

# Systemic Acquired Resistance

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## INTRODUCTION

Systemic acquired resistance (SAR) refers to a distinct signal transduction pathway that plays an important role in the ability of plants to defend themselves against pathogens. After the formation of a necrotic lesion, either as a part of the hypersensitive response (HR) or as a symptom of disease, the SAR pathway is activated. SAR activation results in the development of a broad-spectrum, systemic resistance (Hunt and Ryals, 1996; Neuenschwander et al., 1996). Although SAR is interesting as a paradigm for signal transduction, it may have practical value as well. An understanding of the biochemical changes leading to the resistance state could enable the development of either genetically engineered plants with enhanced disease resistance or novel mode-of-action plant protection chemicals that act by stimulating the plant's inherent disease resistance mechanisms.

SAR can be distinguished from other disease resistance responses by both the spectrum of pathogen protection and the associated changes in gene expression. In tobacco, SAR activation results in a significant reduction of disease symptoms caused by the fungi *Phytophthora parasitica*, *Cercospora nicotianae*, and *Peronospora tabacina*, the viruses tobacco mosaic virus (TMV) and tobacco necrosis virus (TNV), and the bacteria *Pseudomonas syringae* pv *tabaci* and *Erwinia carotovora* (Vernooij et al., 1995). However, the protection is not effective against all pathogens. For example, there is no significant protection against either *Botrytis cinerea* or *Alternaria alternata*. Thus, SAR provides resistance against seven of nine tobacco pathogens, establishing a distinctive fingerprint of protection.

Associated with SAR is the expression of a set of genes called SAR genes (Ward et al., 1991). However, not all defense-related genes are expressed during SAR, and the particular spectrum of gene expression therefore distinguishes the SAR response from other resistance responses in plants. Tobacco is perhaps the best characterized model for SAR, but other plants respond similarly. For example, in *Arabidopsis*, SAR is effective against *P. parasitica*, *P. syringae* pv *tomato* DC3000, and turnip crinkle virus, and the associated SAR genes are a subset of those expressed in tobacco (Uknes et al., 1992).

The SAR signal transduction pathway appears to function as a potentiator or modulator of other disease resistance mechanisms. When SAR is activated, a normally compatible plant-pathogen interaction (i.e., one in which disease is the normal outcome) can be converted into an incompatible one (Uknes et al., 1992; Mauch-Mani and Slusarenko, 1996). Conversely, when the SAR pathway is incapacitated, a normally incompatible interaction becomes compatible (Delaney et al., 1994; Mauch-Mani and Slusarenko, 1996). The mechanism by which this modulation occurs is not understood; however, at least part of the resistance response could be due to expression of the SAR genes.

Several comprehensive literature reviews have been published recently (Chen et al., 1995; Hunt and Ryals, 1996; Neuenschwander et al., 1996; Shirasu et al., 1996), so in this article we review recent findings that relate to specific steps in the SAR signal transduction pathway. In particular, we address progress in the identification of biochemical markers for SAR, the role of salicylic acid (SA) in SAR, chemical activators of SAR, and progress in establishing genetic systems to further elucidate steps in the SAR signaling cascade.

## MOLECULAR MARKERS FOR SAR

SAR has been recognized as a plant response to pathogen infection for almost 100 years (see Chester, 1933). However, most of the early studies were mainly descriptive and lacked quantitative tools to analyze the response. Thus, considerable effort has been devoted to identifying and isolating biochemical markers for SAR that could be used to distinguish it from other inducible plant resistance responses. A number of biochemical and physiological changes have been associated with pathogen infection. These include cell death and the oxidative burst (Low and Merida, 1996), deposition of callose and lignin (Vance et al., 1980; Kaus, 1987), and the synthesis of phytoalexins (Dixon, 1986) and novel proteins (Bol et al., 1990; Bowles, 1990; Linthorst, 1991; see also Dangl et al., 1996; Hammond-Kosack and Jones, 1996, in this issue). Recently, however, marker genes termed SAR genes have been identified

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whose induction is tightly correlated with the onset of SAR in uninfected tissue (Métraux et al., 1989; Ward et al., 1991; Uknes et al., 1992), and these are described in more detail below.

A protein is classified as a SAR protein when its presence or activity correlates tightly with maintenance of the resistance state (Neuenschwander et al., 1996). Analysis of SAR proteins showed that many belong to the class of pathogenesis-related (PR) proteins, which originally were identified as novel proteins accumulating after TMV infection of tobacco leaves (Gianinazzi et al., 1970; Van Loon and Van Kammen, 1970; Van Loon, 1985). In tobacco, the set of SAR markers consists of at least nine families comprising acidic forms of PR-1 (PR-1a, PR-1b, and PR-1c),  $\beta$ -1,3-glucanase (PR-2a, PR-2b, and PR-2c), class II chitinase (PR-3a and PR-3b, also called PR-Q), hevein-like protein (PR-4a and PR-4b), thaumatin-like protein (PR-5a and PR-5b), acidic and basic isoforms of class III chitinase, an extracellular  $\beta$ -1,3-glucanase (PR-Q'), and the basic isoform of PR-1 (Ward et al., 1991). A basic protein family called SAR 8.2 that is induced during the onset of SAR but which shows a pattern of gene expression distinct from that of the other SAR genes has also been described (Ward et al., 1991; Alexander et al., 1992). In Arabidopsis, the SAR marker genes are PR-1, PR-2, and PR-5 (Uknes et al., 1992). The genes encoding these SAR marker proteins have been cloned and characterized and have been used extensively to evaluate the onset of SAR (Ward et al., 1991; Uknes et al., 1992).

