

Genetic dissection of R gene signal transduction pathways

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Mutant screens have identified several genes in tomato, barley and *Arabidopsis* that are required for the function of specific plant disease resistance (R) genes. Two of these genes, *NDR1* and *EDS1*, have recently been cloned from *Arabidopsis*. Most *Arabidopsis* R genes require *NDR1* or *EDS1*, but not both. In a complementary approach, yeast two-hybrid screens have identified several proteins in tomato that interact with the Pto R gene protein, including a kinase and three putative transcription factors. The present data indicate that R gene proteins directly activate multiple signal transduction pathways, and that common defense responses can be activated via independent pathways.

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Abbreviations

EREBP ethylene responsive element-binding protein
LRR leucine-rich repeat
NBS nucleotide binding site
R resistance
SA salicylic acid

Introduction

Despite the recent cloning and characterization of over a dozen plant disease resistance (R) genes (reviewed by J Ellis and D Jones, this issue pp 288–293), the mechanism(s) by which R genes activate defense responses are poorly understood. Studies using various inhibitors indicate that calcium channels, kinases, and phosphatases play important roles in R gene signal transduction pathways (see review by D Scheel, this issue pp 305–310), but the specific genes that encode these proteins have not been identified. In addition, signal transduction components that are unique to plants have undoubtedly been overlooked.

In this review I focus on recent genetic approaches to identifying plant genes required for transduction of the R gene signal. These genetic approaches can be broadly subdivided into three distinct, but complementary strategies: screens for mutants that affect disease resistance, screens for mutants that affect specific defense responses, and yeast two-hybrid screens for proteins that interact with cloned R gene proteins. All three approaches have made significant contributions to our understanding of plant R gene signal transduction pathways. A picture is emerging that suggests R gene signal transduction pathways are highly branched, partially redundant, and likely contain

feedback loops. These features make genetic dissection a particular challenge.

Standard genetic screens for loss of resistance

Perhaps the simplest way to identify R gene signal transduction components is to screen for plant mutants that lose resistance to a specific pathogen. In such a screen one would expect to isolate mutations in a specific R gene, and in any components required for transducing the R gene signal.

Prior to 1993 there were very few published studies in which induced disease-susceptible plant mutants were sought [1,2]. The recovery of mutations in a gene other than an R gene was reported only once [3]. In that study, Torp and Jorgensen screened for induced mutations in barley that suppressed the powdery mildew resistance gene *Mla-12*. Twenty-five susceptible mutants were isolated, but only three were non-*mLa-12* mutants. Subsequent genetic analyses of these three mutations revealed that two were allelic [4], thus only two candidate signal transduction genes were identified among the 25 mutants. These genes were initially designated *Nar-1* and *Nar-2* [4], but have recently been renamed *Rar1* and *Rar2* (required for *Mla* resistance) [5••]. Mutations in *Rar1* and *Rar2* partially suppress resistance mediated by several, but not all, powdery mildew resistance loci [6], indicating that some powdery mildew R genes employ independent signal transduction pathways.

During the early 1990's several groups performed screens for loss of R gene function, often as part of their efforts aimed at cloning plant R genes. Such screens led to the identification of several candidate signal transduction genes in tomato and *Arabidopsis* (listed in Table 1), which will be discussed in detail below. Perhaps the most significant result to come out of these screens, however, was the relative paucity of new genes identified. Several published screens failed to yield a single mutation in genes other than the targeted R gene, despite multiple hits in the R gene [7–9].

There are several plausible explanations for these failures. First, the signal transduction components may be encoded by redundant genes, such that loss of any individual gene produces little to no loss in resistance. Alternatively, some signal transduction components may be essential for plant viability precluding recovery of mutations. A third explanation (and not mutually exclusive) is that R gene signal transduction pathways are highly branched and that blockage of any one branch causes only an intermediate phenotype. Consistent with this hypothesis, the majority of the mutants listed in Table 1 do not produce a complete

