia* (Beer group), and *Ralstonia* (Boucher group). The Hrp genes are generally organized as 23- to 25-Kb clusters of DNA that contain several operons and have been subdivided into group I (*Erwinia* and *Pseudomonas*) and group II (*Xanthomonas* and *Ralstonia*) based on DNA homology (1, 8). Subsequent work revealed that some avirulence genes were actually within or near Hrp loci. It is interesting that it was also revealed that Hrp genes and *avr* genes in *Pseudomonas syringae* are co-regulated. However, how Hrp genes and *avr* genes were functionally related remained a mystery.

A major breakthrough in our understanding of pathogen avirulence genes occurred when the sequencing of the Hrp regions of phytopathogenic bacteria (groups of Ulla Bonas and Christian Boucher) revealed strong homology to type III secretion machinery of mammalian pathogens such as *Yersinia*, *Salmonella*, and *Shigella* (1). Such Type III secretory systems target virulence effector proteins to mammalian host cells, thereby interfering with the ability of the host to resist pathogen attack (3). Several laboratories working in this area have compelling evidence that phytopathogenic bacteria may deliver effector proteins directly to the plant host (13).

It is difficult to conceptually imagine what the selective advantage would be for a pathogen to maintain avirulence genes that are recognized by plant hosts. Recent studies have suggested that *Avr* proteins are most likely to be virulence factors that have evolved to alter or suppress host defense mechanisms (7). Only those plants are protected that have co-evolved resistance mechanisms to specifically detect foreign *Avr* proteins delivered into the host cell. Although much had been learned by studying the pathogen avirulence genes, it was becoming clearer that plant molecular pathologists should begin to turn their attention to the plant side of the interaction, and to focus their efforts on cloning and characterizing genetically understood R genes whose cognate avirulence gene had already been identified.

### Table 1. Bacterial disease reactions on soybean cultivars

<table>
<thead>
<tr>
<th>Race</th>
<th>P. syringae glycinea</th>
<th>Harosoy Cultivar</th>
<th>Norchief Cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psg</em> race 6</td>
<td>HR</td>
<td>Susceptible</td>
<td></td>
</tr>
<tr>
<td><em>Psg</em> race 5</td>
<td>Susceptible</td>
<td>HR</td>
<td></td>
</tr>
<tr>
<td><em>Psg</em> race 5 <em>(avrA)</em></td>
<td>HR</td>
<td>HR</td>
<td></td>
</tr>
</tbody>
</table>

**ISOLATION AND MOLECULAR CLONING OF PLANT DISEASE RESISTANCE GENES**

The identification of resistant germplasm in wild species of agronomic crops and the subsequent introgression into commercial cultivars has been the major focus of many plant breeders for the better part of the 20th century. Furthermore, most plant breeders chose to employ single dominant and semi-dominant genes in their breeding programs because of the ease and efficacy in which they could be introduced into agronomically acceptable cultivars. Thus as little as 10 years ago, the “Holy Grail” in the field of molecular plant pathology was the cloning and characterization of plant R genes. The fact that most characterized resistance genes were inherited as single genetic loci allowed plant biologists to employ genetic strategies to clone R genes. The development of transposon tagging and map-based cloning strategies to clone genes in the 1980s seemed like the best method of choice to clone R genes from plants.

A major breakthrough was the cloning and characterization of the maize Hm1 disease resistance gene (6). The *Hm1* gene controls resistance to certain isolates of *Cochliobolus carbonum* and was cloned by transposon tagging employing the maize transposon *Mu*. The characterization of the *Hm1* gene revealed that it encoded an NADPH-dependent reductase that inactivates the toxin produced by the invading fungus. In the next year a second R gene was isolated by Greg Martin and colleagues while he was working as a post-doctoral student in the Tanksley laboratory at Cornell University. The Cornell group employed a map-based cloning strategy to identify the *Pto* resis-
disease gene from tomato (11). This gene encodes a Ser/Thr kinase activity and is unrelated to the Hm1 gene. Thus at this time, there was no indication of a common relationship between R genes from different plants that controlled unrelated types of pathogens. The pace of cloning R genes increased beginning in 1994 with the cloning and characterization of R genes for resistance to several classes of pathogens, including viral, bacterial, and fungal pathogens. The Arabidopsis RPS2 gene was cloned in my group and the group of Fred Ausubel, the tobacco N gene in the group of Barbara Baker, the tomato Cf9 gene in the group of Jonathan Jones, and the L6 gene of flax in the group of Jeff Ellis (14). Subsequent analyses of the predicted polypeptides revealed that all four proteins contain Leu rich repeat motifs, suggesting that plants may share common mechanisms for disease resistance to diverse pathogens (see Fig. 1). In the following year, the groups of Jeff Dangl and Roger Innes reported the cloning of the Arabidopsis RPM1 gene (4). The sequencing of this gene further validated the idea that disease resistance genes share common protein motifs. This was an extremely exciting time in plant biology: a major breakthrough was achieved that could not have been predicted based on previous knowledge (5). Furthermore, a comparison of the N, RPS2, RPM1, and L6 proteins revealed further similarities as significant homologous stretches of amino acids could be found throughout the proteins. Prominent among these is the presence of a nucleotide binding site (NBS) and a Leu zipper (LZ) or homology to the toll and interleukin 1 receptor (TIR) domains in the N terminus of these proteins (see Fig. 1). These analyses also suggested that additional R genes could be identified by employing PCR with degenerate oligonucleotides and that putative R genes could be inferred from DNA sequencing projects. These predictions have been validated, as at 481 sequences have been identified that are putative R genes containing NBS/LRR domains from three sources: cloned R genes, homologous sequences in public databases, and degenerate PCR cloning (12).

**MAJOR UNANSWERED QUESTIONS AND FUTURE DIRECTIONS**

Today’s “Holy Grail” is to define the molecular basis of disease resistance specificity. We also need to elucidate the biochemical functions of pathogen avirulence proteins and plant R proteins. Moreover, we must focus on better understanding what controls the defense signal transduction pathways in the host leading to the expression of resistance. During the course of this work we expect to uncover the answer to the question: Do resistance proteins bind directly or indirectly to pathogen avirulence proteins or are there protein complexes that form that contain several yet to be identified proteins in addition to R and Avr proteins? The answer to this question is expected to come in the next few years as we begin to define...
the precise mechanisms involved in plant disease resistance. The major advances in this field up until this time have been shaped by genetic approaches, but it is also quite clear that a concomitant biochemical and cell biological effort will also be necessary to unravel the molecular complexities to understand the mechanisms involved in plant disease resistance.

A major future goal is to be able to understand the molecular basis of disease resistance in enough detail to make precise predictions about engineering plants to express resistance proteins that can recognize pathogen molecules essential for pathogenicity. In this manner it is anticipated that biotechnological approaches can engineer durable disease resistance in agricultural crops.

LITERATURE CITED