Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 7137–7139, August 1995

Commentary

Finding the missing pieces in the puzzle of plant disease resistance

Xinnian Dong

Developmental, Cell and Molecular Biology Group, Department of Botany, P.O. Box 91000, Duke University, Durham, NC 27708-1000

The molecular mechanisms involved in protecting plants from microbial infection have long remained a mystery. Intrigued by this fundamental biological question and the obvious benefits that the answers may have for improving agriculture and environmental protection, scientists have applied molecular, biochemical, and genetic approaches in studies of a number of plant systems to make significant progress in recent years in the field of plant pathology.

The interaction between a potential phytopathogen and a plant can have different consequences. When a virulent pathogen infects a plant, it proliferates *in planta*, intracellularly or intercellularly, and generates disease symptoms. During the infection, plant genes designated as pathogenesis-related (PR) genes can be activated, and they may function to prevent the spread of the pathogen. However, when an avirulent pathogen infects a plant, it often triggers rapid death of cells and synthesis of antimicrobial compounds at the site of infection, which restricts the growth of the pathogen and renders it avirulent. The resulting visible necrosis is called a hypersensitive response (HR; refs. 1 and 2). The onset of an HR not only restricts the growth of the pathogen but also precedes (and may be the direct cause of) the activation of a signaling pathway that leads to systemic expression of a collection of PR genes and enhanced, lasting, and nonspecific resistance to a wide range of pathogens, known as systemic acquired resistance (SAR; refs. 3 and 4). Therefore, a plant exposed to an avirulent pathogen can be "immunized" against a variety of virulent pathogens. The signaling pathways that lead to the specific avirulent pathogen-induced HR and the onset of nonspecific systemic resistance are the focus of intense research (4–6).

The specific interaction between an avirulent pathogen and its corresponding plant host that triggers an HR was described by Flor (7) with the gene-for-gene hypothesis. According to this hypothesis, an HR is induced when the product of an avirulence gene (*avr* gene) in the pathogen is recognized by a corresponding resistance gene (*R* gene) product in the plant host. The rapid HR prevents the growth of the pathogen and makes an otherwise virulent pathogen avirulent. Therefore, an HR is a dominant resistance response over a virulent infection. Several avirulence genes have been cloned from various plant pathogens; however, the biological functions of most of the gene products remain unknown (6). A recent breakthrough in the studies of resistance has been the cloning and analysis of several *R* genes that determine resistance in a number of plant systems to very different fungal, bacterial, and viral pathogens. Sequencing analysis shows that one *R* gene cloned from tomato (*PTO*) contains a serine/threonine protein kinase domain (8) and other *R* genes isolated from *Arabidopsis*, flax, tobacco, and tomato (*RPS2*, L^6, *N*, and *Cf9*, respectively) share leucine-rich repeats (9–13), implicating these *R* gene products in signal transduction. It is intriguing that consensus regions are found in these presumed receptors that interact specifically with ligands produced by very different avirulence genes of very different pathogens; this suggests a possible common mechanism for the function of these receptors.

The molecular events that occur after the specific recognition of a pathogen-produced ligand by the corresponding plant receptor appear to be nonspecific. The rapid cell death that follows pathogen recognition is a genetically determined event, since mutants have been isolated that spontaneously form HR-like necrotic lesions in the absence of pathogen infection (14, 15). The genes identified by these mutations (*acd*, for accelerated cell death, and *lsd*, for lesions simulating disease) probably function downstream of the *R* genes in triggering an HR. During an HR, a burst of reactive oxygen species, such as H_2O_2, is detected. They may function to directly inhibit microbial growth, to induce programmed plant cell death, to strengthen the walls of uninfected cells by crosslinking the wall structural proteins, and to induce the expression of a battery of PR genes.

After an HR, there is also an increase in the level of salicylic acid (SA) at the site of HR lesions (10- to 50-fold) and throughout the plant (2- to 5-fold) (16, 17). Salicylic acid has been shown to be required for the induction of nonspecific resistance in the systemic tissue (SAR) and expression of those PR genes that are associated with SAR. A transgenic tobacco plant expressing a bacterial salicylate hydroxylase (that oxidizes SA to an inactive compound, catechol) fails to induce SAR after an avirulent pathogen infection and fails to express the PR genes (18). Further, exogenous application of SA can induce SAR and PR gene expression (19).

