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Coordinate Gene Activity in Response to Agents That Induce Systemic Acquired Resistance

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In a variety of plant species, the development of necrotic lesions in response to pathogen infection leads to induction of generalized disease resistance in uninfected tissues. A well-studied example of this "immunity" reaction is systemic acquired resistance (SAR) in tobacco. SAR is characterized by the development of a disease-resistant state in plants that have reacted hypersensitively to previous infection by tobacco mosaic virus. Here, we show that the onset of SAR correlates with the coordinate induction of nine classes of mRNAs. Salicylic acid, a candidate for the endogenous signal that activates the resistant state, induces expression of the same "SAR genes." A novel synthetic immunization compound, methyl-2,6-dichloroisonicotinic acid, also induces both resistance and SAR gene expression. These observations are consistent with the hypothesis that induced resistance results at least partially from coordinate expression of these SAR genes. A model is presented that ties pathogen-induced necrosis to the biosynthesis of salicylic acid and the induction of SAR.

INTRODUCTION

A host of cellular processes enable plants to defend themselves from disease. These processes apparently form an integrated set of resistance mechanisms that is activated by initial infection and then limits further spread of the invading pathogenic microorganism. When the plant recognizes pathogen attack, it responds by inducing several local responses in the cells immediately surrounding the infection site. These include a localized cell death known as the hypersensitive response (reviewed by Slusarenko et al., 1991), deposition of callose (reviewed by Kauss, 1987), the physical thickening of cell walls by lignification (reviewed by Vance et al., 1980), and the synthesis of various antibiotic small molecules (e.g., phytoalexins) (reviewed by Dixon, 1986) and proteins (e.g., cell wall hydrolases) (reviewed by Bowles, 1990). Genetic factors in both the host and the pathogen determine the specificity of these local responses (reviewed by Keen, 1990), which can be very effective in limiting the spread of infection.

In addition to specific, genetically determined resistance, some plants also display nonspecific "immunity" to subsequent infection after initial inoculation by a necrotizing pathogen. Acquired physiological resistance to disease was first documented early in this century and has been thought to "play an important role in the preservation of plants in nature" (Chester, 1933). Many reports of various types of induced resistance in a variety of host/pathogen systems can be found in the literature (reviewed by Chester, 1933; Kuc, 1982). Typically, the resistance is effective against a broad range of pathogens and can last for several weeks to months after the initial, inducing infection. From such studies has emerged the idea that induced resistance mechanisms might provide new strategies for crop protection either through the development of transgenic plants that constitutively express molecular components of induced resistance or through the discovery of new chemical compounds that act by stimulating natural disease resistance mechanisms (Kuc, 1982; Métraux et al., 1991; Ryals et al., 1991). However, progress toward implementing these strategies has been limited by the availability of data concerning the biochemistry and molecular biology of induced resistance.

A clearly defined example of the immunity reaction was established in a series of experiments by Ross (Ross, 1961, 1966). His work demonstrated that tobacco reacting hypersensitively to tobacco mosaic virus (TMV) would induce systemic resistance in uninfected portions of the

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plant, resulting in decreased symptom severity from secondary infections by several different pathogens. This phenomenon of induced resistance to lesion formation was termed systemic acquired resistance (SAR). Subsequently, several exogenously applied chemicals have been shown to induce SAR (Gianinazzi and Kassanis, 1974; White, 1979; Gianinazzi, 1984). One of these compounds, salicylic acid (SA), accumulates in TMV-infected tobacco (Malamy et al., 1990) and has been identified as an endogenous, phloem-mobile compound in cucumber plants induced to a resistant state (Métraux et al., 1990). It has been suggested that SA is the signal compound that induces SAR in these plants (Malamy et al., 1990; Métraux et al., 1990).

In this study, we have characterized the induction of a set of nine classes of mRNAs that are coordinately induced concomitantly with the onset of SAR. The same set of mRNAs was found to be coordinately expressed after the exogenous application of two different chemical agents: SA, the putative resistance-inducing endogenous signal, and methyl-2,6-dichloroisonicotinic acid (INA), a synthetic regulator of induced resistance (Métraux et al., 1991). The timing and degree of resistance induced by TMV and the two chemicals correlated with the timing and amount of transcript accumulation induced by the different treatments.