Both the expression of marker genes for SAR and the activation of SAR can be triggered by a number of viral, bacterial, and fungal pathogens in a variety of dicotyledonous plants (Neuenschwander et al., 1996); however, the identity and relative expression levels of SAR genes vary between different plant species. For example, in cucumber, acidic PR-1 is weakly expressed (Ryals et al., 1992), whereas in tobacco and Arabidopsis, acidic PR-1 is the predominant SAR-related protein. Such species-specific differences may reflect different evolutionary or breeding constraints that have selected for the most effective SAR response against the particular suite of pathogens to which an individual species is subject (Kessmann et al., 1994).

A number of genes homologous with SAR genes from dicots have been identified in monocot species. Homologs of the PR-1 family have been characterized in maize and barley, and additional PR proteins have been identified in maize (Nassuth and Sanger, 1986; White et al., 1987; Nasser et al., 1988). However, it has not been determined whether the induction of these genes correlates with the onset of SAR in these species. Recently, markers for chemically activated SAR (see below) have been described in wheat (Görlach et al., 1996). These wheat chemically induced (WCI) genes encode a novel lipoxygenase, a cysteine proteinase, and three other proteins whose functions are unknown. The WCI genes are coordinately expressed in response to chemical activators of resistance, and the expression pattern of these genes is similar to those of chemically induced SAR genes in dicot species. However, because a biological model for SAR does not yet exist in wheat, it cannot be confirmed that the WCI genes are bona fide SAR genes (Görlach et al., 1996).

Because the SAR genes are strongly expressed when resistance is maintained, the encoded proteins could contribute to resistance. In support of this idea, *in vitro* antimicrobial activity has been described for tobacco PR-1a (Sandoz, 1991), chitinases (PR-3; Schlumbaum et al., 1986),  $\beta$ -1,3-glucanases (PR-2), PR-4 (Ponstein et al., 1994), and osmotin (PR-5; Woloshuk et al., 1991). Furthermore, synergistic activity has been found for chitinases and  $\beta$ -1,3-glucanases (Mauch et al., 1988). *In vivo* studies involving overexpression of PR-1a in tobacco have demonstrated a significant increase in resistance to infection by the two Oomycete pathogens, *P. tabacina* and *P. parasitica* var *nicotianae* (Alexander et al., 1993b). In other experiments, resistance to *P. parasitica* was enhanced in tobacco overexpressing SAR 8.2 (Alexander et al., 1993a), and overexpression of tobacco osmotin partially inhibited growth of *P. infestans* in potato but not in tobacco (Liu et al., 1994). Also, synergistic activity of chitinases and  $\beta$ -1,3-glucanases has been demonstrated in transgenic plants (Zhu et al., 1994; Jach et al., 1995). This evidence suggests that the proteins encoded by the SAR genes are causally associated with disease resistance.

#### ACCUMULATION OF SA IS REQUIRED FOR SAR SIGNAL TRANSDUCTION

A large body of evidence suggests that SA plays a key role in both SAR signaling and disease resistance. Initially, the level of SA was found to increase by several hundred-fold in tobacco or cucumber after pathogen infection, and this increase was shown to correlate with SAR (Malamy et al., 1990; Métraux et al., 1990; Rasmussen et al., 1991). Since these reports, a considerable amount of data has established a correlation between the concentration of SA and the establishment of enhanced disease resistance not only in tobacco and cucumber but in other plants as well (Malamy et al., 1990; Métraux et al., 1990; Rasmussen et al., 1991; Dempsey et al., 1993; Uknes et al., 1993; Yalpani et al., 1993b; Cameron et al., 1994). These data, coupled with the finding that exogenous SA can induce SAR (White, 1979; Ward et al., 1991; Vernooij et al., 1995) and SAR gene expression (Ward et al., 1991; Uknes et al., 1992), led to the suggestion that SA was involved in SAR signaling.

Compelling evidence supporting this idea comes from the analysis of transgenic plants expressing the bacterial *nahG* gene encoding salicylate hydroxylase, an enzyme that catalyzes the conversion of SA to catechol. These plants are not only unable to accumulate free SA, but they are incapable of mounting a SAR response to viral, fungal, or bacterial pathogens (Gaffney et al., 1993; Bi et al., 1995; Friedrich et al., 1995; Lawton et al., 1995), indicating that SA accumulation is required for SAR induction.

