Plant Pathol. J. 17(2) : 83-87 (2001)

The Plant Pathology Journal
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Symposium

Molecular Plant-Microbe Interactions

December 22, 2000, Seoul, Korea

New Aspects of Gene-for-Gene Interactions for Disease Resistance in Plant

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(Received on January 18, 2001)

Disease resistance in plants is often controlled by gene-for-gene mechanism in which avirulence (*avr*) gene products encoding by pathogens are specifically recognized, either directly or indirectly by plant disease resistance (*R*) gene products. Recent studies arising from molecular cloning of a number of *R* genes from various plant species that confer resistance to different pathogens and corresponding *avr* genes from various pathogens resulted in the accumulation of a wealth of knowledge on mode of action of gene-for-gene interaction. Specially, members of the NBS-LRR class of *R* genes encoding proteins containing a nucleotide binding site (NBS) and carboxyl-terminal leucine-rich repeats (LRRs) confer resistance to very different types of phytopathogens, such as bacteria, fungi, oomycetes, viruses, nematodes and aphids. This article reviewed the molecular events that occur up-stream of defense response pathway, specially, bacterial *avr* gene protein recognition mediated by NBS-LRR type *R* gene product in plant based on current research results of well studied model plants.

Keywords : avirulence gene, resistance, R-gene.

A plant's successful defense against invading pathogens depends on early perception of the pathogens and initiation of the appropriate signaling processes to activate the multi-component defense response. Genetic analysis of many different plant-pathogen interactions has led to a model, gene-for-gene interactions, in which plant resistance (*R*) genes are postulated to encode the direct or indirect receptors for products of avirulence (*avr*) genes in the pathogens (Flor, 1971). Because the early recognition of pathogen invasion and initiation of the defense response is crucial for resistance, not only the molecular basis for understanding *R* gene mediated *avr* gene recognition is one of major focus in the disease resistance research, but also plant resistance (*R*) genes are one of the most important class of genes that has been used by breeders for disease control.

Subsequent signal transduction steps that transmit the recognition event and induce the plant defense are becoming increasingly apparent. Intensive genetic screen in model plant *Arabidopsis* showed that some of the activated signaling components appear to be shared, although early triggering step requires specific *avr* and *R* gene products in the gene-for-gene manner (Feys and Parker, 2000). It is quite plausible that Avr-R recognition events have evolved to somehow potentiate the activities of a conserved pre-existing basal resistance machinery. Thus, plants seem to activate their defense response against a variety of bacterial, viral, fungal, and nematode pathogens in similar ways.

Changes in ion fluxes, such as the activation of a Ca^{2+} and H^+ influx or a K^+ and Cl^- efflux, as well as the production of reactive oxygen species (ROS) such as O_2^-, H_2O_2, or nitric oxide (NO), reportedly occur within minutes after the interaction between *avr* and *R* gene products (Richberg, et al., 1998). Specially, ROS and NO synergistically stimulate salicylic acid (SA) biosynthesis, and SA in turn potentiates ROS-NO dependent response. This kind of regulation of ROS-NO production and SA accumulation establishes a binary switch for fine tuning and amplifying the magnitude of the signals that flow through the defense response pathway, and guarantees timely activation of defense response (Dangl, 1998). Such a potentiation of plant defense signals after *R* gene dependent pathogen recognition leads plant to develop a rapid, localized cell death, termed the hypersensitive response (HR), at the site of attempted infection, and induce production of anti-microbial compounds and pathogenesis-related (PR) gene expression. Activation of local responses at the site of infection can be followed by establishment of secondary immunity throughout the plant (systemic acquired resistance), which is long lasting and effective against a broad spectrum of pathogens (McDowell

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and Dangl, 2000).

Recent studies arising from molecular cloning of a number of *R* genes from various plant species that confer resistance to different pathogens and corresponding *avr* genes from various pathogens resulted in the accumulation of a wealth of knowledge on both mode of action of gene-for-gene interaction and evolution of *R* genes (Hammond-Kosack and Jones, 1997; Ellis and Jones, 1998). This article focuses on the molecular events that occur upstream of defense response pathway, pathogen recognition. Specially, bacterial *avr* gene protein recognition mediated by NBS-LRR type *R* gene product in plant will be discussed.

