Genes controlling expression of defense responses in *Arabidopsis*

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In the past year, two regulatory defense-related genes, *EDS1* and *COI1*, have been cloned. Several other genes with regulatory functions have been identified by mutation, including *DND1*, *PAD4*, *CPR6*, and *SSI1*. It has become clear that jasmonate signaling plays an important role in defense response signaling, and that the jasmonate and salicylic acid signaling pathways are interconnected.

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Abbreviations

avr	avirulence
HR	hypersensitive response
ISR	induced systemic resistance
JA	jasmonic acid
LRR	leucine rich repeat
LZ	leucine zipper
NBS	nucleotide binding site
PR	pathogenesis related
R	resistance
SA	salicylic acid
SAR	systemic acquired resistance

Introduction

Plants are capable of activating a large array of defense mechanisms in response to pathogen attack. A crucial factor determining the success of these mechanisms is the speed of their activation. Consequently, there is considerable interest in understanding how plants recognize pathogen attack and control expression of defense mechanisms.

Some potential pathogens trigger a very rapid resistance response called gene-for-gene resistance. This occurs when the pathogen carries an avirulence (*avr*) gene that triggers specific recognition by a corresponding host resistance (*R*) gene. *R* gene specificity is generally quite narrow, in most cases only pathogens carrying a particular *avr* gene are recognized. Recognition is thought to be mediated by ligand–receptor binding. *R* genes have been studied extensively in recent years and several excellent reviews are available [1–3].

One of the defense mechanisms triggered by gene-for-gene resistance is programmed cell death at the infection site. This is called the hypersensitive response, or HR. Pathogens that induce the HR, or cause cell death by other means, activate a systemic resistance response called systemic acquired resistance (SAR). During SAR, levels of salicylic acid (SA) rise throughout the plant, defense genes such as pathogenesis related (PR) genes are expressed, and the plant becomes more resistant to pathogen attack. SA is a crucial component of this response. Plants that cannot accumulate SA due to the presence of a transgene that encodes an SA-degrading enzyme (*nahG*), develop an HR in response to challenge by avirulent pathogens, but do not exhibit systemic expression of defense genes and do not develop resistance to subsequent pathogen attack [4]. The nature of the systemic signal that triggers SAR is a subject of debate [5,6]. SA clearly moves from the site of the HR to other parts of the plant, but if this is the signal, it must be effective at extremely low concentration [7].

SAR is quite similar to some reactions that occur locally in response to attack by virulent (those that cause disease) or avirulent (those that trigger gene-for-gene resistance) pathogens. In general, activation of defense gene expression occurs more slowly in response to virulent pathogens than in response to avirulent pathogens. Some pathogens trigger expression of defense genes through a different signaling pathway that requires components of the jasmonic acid (JA) and ethylene signaling pathways [8]. The SA and JA pathways interact in a complicated manner that is poorly understood.

One approach to understanding the signal transduction networks that control defense mechanisms is to use genetic methods to identify signaling components and determine their roles within the network. Considerable progress has been made using this approach in *Arabidopsis*–pathogen model systems. This review will focus on recent (published in 1998 and early 1999) progress in identifying *Arabidopsis* genes that affect regulation of defense gene expression, and on what is known about their roles and relative positions in the signal transduction network. Figure 1 shows a model of how the network might be arranged (see [9], for a discussion on earlier work). Due to space limitations, *R* genes, genes studied in other plant species, and insights gained from other types of analysis will not be discussed in detail.