Table 1

Mutations that suppress known R genes.		
Mutation (plant)	R genes suppressed* (pathogen)	Reference
<i>ndr1</i> (<i>Arabidopsis</i>)	<i>RPS2</i> , <i>RPM1</i> , <i>RPS5</i> (<i>P. syringae</i>) <i>RP P2</i> , <i>RPP4</i> , <i>RPP7</i> (<i>P. parasitica</i>)	[33,35**]
<i>eds1</i> (<i>Arabidopsis</i>)	<i>RPP1</i> , <i>RPP10</i> , <i>RPP12</i> , <i>RPP14</i> (<i>P. parasitica</i>); <i>RPS4</i> (<i>P. syringae</i>)	[38]
<i>pad1</i> [†] , <i>pad2</i> [†] , <i>pad3</i> [†] (<i>Arabidopsis</i>)	<i>RPP2</i> , <i>RPP4</i> , <i>RPP19</i> (<i>P. parasitica</i>)	[14**]
<i>pad4</i> (<i>Arabidopsis</i>)	<i>RPP2</i> , <i>RPP4</i> , <i>RPP19</i> (<i>P. parasitica</i>)	[14**]
<i>prf</i> (tomato)	<i>Pto</i> (<i>P. syringae</i>)	[29,31]
<i>rcr1</i> , <i>rcr2</i> (tomato)	<i>Cf9</i> (<i>C. fulvum</i>)	[39]
<i>rar1</i> , <i>rar2</i> (barley)	<i>Mla-12</i> , <i>Mla-13</i> , <i>Mlat</i> , <i>Mla-23</i> (<i>E. graminis</i> f.sp. <i>hordei</i>)	[3,4,5**,6]
<i>npr1/nim1</i> [‡] (<i>Arabidopsis</i>)	<i>RPP12</i> , <i>RPP14</i> (<i>P. parasitica</i>)	[20,21]

*Includes examples of partial suppression as well as complete suppression. †These mutations do not significantly suppress resistance, except in double mutant combinations with each other. ‡This mutation enhances hyphal growth of avirulent *P. parasitica* strains, but no sporulation is observed; thus, the indicated R genes are only mildly suppressed.

loss of resistance. It is likely that weakly susceptible mutants have been overlooked in most screens in favor of the fully susceptible R gene mutants.

To put the branched pathway hypothesis in context, one must consider what is known about R gene-activated defense responses. There are several distinct responses [10], including formation of active oxygen species, formation of antimicrobial secondary metabolites called phytoalexins, synthesis and secretion of hydrolytic enzymes (and other proteins of unknown function), general strengthening of the cell wall via crosslinking of cell wall components and deposition of phenolics, and programmed cell death. Thus, branching of signaling pathways must occur at some level. If branching occurs at, or very close to, the R gene product, there may be few mutations that can block more than a small subset of R gene-mediated defenses; thus, there may be few mutations outside of R gene mutations that cause dramatic decreases in resistance.

Screening for mutations that affect specific defense responses

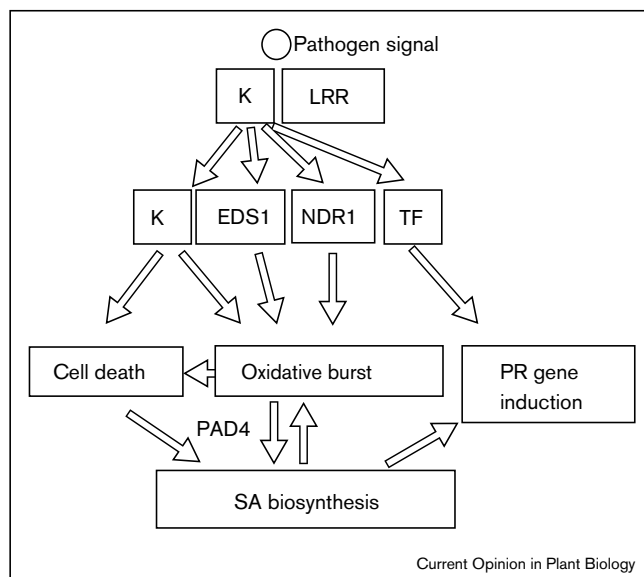
An alternative to screening for loss of resistance is to screen for loss of a specific defense response. Mutants recovered from such screens can subsequently be assayed for their effect on disease resistance and intermediate levels of resistance can be carefully quantified. Additive effects can be assayed by constructing double mutants.