Avirulence (*avr*) gene products have dual functions. By definition, an *avr* gene of pathogen encodes an antigenic molecule that, after recognition by a cognate *R* gene product in a particular plant genotype, triggers hypersensitive response (HR) and other defense responses which limit pathogen growth in plant (Hammond-Kosack and Jones, 1997). This definition, however, does not imply that the *avr* gene has an intrinsic biological function as important mediator of the interaction between pathogen and host plant. Then, why does pathogen have detrimental *avr* gene? and if *avr* gene is useful for pathogen, what is the intrinsic biological function of *avr* gene? The paradoxical presence of *avr* genes in pathogens is resolved by studies demonstrating that many *avr* genes have dual functions, having a role not only as virulence factors but also as avirulence factors on host plants lacking the corresponding *R* gene (Kjemtrup, et al., 2000). Therefore, *avr* genes may originally have been evolved as virulence factors. However, they can become *avr* genes as soon as their products are recognized by the surveillance system (*R* genes) of plant that evolves to catch up with pathogen.

Once *avr* gene is recognized by the surveillance system of plant, pathogen may not need *avr* gene any longer. Consistent with this hypothesis, most of published *avr* genes are associated with IS elements and transposase terminal repeats (Kjemtrup et al., 2000). It therefore seems that pathogens utilize transposon-based mechanism for removing or inactivating *avr* genes. According to unpublished results from Dangl's laboratory, *P. syringae.* pv *maculicola* strain M6 inactivates chromosomal *avr* gene by transposition and plasmid excision mechanisms. This event occurs more frequently in response to selective pressure initiated by *R* gene mediated host defense response.

Subcellular location of Avr proteins in plant. Although not all *avr* gene products are necessarily delivered via the type-III secretory pathways, many of bacterial *avr* gene products are delivered into the plant cells by the *Hrp*-associated type III secretion pathways (Collmer, 1998). Once *avr* gene products are inside plant cell, some Avr proteins are directed to specific subcellular localization, which might be relevant to the localization where *R* gene mediated *avr* gene recognition takes place as well as the localization of respective host targets of *avr* gene product as virulence factor in plant (Nimchuk et al., 2000).

Members of *Xanthanonas avrB3* family, including both *avrBs3* and *avrXa10*, contain functional nuclear localization sequence (NLS) and target to the nucleus in plant cell, suggesting that recognition of these Avr proteins may occur in the nucleus (Bonas and Van der Ackerveken, 1999). Whereas, classes of *P. syringae* Avr proteins, such as AvrB, AvrC, AvrPphB, AvrRpm1, and AvrPto that have fatty acylation sites at their amino termini, target to the plasma membrane in the plant cell (Nimchuk et al., 2000; Shan, et al., 2000). AvrRpt2 of *P. syringae*, however, is known to localize in cytoplasm in the plant cell (Mudgett and Stasskawicz, 1999). This variation could favor evolution of host recognition complexes, anchored by the relevant R protein, with different subcellular localization. Consistent with this hypothesis, the corresponding *R* gene of *avrRpm1* and *avrB*, *RPM1* is a peripheral plasma membrane protein (Boyes et al., 1998) whereas the corresponding *R* gene of *avrRpt2*, *RPS2* is a cytoplasmic protein (Leister et al., 1996).

The classes of resistance (*R*) gene products. Most of *R* genes encode proteins that carry a structural motif with a repeating pattern of 20 to 30 amino acids called a leucine-rich repeat (LRR). LRR-containing *R* genes can be subdivided into two broad classes (Pan, et al., 2000; Richter and Ronald, 2000). First LRR type *R* genes in which the predicted gene product contains an amino terminal, extracellular LRR and a membrane anchor include *Cf-4* and *Cf-9* that confer resistance against *Cladosporium fulvum* in tomato. *Xa21* conferring resistance against *Xanthmonas orysae* in rice is a combined type of *R* gene, which contains *Cf-4*/*Cf-9* type extracellular LRR and a membrane anchor and *Pto* type kinase domain in cytoplasm. Second LRR type *R* gene products are predicted to be cytoplasmic. In general, cytoplasmically located *R* gene products are characterized by the presence of a conserved region containing a NBS and carboxy terminal LRR region. These NBS-LRR type *R* gene product can be grouped again into two distinct classes; those with toll/interleukin-1 receptor homologous amino termini to the NBS (TIR class) and those with leucine zipper homologous amino termini to NBS (LZ class). The TIR class of *R* genes include *N* from tobacco, *M* and *L6* from flax, and *RPP5* and *RPS4* from *Arabidopsis*. The LZ class of *R* genes include *RPS2* and *RPM1* from *Arabidopsis*, and *I2*, *Mi*, and *Prf* from tomato as well as *Dm3* from lettuce (Michelmore, 2000; Pan, et al., 2000; Richter and Ronald, 2000).