RESULTS

Induction of SAR

To correlate the onset of SAR with the induction of gene expression, tobacco plants were treated with various inducers, and subsets of the plants were either scored for resistance or harvested for RNA isolation. For experiments using TMV, plants were inoculated on lower leaves and then harvested at 3-day intervals after the inducing infection. Both infected lower leaves (primary leaves) and uninfected upper leaves (secondary leaves) were sampled for RNA. In parallel, secondary leaves of identically treated plants were challenge inoculated with TMV and scored for resistance 7 days later.

For experiments involving SA treatment, the compound was applied by spraying entire plants to minimize the effects of translocation. It was clear from previous experiments that exogenously applied salicylate is either metabolized or sequestered so that only a small fraction of the initially applied compound is detectable in nontreated leaves (Métraux et al., 1990). In addition, because preliminary experiments had indicated that gene expression was strongly induced within 1 day after SA treatment, the plants were sampled for RNA isolation repeatedly over a 48-hr period. INA was injected into leaves because injection resulted in much greater efficacy of the compound at a given concentration compared with spray application (data not shown).

In all experiments, the onset of SAR was assayed as a decrease in the size of the TMV lesion. Previous work had shown that the decrease in TMV lesion size parallels an increase in resistance to *Cercospora nicotianae*, *Peronspora tabacina*, *Phytophthora parasitica* var *nicotianae*, and *Pseudomonas syringae* pv *tabaci* (P. Ahl-Goy, manuscript in preparation). Because the TMV assay is easier both to manipulate and to contain in the greenhouse, it was chosen as an indicator for the onset of SAR.

Figure 1 illustrates typical biologically induced SAR. The data in Table 1 show that resistance in response to TMV induction was detected in systemic tissue within 6 days, in accordance with previous reports (Ross, 1961). The two chemical compounds were found to induce resistance to varying extents (Table 1). SA induced resistance within 2 days and was accompanied by phytotoxicity that was first detectable approximately 2 days after treatment. SA was also effective at lower concentrations, but its action was less dramatic (data not shown). In these experiments, the INA compound had little effect in tobacco at 2 days after injection (Table 1). At 4 days after treatment, however, significant reduction in lesion size was observed. INA did not cause noticeable phytotoxicity at the concentration used.

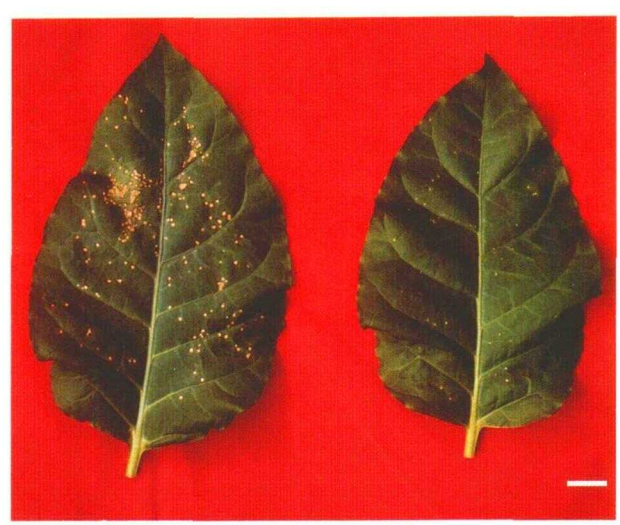


Figure 1. The SAR Response.

Secondary leaves, challenged with TMV on day 7 after primary leaf treatments, are shown 7 days after challenge.
(Left) Secondary leaf from a plant mock inoculated on primary leaves with buffer and carborundum.
(Right) Secondary leaf from a plant inoculated on primary leaves with TMV.
Bar = 2 cm.

Table 1. Magnitude of SAR in Response to Biological and Chemical Treatments

Treatment	Duration		
a	0 days	3 days	6 days
Buffer	3 mm	2–3 mm	3 mm
TMV	3 mm	2–3 mm	<0.5 mm
b	0 days	2 days	
H_2O	3 mm	3 mm	
SA	3 mm	1 mm	
c	2 days[a]	4 days	
H_2O	5 mm	4 mm	
INA	4 mm	1 mm	

Plants were treated at time 0 and then inoculated with TMV at the times shown. Values are the average size of TMV lesions, scored at 7 days after inoculation. In part a, uninfected upper leaves were challenge inoculated. In parts b and c, treated leaves were challenged. In part b, plants were subjected to spray treatment. In part c, leaves were injected as described in Methods. SD < 1 mm.