Interestingly, in Arabidopsis, depletion of SA causes a breakdown of both SAR and gene-for-gene resistance. Inoculation

of *nahG*-transformed (NahG) *Arabidopsis* with incompatible races of *P. parasitica* or with strains of *P. syringae* DC3000 carrying an avirulence gene led to the development of severe disease symptoms, which is in contrast to the absence of pathogen growth on isogenic wild-type plants (Delaney et al., 1994). Recently, Mauch-Mani and Slusarenko (1996) used 2-aminoindan-2-phosphonic acid (AIP), an inhibitor of phenylalanine ammonia-lyase (PAL) activity, to block general phenylpropanoid metabolism, which is thought to include the biosynthetic pathway of SA. Pretreatment of *Arabidopsis* ecotype Col-0 with AIP converts the interaction with *P. parasitica* isolate EMWA from incompatible to compatible. Interestingly, the AIP effect is suppressed by the exogenous application of SA (Mauch-Mani and Slusarenko, 1996). These inhibitor experiments indicate that an important function of PAL activity in plant disease resistance is to provide precursors for the production of SA. Both the phenotype of NahG plants and the AIP experiments suggest that SA is a signal in resistance (*R*) gene-mediated resistance responses and that the ability of the plant to rapidly produce high levels of SA modulates disease resistance.

### IS SA THE TRANSLOCATED SIGNAL?

Pathogen infection results in significant amounts of SA in the phloem sap of both cucumber and tobacco (Métraux et al., 1990; Yalpani et al., 1991). Additionally, *in vivo* SA-labeling studies provide evidence that SA produced in the leaves of TMV-infected tobacco or TNV-infected cucumber is transported throughout the plant and accumulates in uninfected tissues (Shulaev et al., 1995; Mölders et al., 1996). In fact, as much as 70% (tobacco) and 50% (cucumber) of the increase in SA in uninfected tissue of pathogen-inoculated plants results from SA translocation from infected leaves to uninfected leaves.

These data support the contention that SA may be the signal that translocates from an infection site to activate SAR elsewhere in the plant. However, two lines of evidence suggest that SA is not the long distance signal. First, in cucumber, primary leaves infected with *P. syringae* can be removed 6 hr after inoculation, which is before SA accumulates in the phloem, without affecting the systemic increase of SA or SAR gene expression (Rasmussen et al., 1991). Second, in grafted tobacco plants, TMV inoculation of NahG rootstocks resulted in very little SA accumulation in infected tissue, compared with a 185-fold increase for wild-type (Xanthi) plants. However, transmission of the systemic signal out of the NahG rootstocks appeared to be unaffected because the grafted wild-type scions had elevated levels of both SAR gene expression and induced resistance equivalent to those seen in ungrafted wild-type plants (Vernooij et al., 1994).

These results suggest that either SA is not the long distance signal or very small amounts of SA in infected leaves are sufficient for full SAR induction. We have recently found that maximal induction of SAR occurs only at high concentrations

of SA in the infected leaf (M.G. Willits and J.A. Ryals, unpublished data). Also, Beffa et al. (1995) have found that transgenic tobacco plants producing cholera toxin form spontaneous lesions, accumulate high levels of SA, and display enhanced disease resistance. In grafting experiments in which these plants are used as rootstocks, the wild-type scions are not induced for SAR, again providing evidence that SA is not the translocated signal.

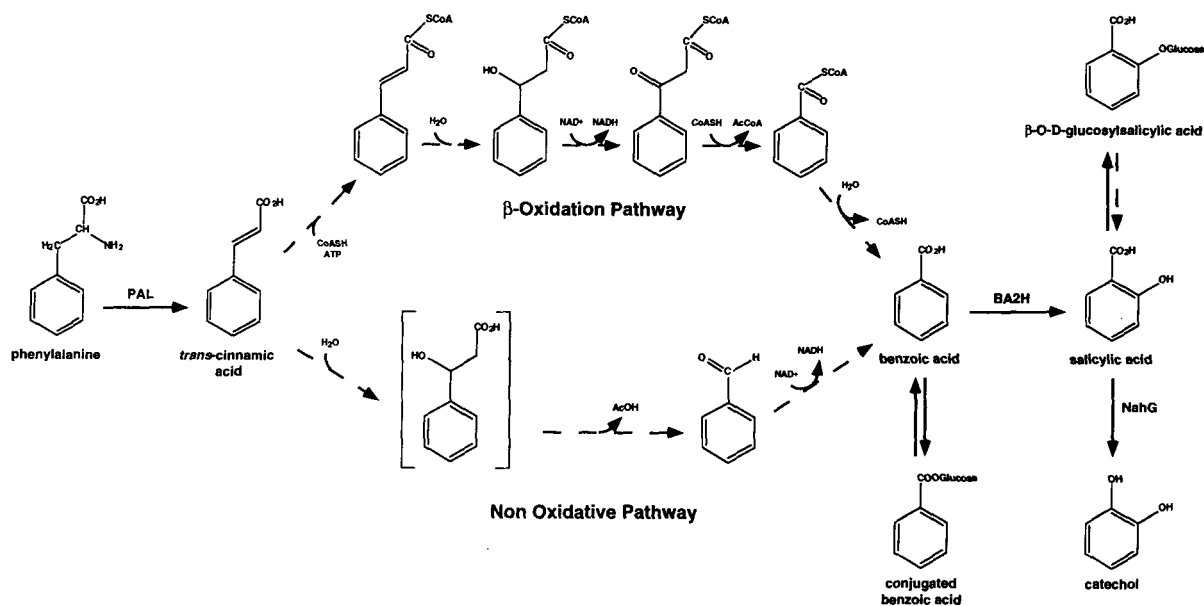
Even though SA is not likely to be the translocated signal that triggers SAR in distal plant organs, it is essential for SAR signal transduction. Inoculation of wild-type rootstocks with TMV leads to the induction of SAR in wild-type scions but not in NahG scions, demonstrating that the induction of SAR in systemic tissues is SA dependent (Vernooij et al., 1994). These findings indicate that SA is an essential signal in SAR and that it is required downstream of the long distance signal.