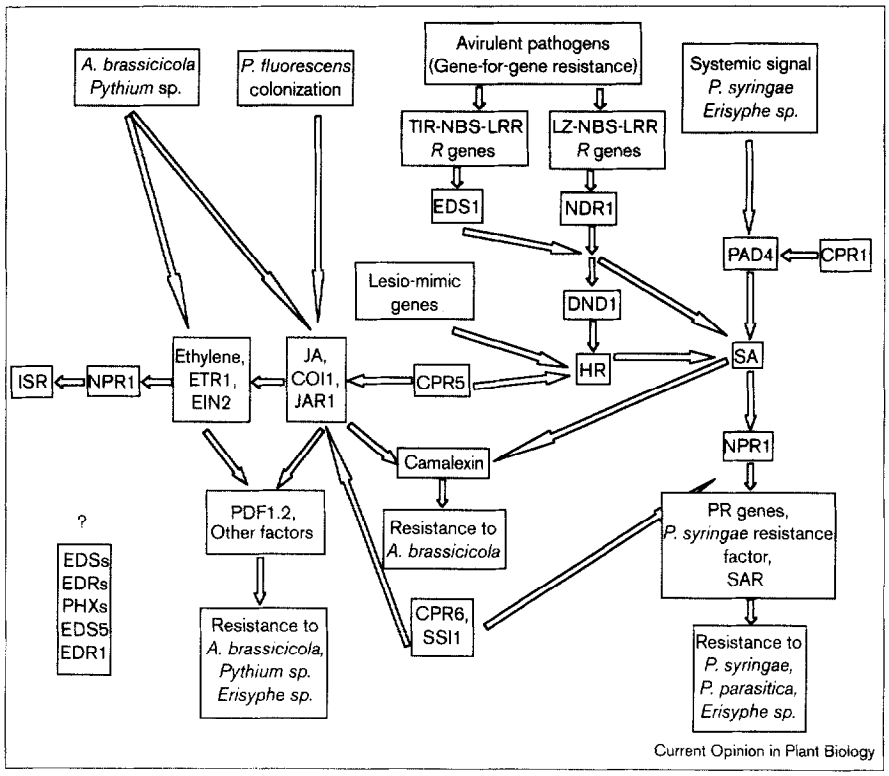
R gene signal transduction

Genes such as *NDR1*, *EDS1*, *DND1*, and the lesion-mimic genes probably act in signal transduction pathways downstream from *R–avr* recognition.

NDR1 and *EDS1* are required for gene-for-gene mediated resistance to avirulent strains of the bacterial pathogen *Pseudomonas syringae* and the oomycete pathogen *Peronospora parasitica*. Curiously, *ndr1* mutants are susceptible to one set of avirulent pathogens, whereas *eds1* mutants are susceptible to a non-overlapping set [10••]. The five cloned *R* genes that require *EDS1* all belong to the subset of the nucleotide binding site–leucine rich repeat (NBS–LRR) class of *R* genes

Figure 1

A model of the defense response signaling network showing the relative sites of action of genes discussed in this review. This model is almost certain to be found incorrect before this article is published, and is intended only as a means to stimulate discussion. The SA amplification loop is not shown, as it is not clear which genes might be involved in this. The mutual inhibition between the JA and SA pathways is not shown for the same reason. The rationale for the arrangement of genes in the network is presented in the text. This figure is adapted from Figure 1 of last year's review of this topic [9], with alterations to incorporate results reported in the last year.



that contain sequences similar to the cytoplasmic domains of *Drosophila* Toll and mammalian interleukin 1 transmembrane receptors. The two genes that require *NDR1* belong to the leucine-zipper (LZ) subclass of NBS–LRR genes. There is another LZ–NBS–LRR gene that does not require *EDS1* or *NDR1*, so the correlation between R gene structure and requirement for *EDS1* or *NDR1* is not perfect. Nevertheless, these results show that *R* genes differ in their requirements for downstream factors and that these differences are correlated with *R* gene structural type.

NDR1 encodes a protein with two predicted transmembrane domains [11]. RPM1, which requires NDR1 to mediate resistance, is membrane-associated, despite the fact that its primary sequence does not include any likely membrane-integral stretches [12]. It is possible that part of the function of NDR1 is to hold *R* proteins close to the membrane. *EDS1* encodes a protein with blocks of homology to triacyl glycerol lipases [13••]. The significance of this homology is not known, but it is tempting to speculate that EDS1 is involved in synthesis or degradation of a signal molecule. EDS1 expression is inducible by SA and pathogen infection, suggesting that EDS1 may be involved in signal amplification [13••].

It has been extremely difficult to isolate mutations in genes other than the *R* genes that are required for gene-for-gene resistance. McNellis *et al.* have devised a selection procedure on the basis of precisely controlled inducible expression of the *avr* gene *avrRpt2* in plants carrying the corresponding resistance gene *RPS2* [14•]. Expression of *avrRpt2* in this background is lethal, as it triggers a systemic HR. It is now possible to select for mutants with subtle defects in gene-for-gene signaling by requiring growth on a concentration of inducer slightly higher than the lethal dose. This is a very promising approach for identifying loci involved in gene-for-gene resistance and/or the HR.