[a] Scored after 10 days.

Gene Expression Associated with SAR

To define the onset of SAR at the molecular level, we previously characterized the structure of 29 distinct genes putatively induced during the onset of resistance. These genes were isolated using several methods, including isolation and characterization of proteins followed by cDNA identification with oligonucleotides, cross-hybridization of known cDNA clones to previously unknown related genes, and differential screening of cDNAs expressed in tissue rendered systemically resistant by TMV infection. The cDNA clones fall into 13 distinct gene families that encode the 10 pathogenesis-related (PR) proteins from tobacco and their basic counterparts (reviewed by Carr and Klessig, 1989; Bol et al., 1990; Ryals et al., 1991) as well as several previously uncharacterized proteins. Table 2 summarizes the gene families and the cDNA clones used as probes in this study.

The steady-state levels of the mRNAs from these gene families in tobacco plants infected with TMV were examined by RNA gel blot hybridization to determine which gene families were closely associated with the onset of resistance. Figure 2 shows the data obtained for primary (TMV-infected) leaf tissue and secondary (uninfected, systemic) tissue from the same plants. In primary leaves, mRNAs detected by all of the probes except the acidic peroxidase were induced by TMV. The timing of maximal induction varied between the different genes, but most reached a maximum of steady-state RNA at 6 days after infection.

Table 2. Gene Families and cDNA Clones Used as Probes

Probe	Relevant Properties of Encoded Protein	Reference
PR-1	Acidic, extracellular; function unknown; most abundant PR protein in tobacco; >90% identical to PR-1b and PR-1c	Payne et al. (1988b)
PR-2	Acidic, extracellular β-1,3-glucanase; >90% identical to PR-N and PR-O	Ward et al. (1991)
PR-3	Acidic, extracellular chitinase; also known as PR-Q; >90% identical to PR-P	Payne et al. (1990a)
PR-4	Acidic, extracellular; unknown function; homologous to C-terminal domain of Win1 and Win2 of potato	Friedrich et al. (1991)
PR-5	Acidic, extracellular; homologous to thaumatin and bifunctional amylase/proteinase inhibitor of maize; also known as PR-R or PR-S	Payne et al. (1988a)
PR-1 basic	Basic isoform of acidic PR-1	Payne et al. (1989)
Basic class III chitinase	Homologous to cucumber chitinase (Métraux et al., 1989); structurally unrelated to PR-3	K.A. Lawton, E. Ward, G. Payne, M. Moyer, S. Williams, and J. Ryals, manuscript in preparation
Acidic class III chitinase	Extracellular; approximately 60% identical to basic isoform	K.A. Lawton, E. Ward, G. Payne, M. Moyer, S. Williams, and J. Ryals, manuscript in preparation
PR-Q′	Acidic, extracellular β-1,3-glucanase; approximately 55% identical to PR-2 group	Payne et al. (1990b)
Basic glucanase	Vacuolar; approximately 55% identical to PR-2 group and PR-Q′	Shinshi et al. (1988)
Basic chitinase	Vacuolar; approximately 65% identical to PR-3 group	Shinshi et al. (1987)
SAR 8.2	Unknown function; cloned by +/− screen of cDNA library from secondary leaves of TMV-infected plants	D. Alexander, J. Pear, J. Stinson, C. Glascock, R. Goodman, and J. Ryals, manuscript in preparation
Acidic peroxidase	Extracellular; lignin-forming	Lagrimini et al. (1987)