The NBS-LRR class of *R* genes is the largest among the four known *R* gene classes. Members of this class have

been identified in dicots and monocots (Hammound-Kosack and Jones, 1997; Pan et al., 2000) and can confer resistance to very different types of phytopathogen, such as bacteria, fungi, oomycetes, viruses, nematodes and aphids (Pan et al., 2000). No other *R* gene class appears to confer resistance to such a diverse set of pathogens.

Plants have abundant reservoirs to evolve new NBS-LRR type *R* genes. The present genome-wide sequencing projects in plants are facilitating the investigation on the numbers and organization of disease resistance genes (Michelmore, 2000). The analysis of 67Mb representing > 50% of the *Arabidopsis* genome detected 120 predicted gene products with similar to the NBS domain encoded by plant NBS-LRR type *R* genes. Assuming a similar distribution of genes in the remaining 50% of the genome, about 200-300 NBS-encoding genes are present in *Arabidopsis* (Meyers et al., 1999). This would represent close to 1-2% of all *Arabidopsis* genes. In rice, the estimates are even higher with values as high as 1500, which represent about 5% of rice genome (Meyers et al., 1999). In addition, NBS-LRR-like sequences tend to be clustered in various plant genomes. The fact that considerable haplotype diversity was observed within theses clusters was interpreted as indicative of high rates of instability (Michelmore, 2000). It is clear that a variety genetic mechanisms, such as point mutation, unequal recombination, and gene conversion, generate diversity in NBS-LRR type *R* gene clusters (Meyers et al., 1999; Pan et al., 2000). The prevalence of NBS-LRR proteins in various plant species and generation of various homologs in NBS-LRR type R gene clusters are consistent with their proposed function as adoptable surveillance molecules for rapidly evolving pathogens.

Mode of action for *avr* gene-for-*R* gene interaction. The genetically defined specificity of *avr* gene recognition coupled with recent findings that Avr proteins are able to generate *R* gene dependent resistant-like responses when expressed inside plant cells. The simplest interpretation of this fact is that NBS-LRR proteins are receptors that directly interact with their cognate *avr* gene product in plant. However, it has not been able to demonstrate direct interaction between *avr* gene products and *R* gene product through yeast two hybrid analysis. Only the product of the tomato *R* gene, *Pto*, which encodes a serine/threonine protein kinase, has been shown to interact with the AvrPto pro-