Characterization of *dnd1* mutants has provided genetic evidence that the HR is separable from gene-for-gene resistance [15••]. When *dnd1* plants are infected with avirulent pathogens, no HR occurs, but the level of resistance is comparable to that in wild-type plants. One possibility is that DND1 is a regulator of cell death. However, *dnd1* mutants also have elevated SA levels and constitutively express the defense gene *PR1*, raising the possibility that SAR activation leads indirectly to suppression of cell death. This idea could be tested by constructing a *dnd1 nahG* line.

Lesion-mimic mutants develop HR-like lesions, have high levels of SA, and express defense genes, all in the absence of pathogen attack. It is likely that some of the lesion-mimic gene products have important roles in regulation of the HR. These mutants have been studied quite extensively, but few results have been reported in the last year. The reader may refer to recent reviews describing this interesting class of mutants [16,17].

SA-dependent signaling

SA levels increase locally in response to pathogen attack, and systemically in response to the SAR-inducing signal. SA is necessary and sufficient for activation of PR gene expression and enhanced disease resistance. Physiological analyses and characterization of certain lesion-mimic mutants strongly suggest that there is a positive autoregulatory loop affecting SA concentrations [18–20]. Several mutants with defects in SA signaling have been characterized. These include *npr1*, in which expression of *PR* genes in response to SA is blocked; *cpr1*, *cpr5*, and *cpr6*, which constitutively express *PR* genes; the *npr1* suppressor *ssi1*; *pad4*, which has a defect in SA accumulation; and *eds5*, which has a defect in *PR1* expression.

Expression of the defense genes *PR1*, *BG2*, and *PR5* in response to SA treatment requires a gene called *NPR1* or *NIM1*. Mutations in *npr1* abolish SAR, and cause enhanced susceptibility to infection by various pathogens [21–24]. NPR1 appears to be a positive regulator of *PR* gene expression that acts downstream from SA. *NPR1* encodes a novel protein that contains ankyrin repeats (which are often involved in protein–protein interactions [25,26]), and that is localized to the nucleus in the presence of SA [9]. Consequently, it is unlikely that NPR1 acts as a transcription factor to directly control *PR* gene expression, but its nuclear localization suggests that it may interact with such transcription factors.

The *cpr1*, *cpr5*, and *cpr6* mutations cause elevated SA levels, constitutive expression of *PR1*, *BG2*, and *PR5*, and resistance to *P. syringae* and *P. parasitica* [27,28,29••]. In all cases, *cpr nahG* plants do not exhibit elevated gene expression or resistance to *P. syringae*, suggesting that the *CPR* genes act upstream from SA. In *cpr5 npr1* double mutants, defense gene expression and resistance to *P. syringae* are abolished, confirming that CPR5 is acting upstream from NPR1 [28]. The case of *cpr6* mutants is more complicated. The *cpr6* mutation is dominant, so it is likely that the mutant phenotype represents a gain of function rather than a loss of function [29••]. In *cpr6 npr1* plants, constitutive expression of *PR1*, *BG2*, and *PR5* is retained, but resistance to *P. syringae* is lost [29••]. This result leads to two interesting conclusions. First, there must be an SA-dependent, NPR1/NIM1-independent mechanism for activation of *PR1*, *BG2*, and *PR5* [29••]. This could explain the observation that in *npr1* plants infected with *P. syringae*, expression of *PR1* is reduced but not abolished, and expression of *BG2* and *PR5* is wild-type [23]. Second, the factor responsible for *P. syringae* resistance in *cpr6* plants is not *PR1*, *BG2*, or *PR5*, implying that the relationship between expression of these genes and *P. syringae* resistance is merely correlative, not causal [29••]. The challenge now is to find a defense mechanism that is constitutively expressed in *cpr6* in an NPR1-dependent manner, and to determine if this mechanism confers resistance to *P. syringae*.