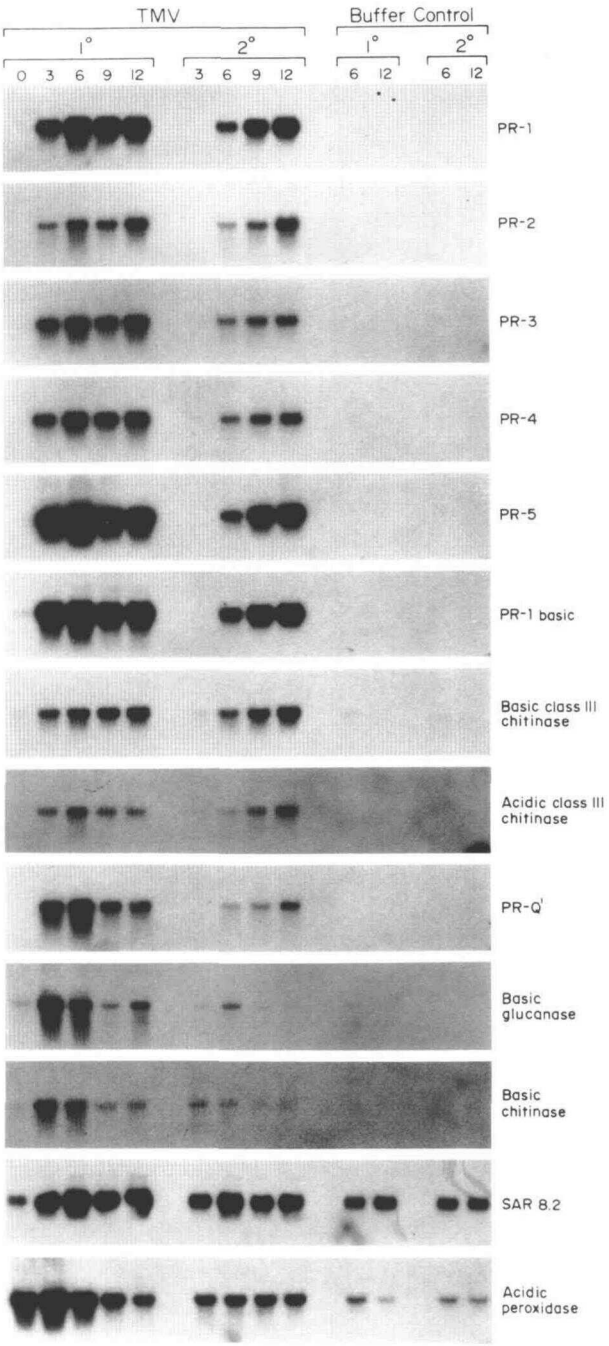


Figure 2. Gene Expression Correlated with SAR.

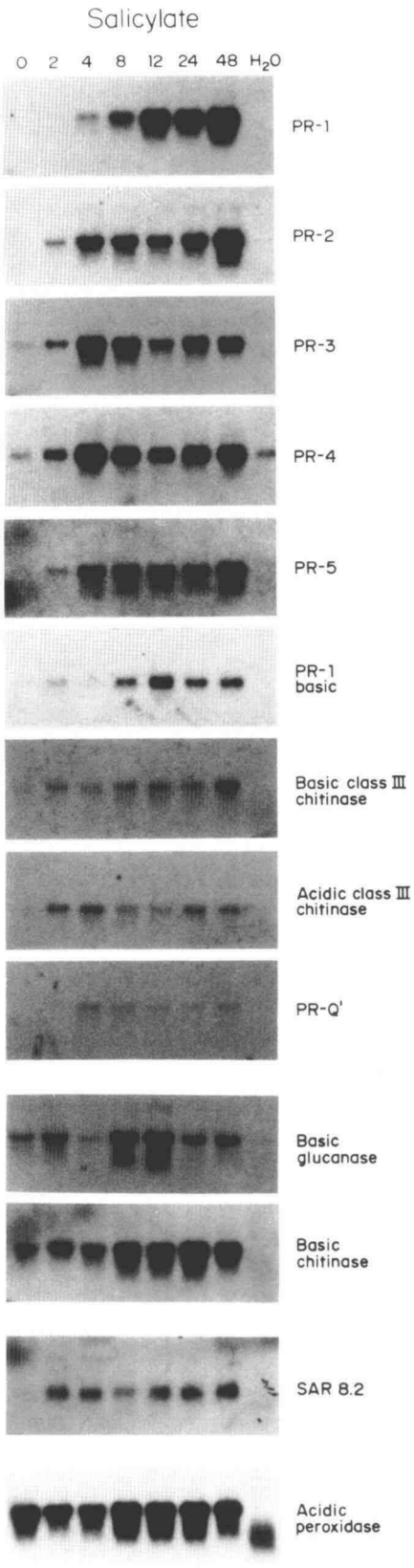
Time course of mRNA accumulation (in days) in primary (1°) and secondary (2°) leaf tissue of plants inoculated with TMV or mock inoculated with buffer and carborundum. The probe labels correspond to the DNAs described in Table 2.