### BIOSYNTHESIS OF SA

Given the importance of SA in disease resistance, the pathway of SA biosynthesis may represent a major control point in plant defense responses. The biosynthetic pathway of SA appears to begin with the conversion of phenylalanine to *trans*-cinnamic acid (*t*-CA) catalyzed by PAL, as shown in Figure 1. The conversion of *t*-CA into SA has been proposed to proceed via chain shortening to produce benzoic acid (BA), followed by hydroxylation at the C-2 position to derive SA (Yalpani et al., 1993a). The latter step is likely catalyzed by a cytochrome P450 monooxygenase, called benzoic acid 2-hydroxylase (BA2H), the activity of which is induced by either pathogen infection or exogenous BA application (León et al., 1993b). Because exogenous BA causes SA accumulation but *t*-CA does not (Yalpani et al., 1993a), it seems plausible that the rate-limiting step in SA biosynthesis is the conversion of *t*-CA to BA, although other possibilities exist.

The mechanism of BA production from *t*-CA is unknown, but it may occur in a manner similar to the  $\beta$ -oxidation of fatty acids. Evidence for  $\beta$ -oxidation of *t*-CA to BA comes from studies on *Quercus pedunculata*, showing that acetyl-CoA and ATP stimulate the formation of SA from *t*-CA in cell-free extracts (Alibert and Ranjeva, 1971). However, other studies have suggested a nonoxidative mechanism; in suspension cultures of *Lithospermum erythrorhizon* and *Daucus carota*, the conversion of *p*-coumarate (differing from *t*-CA by an additional 4-hydroxyl group) to *p*-hydroxybenzoic acid appears to be independent of acetyl-CoA as a cofactor (Yazaki et al., 1991; Schnitzler et al., 1992). In this case, *p*-hydroxybenzaldehyde is formed as an intermediate, which is also inconsistent with  $\beta$ -oxidation. Both possibilities are illustrated in Figure 1.

Interestingly, both BA and SA can be conjugated to glucose, and regulation of SA levels through SA or BA conjugation may be important. In healthy tobacco plants, there is a large pool of conjugated BA that decreases transiently in size after pathogen infection. The decrease in conjugated BA levels correlates



**Figure 1.** Proposed SA Biosynthetic Pathways in Plants.

Oxidative and nonoxidative pathways for the conversion of *t*-CA to BA, leading to formation of SA, are shown. Solid arrows indicate established biochemical reactions, whereas broken arrows indicate possible steps not yet described (see Yazaki et al., 1991).

with an increase in free BA and SA (Yalpani et al., 1993a). Once SA accumulates, it is rapidly converted to  $\beta$ -O-D-glucosylsalicylic acid (SAG), which apparently is not active in disease resistance (León et al., 1993a). Conversion of SAG to free SA represents another potential mechanism for increasing levels of free SA (Figure 1). In summary, whereas SA appears to play an important role in both SAR and *R* gene-mediated resistance, little is known about its synthesis and degradation.

#### MODES OF ACTION OF SA

The mechanism by which SA induces SAR is unknown; however, it has been proposed that  $H_2O_2$  acts as a second messenger of SA in SAR signaling. An SA binding protein was identified as catalase; SA was found to inhibit the catalase activity of this protein, leading to elevated levels of  $H_2O_2$ . Furthermore,  $H_2O_2$  was found to cause induction of PR-1 gene expression and was postulated to induce SAR (Chen et al., 1993, 1995).

Recent reports, however, indicate that  $H_2O_2$  is not a second messenger of SA in SAR signaling (Bi et al., 1995; León et al., 1995; Neuenschwander et al., 1995b; Summermatter et al., 1995). For  $H_2O_2$  to function as a signaling agent of SA,  $H_2O_2$  levels should increase in uninfected leaves of tobacco plants during SAR activation. This was tested by inoculating leaves of tobacco with TMV and monitoring the accumulation

of  $H_2O_2$ , PR-1 mRNA, and the establishment of SAR. In the uninfected leaves of inoculated plants, SAR gene expression and the establishment of SAR did not correlate with an increase in  $H_2O_2$  levels. In addition, induction of PR-1 expression by  $H_2O_2$  was directly tested by infiltration of tobacco with  $H_2O_2$ . Substantial PR-1 mRNA accumulation resulted after infiltration of 1 M  $H_2O_2$ , a concentration that also caused severe tissue damage. However, in NahG plants, 1 M  $H_2O_2$  did not induce PR-1 significantly, indicating a requirement for SA in  $H_2O_2$ -mediated PR-1 expression (Neuenschwander et al., 1995b). In fact, high concentrations of  $H_2O_2$  were found to induce SA synthesis in both tobacco (León et al., 1995; Neuenschwander et al., 1995a) and *Arabidopsis* (Summermatter et al., 1995), suggesting that  $H_2O_2$  may induce PR-1 accumulation through induction of SA. Taken together, these results indicate that  $H_2O_2$  is not a second messenger of SA in the signal cascade leading to establishment of SAR.