The systemic signal that links the local HR with the activation of SAR has yet to be found. Grafting experiments between transgenic tobacco plants expressing salicylate hydroxylase and the wild-type plants showed that SA is probably not the systemic signal that moves from the site of the primary inoculation to the systemic tissues but that it is a local signal required for the induction of SAR (20). Also unknown is the mechanism(s) by which SA induces SAR and PR gene expression. Recent studies conducted in the laboratory of Daniel F. Klessig at Rutgers University have shed light on this question. By using ^{14}C-labeled SA, Chen and Klessig (21) purified a soluble SA-binding protein (SABP) from tobacco that binds to SA with high affinity and specificity, suggesting that it is a receptor for SA (21). The protein has been identified as a catalase whose enzymatic activity, breaking down H_2O_2 to H_2O and O_2, is inhibited by SA (22). Chen *et al.* (22) have proposed that SA functions by increasing the intracellular level of reactive oxygen species, such as H_2O_2, and that reactive oxygen species may be involved in the activation of PR genes and in the induction of SAR. In this issue of the *Proceedings*, Conrath *et al.* (23) further strengthen this argument by showing that another chemical inducer of SAR, 2,6-dichloroisonicotinic acid (INA; ref. 24) also binds to catalase and inhibits its enzymatic activity with a dose–response curve similar to that of SA. Conrath *et al.* (23) have tested a number of INA analogs and SA derivatives and found correlation between the ability of these compounds to bind catalase and inhibit its activity with their ability to activate PR gene expression and to induce resistance. Furthermore, Conrath *et al.* (23) have shown that application of antioxidants suppresses INA-mediated PR gene expression. These data not only identify a cellular target for the action of INA but also strongly suggest

Abbreviations: PR, pathogenesis-related; HR, hypersensitive response; SAR, systemic acquired resistance; SA, salicylic acid; INA, 2,6-dichloroisonicotinic acid; SABP, SA-binding protein; MTD, mannitol dehydrogenase.

an important role for reactive oxygen species in activating PR genes and inducing resistance.

There is convincing experimental evidence showing that SA is required for the induction of SAR, that SA and INA are inhibitors of catalase, and that reactive oxygen species play an important role in certain plant defense responses. However, whether and how inhibition of catalase is directly involved in the induction of SAR has yet to be shown. Attempts to induce SAR by using H_2O_2 have not been successful (25). It is possible that SABP/catalase is not the only receptor for SA. As pointed out by Conrath *et al.* (23), SABP/catalase may be involved in the initial oxidative burst associated with HR, and other factors such as SA compartmentation or the intracellular redox state may be involved in the induction of SAR. In a seemingly similar case in mammalian cells, separate oxidant-initiated and redox-regulated mechanisms have been proposed for the signal transduction pathways that activate the mammalian NF-κB transcription factor, which regulates the mammalian inflammatory response genes (26). It will be extremely interesting to examine the phenotypes of transgenic plants with altered expression of the SABP/catalase gene and study the effects on the different resistance responses. In addition to molecular and biochemical approaches, genetic studies may also provide insight as to how the SA signal is transduced, leading to the expression of PR genes and the onset of SAR. An *Arabidopsis* mutant that is insensitive to both SA and INA induction of SAR has recently been identified and characterized (27). This mutant, designated *npr1* (nonexpresser of PR genes), carries a single recessive mutation that abolishes SA-, INA-, and avirulent pathogen-induced PR gene expression and SAR. Cloning of the *NPR1* gene may help to identify a regulatory component that transduces the SA signal.