Glazebrook and colleagues were among the first to pursue this strategy. They screened for mutations in *Arabidopsis* that reduced induction of the phytoalexin camalexin [11]. Five phytoalexin deficient (*pad*) mutants were identified [12,13,14**], but only the *pad3* mutant appeared to completely lack camalexin. The mutations in the other four mutants likely affect the regulation of camalexin production (directly or indirectly), rather than synthesis *per se*. None of the *pad* mutations affected resistance to avirulent *P. syringae* strains, thus, camalexin is not necessary for R gene-mediated resistance against *Pseudomonas syringae* [12,13,14**]. The *pad1*, *pad2*, and *pad3* mutations also had little to no effect on resistance to avirulent *P. parasitica* strains. Interestingly, however, all combinations of double mutants between *pad1*, *pad2*, and *pad3* displayed significant decreases in resistance to *P. parasitica* [14**]. These data indicate that these three *PAD* genes may be functioning in different pathways that are partially redundant for resistance to *P. parasitica*. In contrast to the other *pad* mutations, *pad4* appeared to completely suppress resistance mediated by the *P. parasitica* R genes *RPP2* and *RPP4*. Thus, the *PAD4* gene may function in a pathway that is critical for resistance to at least some *P. parasitica* strains.

Recent physiological analyses indicate that *pad4* plants are inhibited in salicylic acid (SA) accumulation, at least in response to a virulent strain of *P. syringae* (J Glazebrook, personal communication). SA is known to induce several pathogenesis related (*PR*) defense genes in *Arabidopsis* [15], and also functions in a positive feedback loop that boosts hydrogen peroxide production [16•], camalexin synthesis [17], and its own synthesis [18]. Reduction of endogenous SA levels in plants via introduction of a bacterial salicylate hydroxylase gene (*nahG*) enhances susceptibility to both virulent and avirulent pathogens [19]. Thus, SA plays a central role in multiple defense responses (Figure 1).

How the *pad4* mutation reduces SA levels is not known, nor is it known whether SA is reduced during responses to pathogens other than virulent strains of *P. syringae*. The *pad4* mutation does not reduce resistance mediated by the *P. syringae* resistance gene *RPS2* [13]. This observation suggests that *RPS2* can induce SA accumulation by a *pad4*-independent pathway, as reduction of SA levels by expression of *nahG* significantly reduces *RPS2*-mediated

Figure 1



Components of R gene signal transduction pathways. This model summarizes the genetic and yeast two-hybrid data discussed in the text, and is not meant to represent the situation for all R genes, or even a specific R gene. Recognition of the pathogen-derived molecule is mediated by a kinase (K), or a leucine-rich repeat (LRR)-containing protein, or possibly a complex containing both. Yeast two-hybrid data indicate that the kinase component may then interact with several different proteins, including other kinases, and transcription factors (TF). EDS1 and NDR1 are additional downstream factors identified by mutational screens, and are required by non-overlapping sets of R genes in *Arabidopsis*. Because of this specificity, I have positioned them close to the R gene complex. NDR1, EDS1, and the kinase component are depicted as activating the oxidative burst and cell death responses, either directly or indirectly. These responses in turn likely enhance synthesis of salicylic acid (SA), which acts to potentiate the oxidative burst and expression of PR genes. The *pad4* mutation of *Arabidopsis* may be inhibiting the SA induction signal.

resistance [19]. The different effects of *pad4* on *RPP2* and *RPP4* relative to *RPS2* suggest that the signal transduction pathways employed by these R genes are at least partly independent.

Screening for mutations that block defense gene induction

A second approach to screening for defense response mutants is to screen for loss of defense gene induction. This can be accomplished by fusing a promoter of interest to an easily assayed reporter gene such as β -glucuronidase (GUS), then screening for plants that fail to induce the reporter gene upon exposure to an avirulent pathogen, or other appropriate stimulus.