If  $H_2O_2$  is not directly involved in SAR signaling, what is the biological significance of the inhibition of catalase by SA? One possibility is that this finding is of little relevance for plant-pathogen interactions. For example, recent reports suggest that very high levels of SA (1 mM) inhibit the *in vitro* activity of a variety of heme-iron-containing enzymes, including catalase (Chen et al., 1993), ascorbate peroxidase (Durner and Klessig, 1995), and the mitochondrial enzyme aconitase, possibly as a consequence of the reported ability of SA to chelate iron (Rueffer et al., 1995). This effect of SA on enzyme inhibition may have no real biological significance. Alternatively, the inhibition of

catalase and peroxidase could be very important for lesion formation. The  $K_d$  for SA binding to catalase and ascorbate peroxidase was reported as 14  $\mu\text{M}$  (Chen and Klessig, 1991) and 78  $\mu\text{M}$  (Durner and Klessig, 1995), respectively. This concentration of SA occurs immediately adjacent to a pathogen-induced lesion but not in uninfected leaves in which SA concentrations are 10- to 100-fold lower (Enyedi et al., 1992; Neuenschwander et al., 1995b). Therefore, the biological significance of SA-mediated inhibition of oxidoreductases may be restricted to local responses in infected tissue.

Interestingly, in parsley cell cultures and cucumber cotyledons, SA pretreatment was recently found to increase dramatically the competence of the tissue to trigger a burst of  $\text{H}_2\text{O}_2$  in response to subsequent elicitor treatment. This conditioning of cells by SA was dependent on protein synthesis and correlated with enhanced resistance of cucumber cotyledons to the fungal pathogen *Colletotrichum lagenarium*. Moreover, the increase in  $\text{H}_2\text{O}_2$  levels was not due to a decrease in the rate of degradation but to an increase in  $\text{H}_2\text{O}_2$  synthesis (Kauss and Jeblick, 1995; Fauth et al., 1996).

Thus, the data now available suggest the existence of more than one mode of action of SA in resistance responses. In uninfected leaves, a high-affinity receptor for SA could mediate the induction of SAR gene expression. After establishment of SAR, the tissue becomes competent for rapid elicitation of an oxidative burst at the site of pathogen attack, as opposed to a slower response in tissues in which SAR has not been established. In infected leaves, high concentrations of SA around the site of infection may inhibit catalase and other oxidoreductases. Inhibition of catalase activity could prolong the half-life of  $\text{H}_2\text{O}_2$  and lead to an amplification of the oxidative burst. The oxidative burst may trigger a variety of local defense responses (Mehdy, 1994; Levine et al., 1996; see also Hammond-Kosack and Jones, 1996, in this issue), including programmed cell death during the HR as well as defense gene expression and synthesis of SA in adjacent cells. This would create a runaway cycle leading to high levels of both SA and  $\text{H}_2\text{O}_2$  at the site of pathogen attack. In this model, inhibition of oxidoreductases represents a low-affinity perception mechanism that transduces high local SA levels into local defense responses.

## CHEMICAL ACTIVATORS OF SAR

SAR was first described as a response to pathogen infection. Subsequently, it has been found that treatment of plants with low molecular weight molecules can also induce SAR. The use of chemicals to activate SAR provides novel alternatives for disease control in agronomic systems as well as tools for the elucidation of the SAR signal transduction cascade (Neuenschwander et al., 1995a). To be considered an activator of SAR, a chemical should exhibit three characteristics (Kessmann et al., 1994): first, the compound or its significant metabolites should not exhibit direct antimicrobial activity; second, it should induce resistance against the same spectrum of pathogens as in biologically activated SAR; and third, it

should induce the expression of the same marker genes as evident in pathogen-activated SAR.

Several chemicals or extracts, including silicon, phosphate, 2-thiouracil, polyacrylic acid, nucleic acids, and fosethyl-Al, have been reported as potential activators of resistance but have failed to fulfill the above criteria (Kessmann et al., 1994). Other compounds, such as DL-3-aminobutanoic acid or probenazole, have been shown to slightly induce either PR-1 gene expression or resistance against one or two pathogens, but activation of bona fide SAR has not been demonstrated (Iwata et al., 1980; Asselin et al., 1985; Cohen et al., 1993).

To date, SA is the only plant-derived substance that has been demonstrated to be an inducer of SAR (White, 1979; Antoniw and White, 1980; Ward et al., 1991). The chemicals 2,6-dichloroisonicotinic acid and its methyl ester (both referred to as INA) were the first synthetic compounds shown to activate SAR, thus providing broad-spectrum disease resistance (Métraux et al., 1991; Vernooij et al., 1995). However, both SA and INA were insufficiently tolerated by some crop plants to warrant practical use as plant protection compounds. Recently, the synthetic chemical benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) was demonstrated to be a potent SAR activator (Friedrich et al., 1996; Görlach et al., 1996; Lawton et al., 1996) that supplies protection in the field against a broad spectrum of diseases in a variety of crops. Thus, BTH is an attractive compound for practical agronomic use. The resistance observed in the plants after treatment with INA or BTH is not due to a direct action of the compounds on the pathogen, because neither the compounds nor their significant metabolites exhibit *in vitro* antibiotic activity (Métraux et al., 1991; Friedrich et al., 1996). Moreover, in tobacco, Arabidopsis, and wheat, INA and BTH induce the same set of SAR genes that is induced by SA (Ward et al., 1991; Uknes et al., 1992; Friedrich et al., 1996; Görlach et al., 1996; Lawton et al., 1996).