At the ends of the signaling pathways are the effector PR genes that presumably determine the various resistance responses, such as HR, SAR, and the local resistance that serves to prevent the spread of infection. These genes are identified by their induced expression after a pathogen infection. In attempts to understand the molecular basis of disease resistance, many of these genes have been cloned and speculations have been made about their roles based mainly on the sequences of the proteins they encode. For those characterized PR genes, various inducers, distinctive induction kinetics, and diverse biological functions have been found (1, 2, 4). Some PR genes are induced rapidly by an avirulent pathogen during an HR; others are induced later and systemically, correlating with the onset of SAR. Often associated with an HR are genes encoding the enzymes in the phenylpropanoid pathway, which leads to the biosynthesis of lignin, antimicrobial secondary metabolites (phytoalexins), and SA. SAR-related PR genes include those encoding β-1,3-glucanase and chitinase, which have been shown to inhibit the growth of several fungal pathogens (28, 29). β-1,3-Glucanase has also been suggested to be involved in releasing defense-activating elicitors (30, 31). Moreover, transgenic tobacco plants constitutively producing a chitinase have enhanced resistance to the fungal pathogen *Rhizoctonia solani* (32), and plants constitutively expressing the PR-1a gene (whose function has not been identified) have increased tolerance to the fungal pathogens *Phytophthora parasitica* and *Peronospora tabacina* (33). Conversely, blocking the induction of SA-regulated PR genes by the activity of salicylate hydroxylase or by the SA-insensitive mutation *npr1* coincides with the demolition of the plants' nonspecific defense mechanisms (SAR and local resistance) and results in more severe disease symptoms and further spread of pathogen than in the wild type (18, 27, 34).

ELI3 was isolated as a plant defense gene from parsley (35) and *Arabidopsis* (36), and the ELI3 mRNA was shown to accumulate in *Arabidopsis* leaves in response to inoculation by strains of the phytopathogen *Pseudomonas syringae*. The induction of ELI3 mRNA accumulation was rapid and dramatic when an avirulent strain of *Pseudomonas* was used, and a delayed induction was observed when the plant was challenged with a virulent strain of *Pseudomonas*. Despite its interesting induction kinetics, the function of the gene was unknown. In this issue of the *Proceedings*, Williamson *et al.* (37) report that a mannitol dehydrogenase (MTD) isolated from celery shares 83% amino acid sequence identity and 93% similarity with the previously described ELI3 protein from parsley and *Arabidopsis* and that celery suspension cells grown in the presence of 1 mM SA have 20-fold higher MTD activity than those grown in the absence of SA. MTD is a catabolic enzyme that oxidizes mannitol to mannose. Mannitol not only is an abundant source of sugar alcohol but also is proposed to function as an osmoprotectant and an antioxidant. Therefore, an increase in MTD activity after an avirulent pathogen infection, which leads to reduction in the pool size of mannitol, correlates well with the oxidative burst and the appearance of dry necrotic HR lesions that are often associated with the resistance response against an avirulent pathogen. The association between a basic metabolic enzyme and the plant defense response is intriguing and informative for understanding the molecular basis of disease resistance in plants. Revealing the biological function of ELI3 as a MTD is a pleasant surprise in the challenging endeavor of understanding the biological roles of the many PR genes that orchestrate a plant resistance response.

Plant disease resistance is still an unsolved puzzle with many missing pieces. However, the progress made in recent research, including those described in the two papers published in this issue of the *Proceedings*, has helped to find some of these pieces and to start putting them together.

I thank Dr. Urs Neuenschwander for sharing his results prior to publication and Scott A. Bowling for helpful suggestions on this manuscript.