In secondary, uninfected tissue from inoculated plants, nine of the gene families were coordinately induced from low initial levels to maximal levels at the day 12 time point. The RNA induction patterns for these genes are depicted in the top nine panels of Figure 2. The mRNAs for these genes began to accumulate to high levels at day 6, at which time SAR was first detected in the secondary tissue. The mRNA levels for both the basic form of β-1,3-glucanase and the basic form of chitinase did not accumulate in the uninfected tissue and, therefore, were not associated with the onset of SAR. SAR 8.2 showed an induction pattern distinct from the first nine genes. This mRNA was expressed in healthy, untreated tissue and increased about twofold in uninfected tissue within 3 days of TMV treatment. Therefore, SAR 8.2 was induced to a lesser extent but faster than the other SAR-related genes. The acidic peroxidase mRNA was not induced in systemic tissue after TMV infection.

Gene Expression in Response to Chemical Induction

The steady-state mRNA levels induced by SA treatment are shown in Figure 3. Qualitatively, the same nine genes induced by TMV in uninfected tissue were coordinately induced by salicylate treatment. The mRNA accumulation from these genes reached high levels at a time when the treated tissue had become resistant to further infection by TMV (Table 1). Several of the genes, notably PR-2, -3, and -4, reproducibly displayed small oscillations in the amount of mRNA overlaid on the overall pattern of inducibility. The minima of induced expression of these genes occurred at the 12 hr time point, which coincided with the end of the photoperiod. Conceivably, expression of these genes may respond to the light regime or a circadian rhythm. Irradiance level has previously been shown to affect PR protein accumulation (Asselin et al., 1985). Although the basic chitinase mRNA appeared to accumulate in response to SA in this experiment, neither the basic chitinase, the basic glucanase, nor the acidic peroxidase mRNAs reproducibly accumulated in response to SA in replicated experiments. We observed fluctuation in the levels of mRNA with time but found no clear indication that these RNAs accumulated in response to treatment by the chemical. Expression of the SAR 8.2 gene did increase in response to SA, with an increased rate of accumulation relative to the other nine mRNAs.

Figure 4 shows gene induction by INA at 2 and 4 days after treatment. Injection of leaves with water resulted in a low level of induction of several genes above background. After 2 days, INA treatment induced low levels of accumulation of the same nine mRNAs as TMV and SA, but these were consistently higher than the water-injected controls. At that time point, there was also no indication of SAR in the treated tissue (Table 1). Moreover, as



observed for SA, there was no reproducible increase in either the basic chitinase, basic glucanase, or acidic peroxidase mRNA in response to INA treatment. The SAR 8.2 gene was slightly induced by INA after 2 days' treatment. At 4 days after INA treatment, the expression of the same genes induced after 2 days substantially increased. A significant decrease in TMV lesion size was also apparent in plants challenge inoculated 4 days after chemical treatment (Table 1).

Quantitative Correlation of mRNA Accumulation with SAR

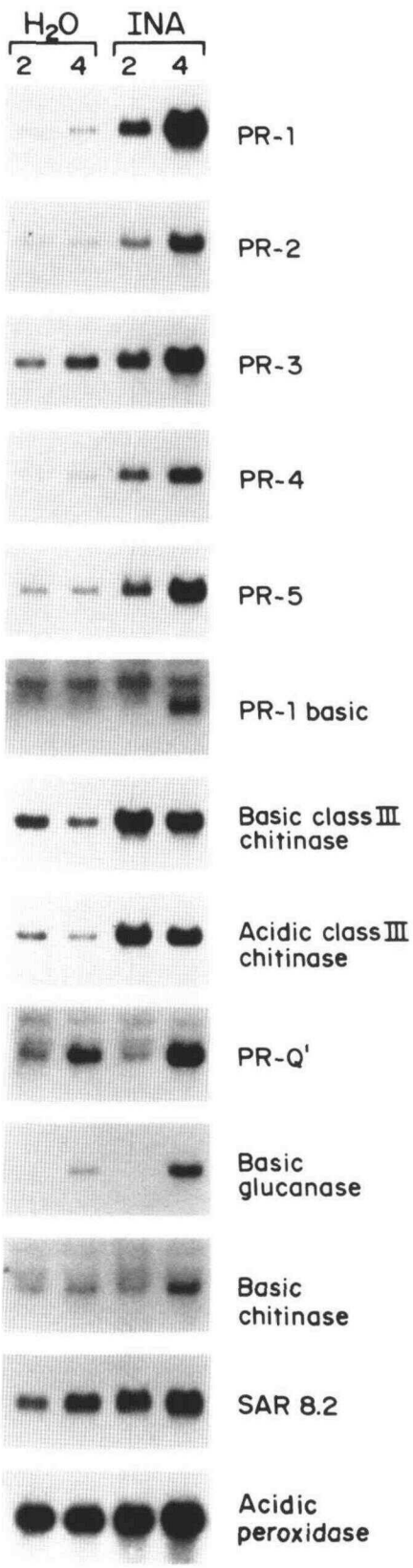
The previous data indicate that TMV, SA, and INA all induce similar patterns of mRNA accumulation but to different extents. Furthermore, the level of SAR increases with the amount of mRNA accumulation. To compare more carefully the amount of gene expression induced by different treatments, we determined the approximate relative abundance of each class of mRNA by quantitatively comparing the hybridization signals on RNA gel blots with hybridization signals on DNA gel blots bearing known standard amounts of cloned DNA. Figure 5 shows the result of such experiments. The 48-hr SA treatment induced accumulation of RNA to a level about one-half of that seen in secondary tissue at 6 days after TMV inoculation, and the 4-day INA treatment induced a level of RNA accumulation similar to that seen in the 48-hr SA treatment. SAR was observed strongly in the secondary TMV tissue and to a lesser extent in the SA- and INA-treated plants, indicating a positive correlation between the level of mRNA accumulation and the degree of SAR.