Neither INA nor BTH treatment causes elevated levels of SA in the plant, and both compounds activate SAR when applied to NahG plants, suggesting that both INA and BTH act independently or downstream of SA in SAR signaling (Vernooij et al., 1995; Friedrich et al., 1996). INA, BTH, and SA are unable to activate SAR gene expression in the *nim1* mutant (noninducible immunity) of Arabidopsis (see below), suggesting that all three compounds activate the SAR signal transduction pathway through the same signaling cascade (Delaney et al., 1995; Lawton et al., 1996). Furthermore, the structural similarities of the three compounds (Görlach et al., 1996) suggest that they may all bind to the same receptor, although direct evidence for this is lacking.

## CONSTITUTIVE SAR MUTANTS

In an effort to identify steps in the SAR signal transduction pathway, several groups have taken a genetic approach. Arabidopsis was chosen as the model plant because it is an established plant system for mutant analysis (Redei and Koncz, 1992) and gene isolation (Meyerowitz, 1992) as well as a facile

system for studying plant–pathogen interactions in general (Dangl, 1993; Kunkel, 1996) and SAR in particular (Uknes et al., 1992; Cameron et al., 1994; Mauch-Mani and Slusarenko, 1994). Mutants that are constitutively activated for SAR or compromised in their ability to launch the SAR response have been identified. Employing these mutants in epistatic analyses is a first step toward elucidating the steps in the pathway leading to SAR activation. A reference compilation of SAR mutants identified to date is provided in Table 1, and a hypothetical schematic of the SAR signal transduction pathway is shown in Figure 2.

SAR is activated by pathogens that trigger or cause a cell death response in plants. The cell death can extend from an HR to disease-related necrosis, but the SAR-specific elements of the death process are not clear. One approach toward understanding the relationship between cell death and SAR is to study mutants that spontaneously exhibit cell death in the absence of pathogens (Langford, 1948; Walbot et al., 1983; Wolter et al., 1993; see also Dangl et al., 1996, in this issue). The identification of these mutants suggests the action of a genetically controlled programmed cell death process. In *Arabidopsis*, seven mutants called lesions simulating disease (*lsd1* to *lsd7*) and one called accelerated cell death (*acd2*) have been described. In addition to their spontaneous lesion formation phenotype, these mutants display elevated SAR gene expression, high concentrations of SA (see Table 1), and resistance to virulent pathogens (Dietrich et al., 1994; Greenberg

et al., 1994; Weymann et al., 1995). These data show that there is a clear link between cell death and SAR induction.

In an effort to determine the epistatic relationship between SA accumulation and cell death in SAR signal transduction, *lsd1*, *lsd2*, *lsd4*, *lsd6*, and *lsd7* were crossed to NahG plants (Weymann et al., 1995; M.D. Hunt, T. Delaney, R. Dietrich, K. Weymann, J. Dangl, and J.A. Ryals, unpublished results; U.H. Neuenschwander, R. Dietrich, J. Dangl, and J.A. Ryals, unpublished results). Consistent with previous reports in which *nahG* expression suppressed SAR gene expression (Delaney et al., 1994; Lawton et al., 1995), progeny of crosses between *lsd2*, *lsd4*, *lsd6*, or *lsd7* and NahG plants did not exhibit PR-1 gene expression. Furthermore, the resistance to a virulent *P. parasitica* isolate evident in the parental mutants was not retained in the progeny of *lsd*/NahG crosses. This is consistent with the lack of SAR activation by necrogenic pathogens that is evident in wild-type plants expressing *nahG* (Gaffney et al., 1993; Delaney et al., 1994; Lawton et al., 1995).

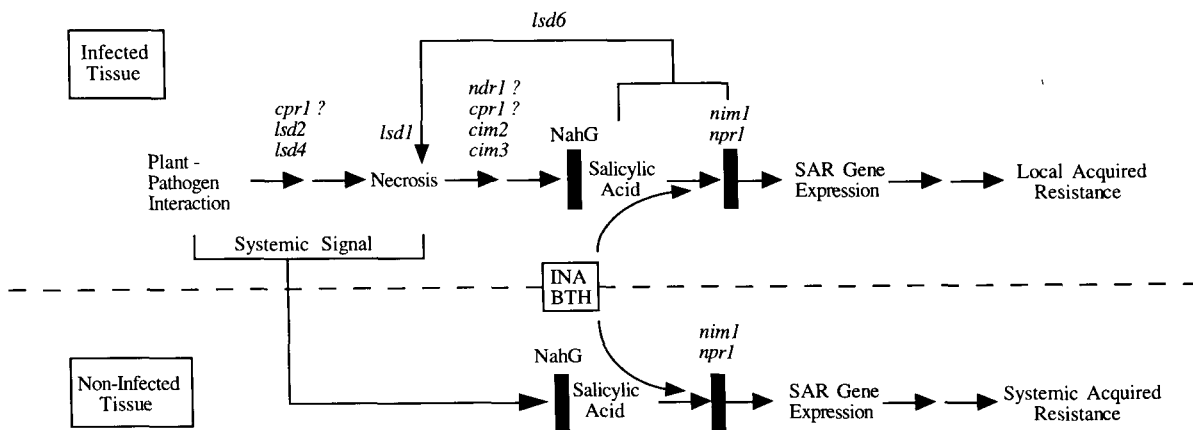
The placement of the lesion mimic mutants in the SAR signal transduction pathway model (Figure 2) was also facilitated by the *nahG* epistasis experiments. Lesion formation remained unchanged for *lsd2* or *lsd4* expressing *nahG* compared with the *lsd2* and *lsd4* parents (M.D. Hunt, T. Delaney, R. Dietrich, K. Weymann, J. Dangl, and J.A. Ryals, unpublished results), indicating that *lsd2* and *lsd4* exhibit cell death that is independent of SA or SA-dependent processes. In contrast, lesion formation was suppressed in *lsd1*, *lsd6*, or *lsd7* mutants ex-