This approach was used to identify the *NPR1* gene of *Arabidopsis* [20]. Mutations in *NPR1* block induction of several different pathogenesis-related (PR) genes by salicylic acid. Interestingly, *npr1* mutants display no significant decrease in resistance to avirulent *P. syringae*

strains [20], and only very minor decreases in resistance to avirulent *P. parasitica* strains [21]. These data indicate that PR gene expression is not necessary for resistance to these pathogens. These data also suggest that SA's role in R gene mediated resistance may have more to do with its function in potentiating hydrogen peroxide production, than in enhancing defense gene expression (Figure 1).

Identifying putative signal transduction components using the yeast two-hybrid system

The cloning of plant R genes has made it possible to use the yeast two-hybrid system [22,23] to 'fish' for proteins that interact with R gene proteins. Martin and colleagues successfully used such a strategy to identify proteins that interact with the Pto protein of tomato [24].

The *Pto* gene confers resistance to *P. syringae* strains that carry the *avrPto* avirulence gene. *Pto* encodes a serine/threonine kinase that is unique among the characterized R gene proteins in that it does not contain a leucine-rich repeat (LRR) domain [25]. This observation is significant because in other R gene proteins, the LRR domain is postulated to bind pathogen-derived ligands (see review by J Ellis, this issue pp 288–293). Nevertheless, the *Pto* and *avrPto* proteins interact in a yeast two hybrid assay [26,27], indicating that *Pto* functions as a specific receptor for *avrPto*.

When *Pto* was used as the bait in a yeast two hybrid screen of a tomato cDNA library, several *Pto*-interacting (*Pti*) genes were identified [24,28•]. *Pti1* encodes a serine-threonine kinase that is specifically phosphorylated by *Pto* [24], indicating that it may participate in a kinase cascade activated by *Pto*. Consistent with this hypothesis, overexpression of *Pti1* in tobacco plants enhances induction of the cell death response induced by *avrPto*. These data must be interpreted with caution, however, as it is not known what gene mediates recognition of *avrPto* in tobacco. In addition, overexpression of PTI1 in tobacco could cause phosphorylation of proteins not normally phosphorylated by PTI1 in tomato.

In addition to *Pti1*, Martin and colleagues identified three putative transcription factors (*Pti4/5/6*) of the EREBP (ethylene responsive element-binding protein) class that interact specifically with *Pto* in the two-hybrid assay [28•]. Evidence that these interactions are biologically relevant is indirect. The *Pti5* and *Pti6* proteins (*Pti4* was not tested) bind to a GCCGCC element found upstream of several PR genes in *Arabidopsis*, tobacco, tomato, potato and bean. Furthermore, expression of the tobacco PR genes is enhanced by overexpression of *Pto*. Finally, a tobacco homolog of the *Pti* genes, EREBP1, is rapidly and highly induced by *P. syringae* strains that contain *avrPto*. The latter observation suggests that the relatively slow kinetics of PR gene induction in response to avirulent *P. syringae* (not detected until 9 hours after infection)

may be because the EREBP transcription factors must be induced first.

Taken together, the above data suggest that Pto activates transcription of *PR* genes via activation of multiple EREBP transcription factors (Figure 1). Several aspects of this model remain to be tested, however; whether Pto phosphorylates the Pti4, Pti5, and/or Pti6 proteins, as would be predicted in such a model, is still under investigation. Also not addressed is how avrPto activates Pto. The interactions between Pto and the Pti proteins do not require avrPto, and neither does the phosphorylation of Pti1 by Pto.

Although the evidence that *Pti1* and *Pti4,5,6* function in *Pto* signal transduction is still circumstantial, the present data fit a model in which Pto activates *PR* gene expression by a pathway that is completely separate from induction of cell death [28••]. This hypothesis is consistent with the branched model of R gene signal transduction proposed above and shown in Figure 1.

Mutational analysis of the Pto R gene pathway

Ideally, the model for Pto signal transduction developed from yeast two hybrid work should be tested through isolation of mutations in the *Pti* genes. Unfortunately, no mutations in the *Pti* genes in tomato have been identified. A screen for mutations in tomato that suppress Pto function, however, did identify one gene that is absolutely required for *Pto*-mediated resistance, *Prf* [29]. Interestingly, *Prf* is a member of the nucleotide binding site (NBS)/LRR class of plant R genes [30•], and is tightly linked to the *Pto* gene [31]. *Prf* is also required for sensitivity to fenthion, an organophosphorous insecticide, the response to which is mediated by the *Fen* gene [32]. *Fen* encodes a kinase that is 80% identical to *Pto* and is part of the *Pto/Prf* cluster [32]. These data suggest that *Prf* is not the primary determinant of specificity, but is required for the recognition of fenthion and avrPto. What role *Prf* serves in the recognition process is unclear.