The phenotypes caused by the dominant *ssi1* mutation superficially resemble those of *cpr* mutants, with the important difference that *ssi1* suppresses *npr1* mutations [30••]. In *ssi1* plants, *PR1*, *BG2*, and *PR5* are constitutively expressed [30••]. In *ssi1 npr1* plants, this expression remains, and unlike *cpr6 npr1* plants, the enhanced sensitivity of *npr1* to *P. syringae* infection is suppressed [30••]. All of the *ssi1* phenotypes are abolished by *nahG*, demonstrating that they are SA-dependent [30••].

PAD4 seems to act upstream from SA. In *pad4* plants infected with a virulent *P. syringae* strain, SA levels, synthesis of the antimicrobial compound camalexin, and *PR1* expression are all reduced [31•]. SA is necessary, but not sufficient, for activation of camalexin synthesis [31•,32]. The camalexin defect in *pad4* plants is reversible by exogenous SA [31•]. Mutations in *pad4* do not affect SA levels, camalexin synthesis, or *PR1* when plants are infected with an avirulent *P. syringae* strain [31•]. Taken together, these results suggest that PAD4 is required for signal amplification to activate the SA pathway in response to pathogens that do not elicit a strong defense response [31•]. The phenotypes of *cpr1 pad4* plants are indistinguishable from those of *pad4* plants, indicating that CPR1 acts upstream from PAD4 to activate *PR* gene expression (N Zhou and J Glazebrook, unpublished data).

Expression of *PR1* is also reduced in *eds5* mutants infected with a virulent *P. syringae* strain [33]. It is likely that EDS5 acts somewhere in the SA pathway. The phenotypes of the various mutants suggest that CPR1 and CPR5 act upstream from SA as negative regulators of SA signaling. CPR6 may also be a positive regulator acting upstream from SA. NPR1 appears to be a positive regulator that functions downstream from SA to activate a subset of SA-dependent responses. SSI1 and EDS5 also affect SA signaling, but their positions in the signal transduction network are not yet clear.

JA-dependent signaling

JA signaling affects diverse processes including fruit ripening, pollen development, root growth, and response to wounding [8]. The *jar1* and *coi1* mutants fail to respond to JA [34,35]. *COI1* has been cloned, and found to encode a protein containing leucine-rich repeats and a degenerate F-box motif [36••]. These features are characteristic of proteins that function in complexes that ubiquitinate proteins targeted for degradation. It follows that COI1 may act by promoting degradation of a factor that exerts a negative regulatory effect in the JA signal transduction pathway.

In the past few years it has become apparent that JA plays an important role in regulation of pathogen defenses. Inoculation of *Arabidopsis* with the avirulent fungal pathogen *Alternaria brassicicola* induces expression of the defensin gene *PDF1.2* [37]. This induction does not require SA or NPR1, but it does require ethylene and JA signaling [37]. Studies of the effect of mutations in *ETR1* (the ethylene receptor), *EIN2* (required for responses to ethylene) or *COI1* on *PDF1.2* expression in response to

A. brassicicola, ethylene, JA, or combinations of JA and ethylene suggest a model in which ethylene and JA are required simultaneously for *PDF1.2* expression [38••].

Like SA signaling, JA signaling has systemic effects. Plants in which only a few leaves were infected with *A. brassicicola* express *PDF1.2* throughout the plant [37]. Although *A. brassicicola* fails to infect wild-type plants, it is able to infect *coi1* mutants, suggesting that JA signaling is required for resistance to *A. brassicicola*. JA-dependent responses are also sufficient to confer resistance to *A. brassicicola*. This was demonstrated using *pad3* mutants, which are unable to synthesize camalexin and are susceptible to *A. brassicicola* [39,40•]. Treatment of *pad3* plants with JA prior to infection greatly reduced *A. brassicicola* growth [40•].