**Table 1.** Arabidopsis SAR Mutants

Mutant	Ecotype	Mutagen	Chromosome— Dominancy/ Recessivity	SA Levels	Comments	References
<b>SAR constitutive</b>						
<i>acd2-2</i>	Col-0	EMS <sup>a</sup>	4—recessive	High	Spontaneous lesions	Greenberg et al. (1994)
<i>cim2</i>	Col-0	EMS	ND <sup>b</sup> —dominant	High	No spontaneous lesions	Lawton et al. (1993)
<i>cim3</i>	Col-0	EMS	ND—dominant	High	No spontaneous lesions	H.-Y. Steiner and J.A. Ryals, unpublished data
<i>cpr1</i>	Col-0	EMS	ND—recessive	High	Lesioned status undetermined	Bowling et al. (1994)
<i>lsd1</i>	Ws-0	T-DNA	4—recessive	High	Spontaneous lesions	Dietrich et al. (1994)
<i>lsd2</i>	Col-0	EMS	ND—dominant	High	Spontaneous lesions	Dietrich et al. (1994)
<i>lsd3</i>	Ws-0	T-DNA	ND—recessive	High	Spontaneous lesions	Dietrich et al. (1994)
<i>lsd4</i>	Ws-0	T-DNA	ND—dominant	High	Spontaneous lesions	Dietrich et al. (1994)
<i>lsd5</i>	Ws-0	T-DNA	ND—recessive	High	Spontaneous lesions	Dietrich et al. (1994)
<i>lsd6</i>	Col-0	EMS	1—dominant	High	Spontaneous lesions	Weymann et al. (1995)
<i>lsd7</i>	Col-0	EMS	ND—dominant	High	Spontaneous lesions	Weymann et al. (1995)
<b>SAR compromised</b>						
NahG	Col-0	NahG	4—dominant	Low	INA rescuable	Lawton et al. (1995)
<i>ndr1</i>	Col-0	Fast neutron	3—recessive	ND	INA rescuable	Century et al. (1995)
<i>nim1</i>	Ws-0	T-DNA	1—recessive	Normal	INA/SA insensitive	Delaney et al. (1995)
<i>npr1</i>	Col-0	EMS	ND—recessive	ND	INA/SA insensitive	Cao et al. (1994)

<sup>a</sup> EMS, ethyl methanesulfonate.

<sup>b</sup> ND, not determined.



**Figure 2.** Signal Transduction in SAR.

Pathogen-induced necrosis triggers the activation of both systemic (bottom) and local (top) acquired resistance. Both signaling cascades are SA dependent and are blocked in NahG plants that are unable to accumulate SA due to the expression of salicylate hydroxylase. Lesion formation in the mutants *lsd1* and *lsd6* is SA dependent, suggesting a feedback loop. NahG plants but not the *nim1* and *npr1* mutants can be "cured" by the two activators INA and BTH. ?, hypothetical placings of mutants in the scheme. Adapted with permission from Hunt and Ryals (1996).

pressing *nahG* (U.H. Neuenschwander, R. Dietrich, J. Dangl, and J.A. Ryals, unpublished results; Weymann et al., 1995), indicating that SA or some SA-dependent process is necessary for spontaneous cell death in these mutants.

The results obtained from the crosses of *lsd1*, *lsd2*, *lsd4*, *lsd6*, or *lsd7* with NahG plants are conflicting because they suggest that lesion formation may be positioned both before and after SA accumulation in the signal transduction pathway. Nevertheless, considerable evidence places lesion formation before SA accumulation. For example, the exogenous application of SA to wild-type plants at levels that effectively activate SAR does not induce lesion formation, although at very high concentrations, SA can cause phytotoxicity (Ward et al., 1991). Furthermore, lesion formation in NahG tobacco and NahG Arabidopsis is not inhibited, even though SA levels are markedly reduced (Gaffney et al., 1993; Delaney et al., 1994; Friedrich et al., 1995). One possible explanation for the suppression of lesion formation in the progeny of *lsd1*, *lsd6*, or *lsd7* and NahG crosses is feedback regulation of lesion formation by SA or SA-dependent events. In support of this hypothesis, the *nahG*-expressing *lsd1* or *lsd6* mutants have been shown to regain lesions after INA treatment, indicating that SA-dependent signaling events may indeed regulate processes such as lesion formation (Weymann et al., 1995). The ability to restore lesions by INA application to progeny of *lsd1* or *lsd6* and NahG crosses is compelling evidence for a feedback loop in the SAR signal transduction pathway (see Figure 2).