Sorting out the relative roles of *Prf* and *Pto* in pathogen recognition has major implications on the function of other plant R genes. Because *Prf* encodes an NBS/LRR protein, one might expect other members of this family to perform a similar function in pathogen recognition. If so, NBS/LRR-type R genes should have kinase partners analogous to *Pto* that control specificity (Figure 1). Kinase partners for other NBS/LRR proteins have yet to be identified, however.

Signal transduction components shared by multiple R genes

The majority of the mutations listed in Table 1 suppress more than one R gene, indicating that R genes share common signal transduction components. It is clear, however, that the overlap is only partial as these mutations

often affect only a subset of R genes. For example, the *ndr1* mutation of *Arabidopsis* strongly suppresses *RPM1*, *RPS2*, and *RPS5* [33], but has no effect on *RPS4* (JE Parker and BJ Staskawicz, personal communication), even though all four R genes confer resistance to *P. syringae*. Interestingly, the *eds1* mutation displays the converse pattern, strongly suppressing *RPS4*, with no effect on *RPM1*, *RPS2*, and *RPS5* (JE Parker and BJ Staskawicz, personal communication). *EDS1* is also required for resistance mediated by some, but not all, *P. parasitica* resistance genes. The requirement for either *EDS1* or *NDR1*, but not both, clearly establishes that different R genes employ different signal transduction pathways. The choice between the *EDS1* pathway versus the *NDR1* pathway may be determined by the structure of the amino terminus of the R gene protein; *RPM1*, *RPS2*, and *RPS5* all contain a putative leucine zipper at the amino terminus, while *RPS4* lacks a leucine zipper, and instead contains a domain with homology to the *Drosophila* Toll protein and the mammalian interleukin-1 receptor (BJ Staskawicz personal communication). Toll/IL-1R domains are also present in the N-terminus of the *P. parasitica* R genes affected by *eds1*, at least those that have been cloned (J Parker, personal communication).

It is unclear whether the above data indicate that different R genes are inducing different defense responses, or simply that they are inducing common defense responses by independent pathways as suggested in Figure 1. Detailed analyses of the resistance responses activated by the *Mla-12* and *Mlg* powdery mildew resistance genes of barley suggest both hypotheses may be true. *Mla-12* mediated resistance is associated with a hypersensitive cell death that is induced after the fungus penetrates the epidermal cell wall and forms a haustorium [4]. In contrast, *Mlg*-mediated resistance acts prior to this stage, preventing successful penetration at most infection sites [34], suggesting that resistance is conferred by a different defense mechanism. In about 20% of the interaction sites on *Mlg* plants, however, the fungus succeeds in forming a haustorium, at which point a cell death response is triggered. Interestingly, The *rar1-2* mutation blocks the cell death response mediated by *Mla-12*, but not the cell death response mediated by *Mlg* [5••]; thus, these two R genes appear to induced the cell death response by different pathways.

Isolation of *NDR1* and *EDS1*

Regardless of how many distinct pathways are activated by R genes, we will not understand how these pathways function until the relevant genes are isolated. Significant progress on this front was made in the past year with the cloning of the *EDS1* and *NDR1* genes from *Arabidopsis* ([35••], JE Parker, personal communication).

The protein encoded by *NDR1* is 219 amino acids long and has no similarity to proteins of known function. Computer modeling predicts that *NDR1* contains two membrane

spanning domains, one near the amino terminus and one at the carboxyl terminus, suggesting that NDR1 is located in a membrane [35••]. NDR1 likely functions downstream of the initial recognition event, as R gene-mediated induction of at least one defense response (the hypersensitive cell death response) still occurs in the *ndr1-1* mutant (a null allele) [33,35••]. The lack of similarity to kinases, phosphatases, or ion channels, however, suggests that NDR1 fulfills a novel role in R gene signal transduction. Further insight into NDR1's function will likely require identification of proteins that interact with NDR1 and localization of NDR1 within the plant cell.