SA signaling and JA signaling pathways are interconnected in complicated ways. Studies in other systems have shown that SA signaling and JA signaling are mutually inhibitory [8,41]. However, synthesis of camalexin in response to *P. syringae* infection is blocked in *nahG* [31•,32] and *coi1* (J Glazebrook, unpublished data) plants, strongly suggesting that camalexin synthesis requires both SA and JA signaling. The *cpr5*, *cpr6*, and *acd2* mutations cause constitutive expression of both *PR1* and *PDF1.2*, suggesting that there may be a common control point for activation of both pathways. [28,29••,37]. *PDF1.2* is also constitutively expressed in *ssi1* plants. Curiously, this expression is SA-dependent, in contrast with wild-type plants, in which activation of *PDF1.2* expression is completely SA-independent [30••]. The proposed explanation for this effect is that *ssi1* acts as a switch between the two pathways [30••]. An alternative possibility is that *ssi1* perturbs the balance of SA-dependent and JA-dependent signaling in a way that shifts *PDF1.2* expression toward SA-dependence.

Induced systemic resistance (ISR)

Some rhizosphere-associated bacteria promote disease resistance [42]. This phenomenon, called ISR, has been studied using *Pseudomonas fluorescens* strain WCS417r to colonize *Arabidopsis* roots [43]. Colonized plants are more resistant to infection by the fungal pathogen *Fusarium oxysporum* f sp *raphani* and *P. syringae* [43]. ISR occurs in *nahG* plants, indicating that it is not an SA-dependent phenomenon [43]. Rather, ISR appears to be JA- and ethylene-dependent. The observation that ethylene can induce ISR in *jar1* mutants led to the hypothesis that ISR requires a JA signal followed by an ethylene signal [44••]. No changes in gene expression associated with ISR have been detected [44••], suggesting that it is different from activation of *PDF1.2* expression by *A. brassicicola*.

Curiously, ISR requires *NPR1* [44••]. This was unexpected in light of the facts that NPR1 was previously known to be involved only in SA-dependent processes, and ISR is SA-independent. This result implies that NPR1 can respond to signals from at least two different sources, one that is SA-dependent and one that is derived from ISR signaling. If the SA-dependent signal is received, NPR1 mediates a resistance response characterized by *PR1* expression, whereas if the ISR signal is received, NPR1 mediates a different resistance response. It is difficult to imagine how this could occur, unless NPR1 is interacting with different 'adapter' molecules to mediate the different signals. The ankyrin repeats found in NPR1 could function in protein–protein interactions between NPR1 and adapter proteins. Identification of proteins that interact with NPR1, and characterization of plants with loss-of-function mutations affecting those proteins, would be very helpful for understanding how NPR1 acts in each pathway. It would also be worthwhile to determine if the *ssi1 or cpr6* mutations suppress the ISR defect of *npr1* mutants.

Relevance to disease resistance

Characterization of the effects of various mutations on resistance to different pathogens has revealed that there is considerable variation in the extent to which pathogens are affected by defense mechanisms. SAR is known to confer resistance to a wide array of pathogens, including bacteria, fungi, oomycetes, and viruses. In *Arabidopsis*, the SA pathway mutants *npr1* and *pad4* show enhanced susceptibility to *P. syringae* and *P. parasitica* [21,22,24,31•,45]. The fungus *Erisyphe orontii* also seems to be sensitive to SA-dependent responses. Among a collection of mutants that display enhanced susceptibility to *P. syringae*, only mutants that had defects in expression of *PR1* were also more susceptible to *E. orontii* [46•]. *P. parasitica* may be inhibited by JA-dependent mechanisms as well as by SA-dependent ones. In *cpr5 npr1* double mutants, the *PR1* expression and resistance to *P. syringae* caused by *cpr5* is abolished, but *PDF1.2* expression and *P. parasitica* resistance are retained, suggesting that activation of the JA pathway is causing *P. parasitica* resistance [28].

JA signaling is important for limiting the growth of certain fungal pathogens. The *fad3-2 fad7-2 fad8* triple mutant is unable to synthesize JA due to an inability to produce linoleic acid, a precursor of JA. These plants and *jar1* plants are much more susceptible to infection by *Pythium* species than wild-type plants are [47•,48•]. JA treatment enhances resistance to *A. brassicicola*, and *coi1* mutants show enhanced susceptibility, whereas the *nahG* transgene and an *npr1* mutation have no effect [40]. These observations suggest that JA signaling is important for resistance to fungi such as *Pythium* species and *A. brassicicola*, while SA signaling has little effect on resistance to *A. brassiciciola*.