1. Dixon, R. A. & Lamb, C. J. (1990) *Annu. Rev. Physiol. Plant Mol. Biol.* **41,** 339–367.
2. Lamb, C. J., Lawton, M. A., Dron, M. & Dixon, R. A. (1989) *Cell* **56,** 215–224.
3. Kuc, J. (1982) *BioScience* **32,** 854–860.
4. Ryals, J., Uknes, S. & Ward, E. (1994) *Plant Physiol.* **104,** 1109–1112.
5. Jones, J. D. G. (1994) *Curr. Biol.* **4,** 749–751.
6. Staskawicz, B. J., Ausubel, F. A., Baker, B. J., Ellis, J. G. & Jones, J. D. G. (1994) *Science* **268,** 661–667.
7. Flor, H. H. (1971) *Annu. Rev. Phytopathol.* **9,** 275–296.
8. Martin, G. B., Brommonschenkel, S. H., Chunwongse, J., Frary, A., Ganal, M. W., Spivey, R., Wu, T., Earle, E. D. & Tanksley, S. D. (1993) *Science* **262,** 1432–1436.
9. Bent, A. F., Kunkel, B. N., Dahlbeck, D., Brown, K. L., Schmidt, R., Giraudat, J., Leung, J. & Staskawicz, B. J. (1994) *Science* **265,** 1856–1860.
10. Mindrinos, M., Katagirl, F., Yu, G.-L. & Ausubel, F. M. (1994) *Cell* **78,** 1089–1099.
11. Ellis, J. G., Lawrence, G. J., Finnegan, E. J. & Anderson, P. A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4185–4188.
12. Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C. & Baker, B. (1994) *Cell* **78,** 1101–1115.
13. Jones, D. A., Thomas, C. M., Hammond-Kosack, K. E., Balint-Kurti, P. J. & Jones, J. D. G. (1994) *Science* **266,** 789–793.
14. Greenberg, J. T., Guo, A., Klessig, D. F. & Ausubel, F. M. (1994) *Cell* **77,** 551–563.
15. Dietrich, R. A., Delaney, T. P., Uknes, S. J., Ward, E. R., Ryals, J. A. & Dangl, J. L. (1994) *Cell* **77,** 565–577.
16. Malamy, J., Carr, J. P., Klessig, D. F. & Raskin, I. (1990) *Science* **250,** 1002–1004.
17. Metraux, J.-P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W. & Inverardi, B. (1990) *Science* **250,** 1004–1006.
18. Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. & Ryals, J. (1993) *Science* **261,** 754–756.
19. White, R. F. (1979) *Virology* **99,** 410–412.
20. Vernooij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H. & Ryals, J. (1994) *Plant Cell* **6,** 959–965.
21. Chen, Z. & Klessig, D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8179–8183.
22. Chen, Z., Silva, H. & Klessig, D. F. (1993) *Science* **262,** 1883–1886.

23. Conrath, U., Chen, Z., Ricigliano, J. R. & Klessig, D. F. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7143–7147.
24. Metraux, J.-P., Ahl Goy, P., Staub, T., Speich, J., Steinemann, A., Ryals, J. & Ward, E. (1991) in *Advances in Molecular Genetics of Plant–Microbe Interactions,* eds. Hennecke, H. & Verma, D. P. S. (Kluwer, Dordrecht, The Netherlands), Vol. 1, pp. 432–439.
25. Neuenschwander, U., Vernooij, B., Friedrich, L., Uknes, S., Kessmann, H. & Ryals, J. (1995) *Plant J.*, in press.
26. Anderson, M. T., Staal, F. J. T., Gitler, C., Herzenberg, L. A. & Herzenberg, L. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11527–11531.
27. Cao, H., Bowling, S. A., Gordon, A. S. & Dong, X. (1994) *Plant Cell* **6,** 1583–1592.
28. Schlumbaum, A., Mauch, F., Vogeli, U. & Boller, T. (1986) *Nature (London)* **324,** 365–367.
29. Mauch, F., Mauch-Mani, B. & Boller, T. (1988) *Plant Physiol.* **88,** 936–942.
30. Keen, N. T. & Yoshikawa, M. (1983) *Plant Physiol.* **71,** 460–465.
31. Mauch, F. & Staehelin, L. A. (1989) *Plant Cell* **1,** 447–457.
32. Broglie, K., Chet, I., Holliday, M., Cressman, R., Riddle, P., Knowlton, S., Mauvais, C. J. & Broglie, R. (1991) *Science* **254,** 1194–1197.
33. Alexander, M., Glascock, C., Weymann, K., Friedrich, L., Maddox, D., Ahl Goy, P., Luntz, T., Ward, E. & Ryals, J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7327–7331.
34. Delaney, T. P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E. & Ryals, J. (1994) *Science* **266,** 1247–1250.
35. Somssich, I. E., Bollman, J., Hahlbrock, K., Kombrink, E. & Schulz, W. (1989) *Plant Mol. Biol.* **12,** 227–234.
36. Kiedrowski, S., Kawalleck, P., Hahlbrock, K., Somssich, I. E. & Dangl, J. L. (1992) *EMBO J.* **11,** 4677–4684.
37. Williamson, J. D., Stoop, J. M. H., Massel, M. O., Conkling, M. A. & Pharr, D. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7148–7152.