The protein encoded by *EDS1* bears no similarity to NDR1 (JE Parker, personal communication). It contains no apparent membrane spanning domains, and is nearly three times larger (623 amino acids). It thus seems likely that EDS1 and NDR1 fulfill different roles in R gene signal transduction. The amino-terminal half of EDS1 has similarity to several eukaryotic lipases, including three well conserved residues in the putative esterase catalytic site. If EDS1 is in fact a lipase, it could be involved in generation of a second messenger derived from a mono-, di-, or triacyl glycerol, or a phospholipid. Several derivatives of linolenic acid (a fatty acid found in plasma membrane lipids) are known to induce plant defense responses [36•], thus R gene-mediated activation of a lipase represents a plausible scenario.

Conclusions

Genetic approaches to dissecting R gene signal transduction pathways are still at an early stage of development. The recent cloning of EDS1 and NDR1 represent significant advances, but there are undoubtedly many other components yet to be identified. The existence of multiple defense responses that have additive effects on resistance may necessitate development of more sophisticated genetic screens. Screening for mutations that enhance or suppress existing mutations that have an intermediate phenotype has proven powerful in other systems, and could be applied to many of the existing mutations listed in Table 1.

Identification of proteins that interact with NDR1 and EDS1 using yeast two-hybrid technology should also provide new leads. In *Arabidopsis*, the biological relevance of such interacting proteins can now be assessed via identification of insertional disruptions in the relevant genes. Large libraries of *Arabidopsis* lines are becoming available that have insertions of T-DNAs or transposons. These libraries can be screened by PCR methodologies to identify lines with insertions in specific genes [37]. This combination of yeast two-hybrid analysis and reverse genetics should greatly accelerate identification of R gene signal transduction components.

Acknowledgements

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Note added in proof

The paper referred to in the text as (Glazebrook, personal communication) has now been accepted for publication [40••]. Also, a recent paper by Aarts *et al.* [41••] reports the use of genetics to show that different R genes confer disease resistance via different signal transduction pathways.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. McIntosh RA: **Nature of induced mutations affecting disease reaction in wheat.** In: *Induced Mutations Against Plant Diseases: Proceedings of the Symposium on the Use of Induced Mutations for Improving Disease Resistance in Crop Plants: 1997 31 Jan-4 Feb; Vienna, Austria: International Atomic Energy Agency; 1977:551-563.*
 2. Torp J, Jorgensen J: **Modification of barley powdery mildew resistance gene *Mla-12* by induced mutation.** *Can J Genet Cytol* 1986, **28**:725-731.
 3. Jorgensen JH: **Genetic analysis of barley mutants with modifications of powdery mildew resistance gene *Mla-12*.** *Genome* 1988, **30**:129-132.
 4. Freialdenhoven A, Scherag B, Hollricher K, Collinge DB, Thordal-Christensen H, Schulze-Lefert P: ***Nar-1* and *Nar-2*, two loci required for *Mla*₁₂-specified race-specific resistance to powdery mildew in barley.** *Plant Cell* 1994, **6**:983-994.
 5. Peterhänsel C, Freialdenhoven A, Kurth J, Kolsch R, Schulze-Lefert P: **Interaction analyses of genes required for resistance responses to powdery mildew in barley reveal distinct pathways leading to leaf cell death.** *Plant Cell* 1997, **9**:1397-1409.
- An important and elegant study that demonstrated at least three distinct pathways for triggering cell death and disease resistance in barley in response to powdery mildew.
6. Jorgensen JH: **Effect of three suppressors on the expression of powdery mildew resistance genes in barley.** *Genome* 1996, **39**:492-498.
 7. Bisgrove SR, Simonich MT, Smith NM, Sattler A, Innes RW: **A disease resistance gene in *Arabidopsis* with specificity for two different pathogen avirulence genes.** *Plant Cell* 1994, **6**:927-933.
 8. Okubara PA, Anderson PA, Oswald E, Michelmore RW: **Mutants of downy mildew resistance in *Lactuca sativa* (lettuce).** *Genetics* 1994, **137**:867-874.
 9. Kunkel BN, Bent AF, Dahlbeck D, Innes RW, Staskawicz BJ: ***RPS2*, an *Arabidopsis* disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*.** *Plant Cell* 1993, **5**:865-875.
 10. Hammond-Kosack KE, Jones JDJ: **Resistance gene-dependent plant defense responses.** *Plant Cell* 1996, **8**:1773-1791.
 11. Tsuji J, Jackson EP, Gage DA, Hammerschmidt R, Somerville SC: **Phytoalexin accumulation in *Arabidopsis thaliana* during the hypersensitive reaction to *Pseudomonas syringae* pv. *syringae*.** *Plant Physiol* 1992, **98**:1304-1309.
 12. Glazebrook JL, Ausubel FM: **Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens.** *Proc Natl Acad Sci USA* 1994, **91**:8955-8959.
 13. Glazebrook J, Rogers EE, Ausubel FM: **Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening.** *Genetics* 1996, **143**:973-982.
 14. Glazebrook J, Zook M, Mert F, Kagan I, Rogers EE, Crute IR, Holub EB, Hammerschmidt R, Ausubel FM: **Phytoalexin-deficient mutants of *Arabidopsis* reveal that *PAD4* encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance.** *Genetics* 1997, **146**:381-392.