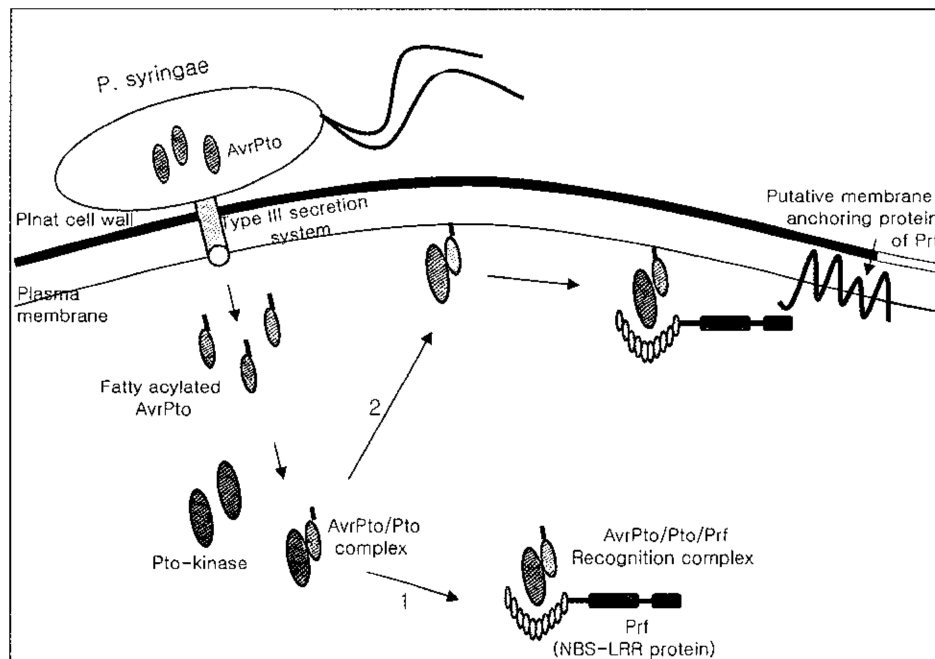

Fig. 1. Models for the AvrPto/Pto/Prf recognition complex that recognizes the bacterial infection and initiates defense response. The facts that AvrPto and Pto localize in the plasma membrane and cytoplasm, respectively and Pto requires Prf to initiate defense response suggest that AvrPto/Pto/Prf complex may be functional recognition complex capable of initiating defense response. The possible subcellular localization of the recognition complex is cytoplasm (1) or plasma membrane (2), depending on localization of Prf. In the case 1, AvrPto may be sequestered to the cytoplasm by cytoplasmic protein Pro kinase upon AvrPto translocation and fatty acylation. AvrPto/Pto complex may be guarded by NBS-LRR type protein, Prf, that is localizing in cytoplasm. Consequently, the subcellular localization of the AvrPto/Pto/Prf recognition complex is in the cytoplasm. In the case 2, Pto may be recruited to the plasma membrane upon AvrPto translocation and fatty acylation, and AvrPto/Pto complex may be guarded by NBS-LRR type protein, Prf, that is localizing in the plasma membrane. As a result, AvrPto/Pto/Prf recognition complex can be located in the plasma membrane.

tein of *P. syringae* in yeast two hybrid analysis (Scofield et al., 1996; Tang et al., 1996). However, *in planta*, Pto function also requires the NBS-LRR type protein, Prf, although the biochemical role that Prf plays in the response is unknown (Salmeron et al., 1996). This excellent genetic analysis suggests that *in vivo* recognition of Avr protein may require corresponding R protein and at least one other plant protein, and that this recognition complex can trigger defense response successfully.

By analogy, NBS-LRR type R protein alone is not sufficient to directly perceive the *avr* signal, and a Pto-like kinase and/ or additional factors may be required to facilitate *avr* signal recognition. Consistent with this hypothesis, A screen for mutations in *Arabidopsis* that suppress NBS-LRR type *R* gene, *RPS5* function did identify one gene that is absolutely required for *RPS5*-mediated resistance, *PBS2* (Warren et al, 1999). Interestingly enough, *PBS2* encodes a member of the Pto-like serine/threonine protein kinase (Innes, unpublished data).

According to Van der Biezen and Jones (1998), a major function of *R* gene products is to guard subcellular pathogenicity targets of Avr proteins as virulence factor. For instance, AvrPto as a virulence factor interacts with pathogenicity target, Pto kinase. Prf, NBS-LRR type protein, could have evolved subsequently to recognize an AvrPto/ Pto complex, resulting in AvrPto/Pto/Prf recognition complex in which AvrPto plays a role as avirulence factor with Pto, and thereby initiate the defense response. Plasma membrane localization of AvrPto protein is essential for virulence and avirulence functions, but not for interaction with Pto kinase (Shan et al., 2000), suggesting that Prf guarding AvrPto/Pto complex may localize in plasma membrane (Fig. 1, case 2). To date, however, there is no direct evidence for this hypothesis. Further biochemical analysis for the functions and localization of these proteins, AvrPto/Pto/Prf and AvrPphB/RPS5/PBS2, will provide compelling evidence for elucidating the mechanisms involved in gene-for-gene interaction.

Conclusions

Despite the recent cloning and characterization of over a thirty plant *R* genes from various plant and identification of a number of *R* gene like genes from plant genomic sequence, the mechanisms by which *R* genes recognize invading pathogens and activate defense response are poorly understood. However, the availability of cloned many *R* genes and *avr* genes in isogenic settings for both host and pathogen and of useful marker genes up-regulated/ down-regulated in a specific interaction manner that are identifying with gene chip techniques will spur genetic, biochemical, and cell biological approaches to dissect *R* gene-mediated signal transduction pathways for disease resistance response. Particularly, based on genetic analysis of gene-for-gene interactions, demonstration of exact subcellular localization of Avr and cognate *R* proteins and recognition complex containing Avr protein, *R* protein, and at least one other plant protein by using elegant biochemical and cell biological approaches will provide new insights for understanding the mechanisms involved in disease resistance response in plant.

Acknowledgment

This work was supported by a grant (PF003106-03) from Plant Diversity Research Center of 21st Frontier Research Program funded by Ministry of Science and Technology of Korean government.

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