Overexpression of rate-limiting defense response regulators may cause the signaling network to respond faster or more strongly to pathogen attack, thereby improving resistance. Overexpression of *NPR1* caused increased resistance to *P. syringae* and *P. parasitica* in a dosage dependent manner [49••]. Importantly, *NPR1*-overexpression had no obvious deleterious effects on plant growth, in contrast to mutations that lead to constitutive overexpression of defense responses, which generally cause dwarfism. In

the future, the effect of overexpression of other cloned regulatory genes, such as *NDR1*, *EDS1*, and *COI1*, should be tested.

Other mutations that may affect signaling

There are several mutants that affect disease resistance that may prove to be involved in control of defense responses, but have not yet been characterized in detail. These include *eds* mutants, that show enhanced disease susceptibility to virulent *P. syringae* strains [23,33,50], *phx* mutants, isolated as suppressors of the lesion-mimic mutant *lsd5* [51], and *edr* mutants, which display enhanced resistance to *P. syringae* and/or *Erisyphe cichoracearum* infection [52•]. *EDR1* almost certainly affects SA signaling, since expression of *PR1* in response to *E. cichoracearum* infection occurs more rapidly in *edr1* mutants than in wild-type plants [52•].

Conclusions

Many genes that function in regulation of defense responses have been identified. Progress has been made in determining the positions of various genes in the signal transduction network. However, current models seem to have little predictive value, in that characterization of new mutants often requires wholesale rearrangements of the existing models in order to explain observed phenotypes. Obviously, the signal transduction network is not well understood.

The field is now in a position to develop second-generation approaches to identify additional components of the signaling networks. These include screening for suppressors and enhancers of known mutations, and using two-hybrid screens to identify proteins that may interact with the products of cloned genes. The biological significance of two-hybrid interactions can be tested using a reverse-genetic approach to obtain loss-of-function mutations in the relevant genes.

For determining the roles of each gene in the signal transduction network, it would be very helpful if all mutants were tested for all phenotypes. It is also important to construct double mutants for epistasis testing. Both of these approaches require free exchange of mutants among various laboratories. The sequencing of the *Arabidopsis* genome, which should be complete in late 2000, will make it possible to apply powerful new techniques to the study of signaling. For example, 'gene chips' could be used to monitor expression levels of every gene simultaneously, so that the effects of mutations on gene expression patterns can be determined completely and efficiently. This will be useful for discovery of pathogen-inducible genes that are not yet known, as well as for elucidation of signal transduction networks.

Acknowledgements

I apologize to scientists whose work I overlooked, or was not able to include due to space limitations.

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33. Rogers EE, Ausubel FM: ***Arabidopsis* enhanced disease susceptibility mutants exhibit enhanced susceptibility to several bacterial pathogens and alterations in *PR-1* gene expression.** *Plant Cell* 1997, **9**:305-316.

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35. Staswick PE, Su W, Howell SH: **Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant.** *Proc Natl Acad Sci USA* 1992, **89**:6837-6840.

36. •• Xie D-X, Feys BF, James S, Nieto-Rostro M, Turner JG: ***COI1*: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility.** *Science* 1998, **280**:1091-1094.

This paper describes isolation of *COI1*. Sequence similarities lead to the hypothesis that protein degradation is involved in JA signaling.

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38. •• Penninckx IAMA, Thomma BPHJ, Buchala A, Metraux J-P, Broekaert WF: **Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*.** *Plant Cell* 1998, **10**:2103-2113.

A nice series of experiments show that jasmonate and ethylene are required concomitantly, rather than sequentially, for activation of *PDF1 2* expression. Confusion about the requirement of ETR1 for *PDF1 2* expression is resolved by using a stronger allele.

39. Glazebrook J, 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.

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46. • Reuber TL, Plotnikova JM, Dewdney J, Rogers EE, Wood W, Ausubel FM: **Correlation of defense gene induction defects with powdery mildew susceptibility in *Arabidopsis* enhanced disease susceptibility mutants.** *Plant J* 1998, **16**:473-485.

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• **jasmonate in pathogen defense of *Arabidopsis*** *Proc Natl Acad Sci USA* 1998, **95**:7209-7214.
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