This paper makes the important observation that double mutant combinations of *pad1*, *pad2*, and *pad3* suppress multiple R genes, while the individual mutations do not, pointing out the likely redundancy of many defense responses.

15. Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD: **Systemic acquired resistance**. *Plant Cell* 1996, **8**:1809-1819.
 16. Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb C: **Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms**. *Plant Cell* 1997, **9**:261-270.
- A seminal paper that demonstrated the synergistic interaction between pathogen signals and SA. Even low levels of SA (50 μM) dramatically enhanced defense responses activated by an avirulent strain of *P. syringae* using a soybean cell suspension system. In addition, H₂O₂ production and cell death could be blocked by inhibitors of SA biosynthesis.
17. Zhao J, Last R: **Coordinate regulation of the tryptophan biosynthetic pathway of indolic phytoalexin accumulation in Arabidopsis**. *Plant Cell* 1996, **8**:2235-2244.
 18. Mauch-Mani B, Slusarenko AJ: **Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of Arabidopsis to Peronospora parasitica**. *Plant Cell* 1996, **8**:203-212.
 19. Delaney TP, Uknes SJ, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, Ryals J: **A central role of salicylic acid in plant disease resistance**. *Science* 1994, **266**:1247-1249.
 20. Cao H, Bowling SA, Gordon S, Dong X: **Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance**. *Plant Cell* 1994, **6**:1583-1592.
 21. Delaney TP, Friedrich L, Ryals JA: **Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance**. *Proc Natl Acad Sci USA* 1995, **92**:6602-6606.
 22. Chien C-T, Bartel PL, Sternglanz R, Fields S: **The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest**. *Proc Natl Acad Sci USA* 1991, **88**:9578-9582.
 23. Fields S, Sternglanz R: **The two-hybrid system: An assay for protein-protein interactions**. *Trends Genet* 1994, **10**:286-292.
 24. Zhou J, Loh Y-T, Bressan RA, Martin GB: **The tomato gene *Pti1* encodes a serine/threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response**. *Cell* 1995, **83**:1-20.
 25. Loh YT, Martin GB: **The *Pto* bacterial resistance gene and the *Fen* insecticide sensitivity gene encode functional protein kinases with serine/threonine specificity**. *Plant Physiol* 1995, **108**:1735-1739.
 26. Scofield SR, Tobias CM, Rathjen JP, Chang JH, Lavelle DT, Michelmore RW, Staskawicz BJ: **Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato**. *Science* 1996, **274**:2063-2065.
 27. Tang X, Frederick RD, Zhou J, Halterman DA, Jia Y, Martin GB: **Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase**. *Science* 1996, **274**:2060-2063.
 28. Zhou J, Tang X, Martin GB: **The Pto kinase conferring resistance to tomato bacterial speck disease interacts with proteins that bind a cis-element of pathogenesis-related genes**. *EMBO J* 1997, **16**:3207-3218.