DISCUSSION

The induction of a physiological immunity reaction in plants infected with necrotrophic pathogens has been described in the literature for many years, but little is known about the molecular biology of this phenomenon. In this report, we have identified a set of nine gene families coordinately induced to high levels of expression during the onset of SAR in tobacco. These gene families include the PR proteins PR-1 (PR-1a, PR-1b, and PR-1c), PR-2 (PR-2a, PR-2b, and PR-2c), PR-3 (PR-3a and PR-3b), PR-4 (PR-4a and PR-4b), and PR-5 (PR-5a and PR-5b), as well as the basic form of PR-1, the acidic and basic forms of class III chitinase, and a recently described extracellular

Figure 3. Time Course of mRNA Accumulation in SA-Treated Tissue (in Hours).

The lane marked "H_2O" contains RNA from control plants treated with water and harvested at 48 hr.



β-1,3-glucanase, PR-Q′. We refer to this collection of gene families as SAR genes because their expression serves as a molecular marker for the onset of an induced resistant state.

Further support for the close association between the expression of the SAR genes and resistance is drawn from the effects of the two chemical inducers. SA has been known to induce both local resistance to TMV and the accumulation of PR proteins (White, 1979). The results presented here demonstrate that SA treatment induces expression of the same set of genes as TMV and that the high-level expression of these genes correlates well with the onset of a resistant state. Furthermore, a synthetic immunization compound, INA, with activity in a number of plant species (Métraux et al., 1991), protected tobacco against TMV and induced the SAR genes. We conclude that expression of the SAR genes coincides with the maintenance of SAR in tobacco.

SAR 8.2 comprises a small gene family (D.C. Alexander, J. Stinson, C. Glascock, E. Ward, R. Goodman, and J. Ryals, manuscript in preparation) that was induced by all of the resistance-inducing stimuli detailed here. The constitutive expression level of SAR 8.2 varied significantly between experiments (see, e.g., Figures 2 and 3). However, even in cases in which the background level was relatively high, the amount of transcript always increased upon induction of resistance. SAR 8.2 may, therefore, represent a second type of SAR-related gene with a distinct pattern of gene induction.

Variable background levels were also seen with the basic chitinase and glucanase mRNAs. However, these genes were not reproducibly induced by any of the agents. Expression of these genes has been shown previously to be coordinately regulated by levels of auxin, cytokinin, and ethylene (Mohnen et al., 1985; Shinshi et al., 1987). Expression of the acidic peroxidase gene analyzed here was not induced under resistance-inducing conditions. However, given the diversity of peroxidase isozymes in tobacco (Lagrimini and Rothstein, 1987), another form of the enzyme may be regulated in a fashion more similar to the SAR genes. For instance, in cucumber, an acidic peroxidase activity is a marker for an induced resistant state (Hammerschmidt et al., 1982).