Arabidopsis mutants also have been identified that possess elevated SA levels and constitutive SAR gene expression in the absence of cell death. Mutants that lack spontaneous cell death but exhibit constitutive SAR are named *cim*, because

they exhibit constitutive immunity that renders them resistant to virulent pathogens (Lawton et al., 1993). *cim3* displays no visible or microscopic lesions after trypan blue staining, possesses elevated levels of both free and conjugated forms of SA, and shows constitutive expression of the SAR biochemical markers PR-1, PR-2, and PR-5 (H.Y. Steiner, S. Uknes, E. Ward, K. Weymann, D. Chandler, S. Potter, and J.A. Ryals, unpublished data). Moreover, *cim3* exhibits heightened resistance to infection with virulent bacterial (*P. syringae* DC3000) and fungal (*P. parasitica*) pathogens. Consistent with previous mutant analyses, inhibition of SA accumulation in *cim3* by expression of *nahG* suppresses both SAR gene expression and resistance to *P. parasitica*. The phenotype of *cim3* indicates that it may encode a gene product that functions at an early step in the signal transduction cascade leading to SAR activation, occurring after cell death but before SA accumulation. Interestingly, Bowling et al. (1994) described and characterized an Arabidopsis mutant that is a constitutive expresser of PR genes (*cpr1*) and that exhibits SAR. However, histochemical staining of leaves to identify the presence of microscopic lesions was not reported, so the classification of this mutant with respect to lesion formation is uncertain.

### SAR-COMPROMISED MUTANTS

In addition to mutants with enhanced disease resistance, Arabidopsis mutants that are compromised in their pathogen defense responses have been identified and characterized. Delaney et al. (1995) identified and characterized six allelic recessive mutants named *nim* that are not responsive to

exogenous application of SA or synthetic SAR activators such as INA. Similarly, Cao et al. (1994) have also isolated and described a recessive Arabidopsis mutant called *npr1* (non-expresser of PR genes), which exhibits compromised activation of SAR. *npr1* may be allelic to *nim1*.

The phenotypes of *nim1* and *npr1* indicate that their block in SAR signaling occurs before SAR gene expression but subsequent to SA accumulation. Evidence for this placement was obtained by analysis of *nim1* plants infected with the avirulent pathogen *P. syringae* DC3000 harboring the cloned *avrRpt2* gene (Whalen et al., 1991). *nim1* plants were shown to accumulate both free and glucose-conjugated SA levels in excess of those in wild-type plants. Therefore, *nim1* plants are able to accumulate SA in response to pathogen infection but appear to be defective in SA perception or in subsequent SA-sensing events. That the *nim1* and *npr1* mutations define genes that act before SAR gene expression was substantiated by the lack of PR-1 induction evident in these plants after INA or SA treatment. Furthermore, *nim1* plants showed reduced and delayed levels of PR-1 mRNA accumulation after infection with the virulent pathogen *P. parasitica* isolate EMWA. Taken together, these results indicate that the *nim1* and *npr1* mutants are compromised in both pathogen-associated and chemical activation of SAR.

In contrast to *nim1* and *npr1*, Century et al. (1995) identified a partial resistance-compromised mutant *ndr1-1* (non-race-specific disease resistance) that is susceptible to several *P. parasitica* isolates (virulent and avirulent) as well as to *P. syringae* DC3000 carrying any one of the cloned bacterial avirulence genes *avrB*, *avrRpm1*, *avrRpt2*, and *avrPph3*. Interestingly, despite its susceptibility to normally avirulent pathovars, the HR of the *ndr1-1* mutant was not substantially different from that in the wild type in response to infection with *P. syringae* harboring *avrB*, *avrRpm1*, or *avrPph3*. However, an HR was not evident in *ndr1-1* after infection with *P. syringae* harboring *avrRpt2*. These results are particularly interesting because of the apparent uncoupling of the HR and disease resistance. *ndr1-1* differs from *nim1* and *npr1* in that resistance is restored upon INA treatment (K. Century and B. Staskawicz, personal communication). Therefore, the *ndr1-1* mutation probably precedes SA accumulation in the SAR signal transduction pathway, whereas *nim1* and *npr1* presumably act subsequent to SA accumulation.

## CONCLUSION

When a pathogen is perceived by a host plant, a series of responses can be activated. Some of these responses, such as the synthesis and release of ethylene, may predispose the plant to further infection and thus contribute to disease susceptibility (Ecker, 1995). Other responses may contribute to the active defense of the host against the pathogen. One of these resistance responses is the SAR signal transduction pathway. Evidence that this response is important for resistance is that

when the pathway is blocked through design (e.g., in NahG transgenic plants) or mutation (e.g., *nim1*, *npr1*, *ndr1*), the plant's defense is compromised. Furthermore, when the pathway is stimulated by exogenous compounds such as BTH or INA, or by mutation (e.g., *lsd*, *acd2*, *cim3*, *cpr1*), the host's defense is strengthened.

Although it is clear that the SAR signal transduction pathway is central to disease resistance, there are still many unanswered questions. What is the identity of the translocated signal? How is SA synthesized after pathogen infection? What is the receptor for SA, INA, and BTH? A detailed understanding of this pathway is important for both practical and theoretical reasons.

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