The first and only paper to show an interaction between an R gene protein and transcription factors. This work has two major implications: signal transduction pathways to the nucleus may be very short, and R gene proteins may directly activate multiple pathways.

29. Salmeron JM, Barker SJ, Carland FM, Mehta AY, Staskawicz BJ: **Tomato mutants altered in bacterial disease resistance provide evidence for a new locus controlling pathogen recognition**. *Plant Cell* 1994, **6**:511-520.
 30. Jones DA, Jones JDG: **The role of leucine-rich repeat proteins in plant defences**. *Adv Bot Res* 1997, **24**:89-167.
- This paper is much more than simply a review. Its insightful analysis has quickly become the paradigm for analyzing the structure and function of R gene LRR domains.
31. Salmeron JM, Oldroyd GED, Rommens CMT, Scofield SR, Kim H-S, Lavelle DT, Dahlbeck D, Staskawicz BJ: **Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster**. *Cell* 1996, **86**:123-134.
 32. Martin GB, Frary A, Wu T, Brommonschenkel S, Chunwongse J, Earle ED, Tanksley SD: **A member of the tomato *Pto* gene family confers sensitivity to fenthion resulting in rapid cell death**. *Plant Cell* 1994, **6**:1543-1552.
 33. Century KS, Holub EB, Staskawicz BJ: ***NDR1*, a locus of Arabidopsis thaliana that is required for disease resistance to both a bacterial and a fungal pathogen**. *Proc Natl Acad Sci USA* 1995, **92**:6597-6601.
 34. Görg R, Hollricher K, Schulze-Lefert P: **Functional analysis and RFLP-mediated mapping of the *Mlg* resistance locus in barley**. *Plant J* 1993, **3**:857-866.
 35. Century KS, Shapiro AD, Repetti PP, Dahlbeck D, Holub E, Staskawicz BJ: ***NDR1*, a pathogen-induced component required for Arabidopsis disease resistance**. *Science* 1997, **278**:1963-1965.
- One of the major advances of 1997 in R gene signal transduction research. This paper describes the cloning of the *NDR1* gene and discusses the predicted amino acid sequence of NDR1. Its novel structure illustrates how much remains to be learned.
36. Creelman RA, Mullet JE: **Biosynthesis and action of jasmonates in plants**. *Annu Rev Plant Physiol Plant Mol Biol* 1997, **48**:355-381.
- An authoritative review on the role of jasmonates in plant signal transduction, including their role in activation of defense responses.
37. McKinney EC, Ali N, Traut A, Feldmann KA, Belostotsky DA, McDowell JM, Meagher RB: **Sequence-based identification of T-DNA insertion mutations in Arabidopsis: actin mutants act2-1 and act4-1**. *Plant J* 1995, **8**:613-622.
 38. Parker JE, Holub EB, Frost LN, Falk A, Gunn ND, Daniels MJ: **Characterization of *eds1*, a mutation in Arabidopsis suppressing resistance to *Peronospora parasitica* specified by several different *RPP* genes**. *Plant Cell* 1996, **8**:2033-2046.
 39. Hammond-Kosack KE, Jones DA, Jones JDG: **Identification of two genes required in tomato for full *Cf-9*-dependent resistance to *Cladosporium fulvum***. *Plant Cell* 1994, **6**:361-374.
 40. Zhou N, Tootle TL, Tsui F, Klessig DF, Glazebrook J: ***PAD4* functions upstream of salicylic acid to control defense responses in Arabidopsis**. *Plant Cell* 1998, **10**:1021-1030.
- A nicely written paper that attempts to position the PAD4 protein in R gene signal transduction pathways. The discovery that *pad4* mutants display significantly reduced levels of salicylic acid further demonstrates the central role SA serves in disease resistance and will likely inspire others to evaluate their disease susceptible mutants for SA levels.
41. Aarts N, Metz M, Holub E, Staskawicz BJ, Daniels MJ, Parker JE: **Different requirements for *EDS1* and *NDR1* by disease resistance genes define at least two R gene mediated signalling pathways in Arabidopsis**. *Proc Natl Acad Sci* 1998, in press.

This important piece of work used genetics to show that different R genes confer disease resistance via different signal transduction pathways.