A relationship between PR protein accumulation and the induced resistant state has been suggested in the literature (Kassanis et al., 1974; Van Loon, 1975; Van Loon and Antoniw, 1982; Gianinazzi, 1984; Tuzun et al., 1989), although questions about such a correlation have arisen (Fraser, 1982; Fraser and Clay, 1983). Five of the nine SAR gene families described here encode the 10 PR

Figure 4. Accumulation of mRNA in Response to INA Treatment.

Times shown are in days. PR-1 basic and PR-Q′ probes showed minor background hybridization to ribosomal bands in this experiment.

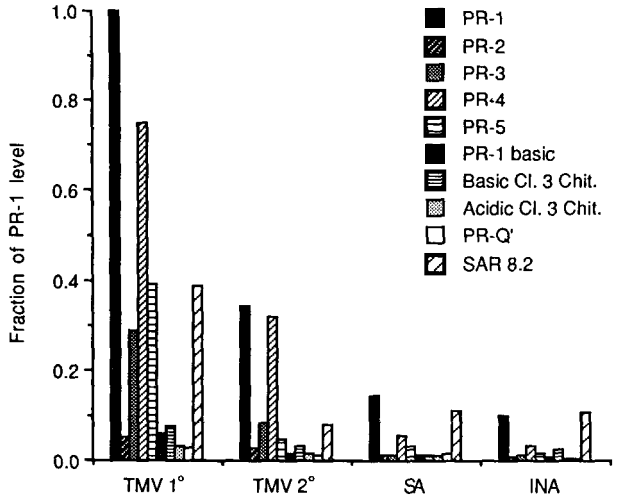


Figure 5. Relative Steady-State Levels of Transcript Induced by Various Resistance-Inducing Treatments.

The bars represent the approximate amount of RNA corresponding to 10 of the probes used in Figures 2 to 4. Values were normalized to the maximum level of PR-1 mRNA detected. TMV 1°, primary leaves from TMV-infected plants at 6 days; TMV 2°, secondary leaves at 6 days; SA, spray-treated leaves at 2 days; INA, injected leaves at 4 days.

proteins of tobacco. The PR-2 proteins PR-2a, PR-2b, and PR-2c (formerly PR-2, PR-N, and PR-O, respectively) have β-1,3-glucanase activity in vitro (Kauffmann et al., 1987). The PR-3 proteins PR-3a and PR-3b (formerly PR-P and PR-Q, respectively) have chitinase activity (Legrand et al., 1987), and the PR-5 proteins PR-5a and PR-5b (formerly PR-R and PR-S) are structurally related to a maize α-amylase/trypsin inhibitor (Richardson et al., 1987). Although the activities of PR-1 and PR-4 are not known, the other proteins all fall into classes of enzymes that have been demonstrated to have in vitro activity against plant pathogens and pests (Mauch et al., 1988; Roberts and Selitrennikoff, 1988; Richardson, 1991; Woloshuk et al., 1991), and it is tempting to speculate that the SAR genes reported here have a causal role in disease resistance. Questions concerning this role in resistance could be addressed using transgenic plants expressing the SAR genes. Recent papers have shown that PR-1 and PR-5 expressed in transgenic tobacco do not confer increased resistance to TMV (Cutt et al., 1989; Linthorst et al., 1989).

The primary mode of action of INA is unknown. Presumably, the compound acts as either an inducer or an analog of the bona fide endogenous signal. SA is an endogenously synthesized compound that may signal activation of the resistant state in cucumber and tobacco (Malamy et al., 1990; Métraux et al., 1990). The experiments presented here show that exogenously applied SA induces both the SAR genes and SAR to levels comparable with biologically induced SAR. The results are, therefore, consistent with the proposed role of SA as a signal molecule. This is a particularly interesting result because it might provide a biochemical basis for the link between pathogen-induced necrosis and the induction of SAR. Figure 6 illustrates two proposed biosynthetic pathways for the synthesis of SA in higher plants (see, e.g., El-Basyouni et al., 1964). In one scheme, *trans*-cinnamic acid is converted first to 2-hydroxycinnamic acid and then through oxidation to SA. In the alternative pathway, *trans*-cinnamic acid is β-oxidized to benzoic acid and then *ortho*-hydroxylated to SA. Which of these pathways is favored in tobacco is unknown; however, it is interesting that both syntheses branch off the phenylpropanoid biosynthetic pathway from *trans*-cinnamic acid. The development of TMV-induced necrosis dramatically increases the activity of phenylalanine ammonia-lyase in tobacco (Fritig et al., 1973; Legrand et al., 1976; Duchesne et al., 1977), and the induction of phenylalanine ammonia-lyase expression is linked to pathogen-induced necrosis in other plant/pathogen interactions (Bell et al., 1986). If enzymes in the dedicated SA biosynthetic pathway are similarly induced during necrosis, then SA would be synthesized and could be released as a signal molecule. Therefore, the plant would respond to pathogen infection by inducing necrosis, or hypersensitivity, which would in turn result in the formation and release of SA and the induction of systemic resistance. The SA biosynthetic pathway, its regulation, and the mechanism by which SA

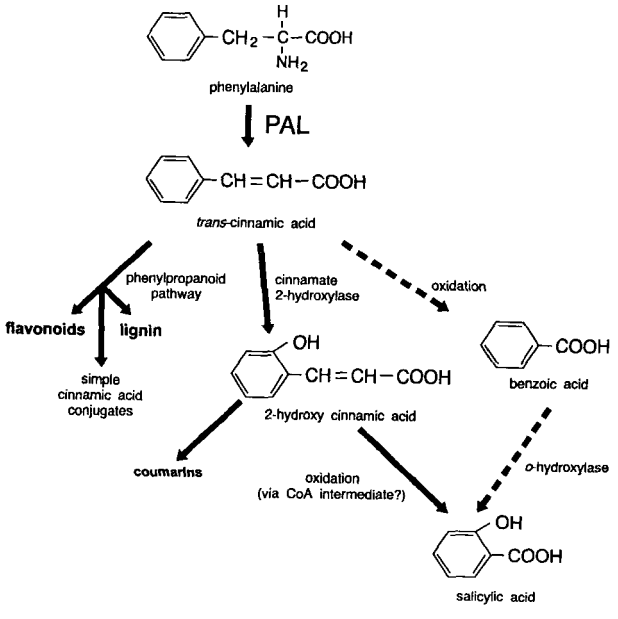


Figure 6. Possible Biosynthetic Pathway for SA.

Limited evidence exists for either of the two routes to the final product. PAL, phenylalanine ammonia-lyase.

triggers SAR gene expression clearly are areas for further study.

METHODS

Plant Material

Nicotiana tabacum cv Xanthi.nc plants were grown in a greenhouse in a 16:8 hr light:dark regimen. At 6 to 7 weeks of age, plants were infected with a suspension of the common strain (U1) of TMV (0.5 mg/mL) in a solution of 10 mM sodium phosphate (pH 7) containing carborundum. Thirty milliliters of virus suspension was gently rubbed onto the adaxial surface of three lower leaves (referred to as the primary leaves). Control plants were mock inoculated in identical fashion with buffer and carborundum only. At each time point, the primary leaves from each of three plants were excised, snap frozen in liquid nitrogen, and pooled for storage. The next leaf acropetal to the infected leaves (referred to as the secondary leaf) was also collected from the three plants in the same fashion. At the time points indicated in Table 1, induction of SAR was assayed by challenge-inoculating a set of three plants on three leaves acropetal to the leaves infected at time 0. The size of 10 lesions per leaf was either visually estimated or measured with a caliper 7 days after challenge inoculation. For time courses of induction by SA, plants were sprayed to the point of impending runoff with 50 mM aqueous solution of SA, sodium salt (Sigma). Three treated leaves from each of four plants were collected at each time point as described above. In addition, resistance was assayed at 48 hr after treatment by infecting three leaves on each of four plants with TMV as described above. For experiments with INA, a suspension of a wettable powder formulation of the methyl ester of 2,6-dichloroisonicotinic acid (final concentration = 1 mM) was injected into leaves under the abaxial epidermis with a 27-gauge needle attached to a syringe. Approximately 3 mL per leaf was injected into two leaves of each plant. Samples for RNA extraction were pooled from three plants, and three additional plants were challenged with TMV.

RNA Extraction and Gel Blot Hybridization

RNA was purified from frozen tissue samples by phenol/chloroform extraction followed by lithium chloride precipitation (Lagrimini et al., 1987). Ten-microgram samples of total RNA were separated by electrophoresis through formaldehyde agarose gels and blotted to nylon membrane (GeneScreen Plus, Du Pont-New England Nuclear) as described by Ausubel et al. (1987). Ethidium bromide was included in the sample loading buffer at 40 μg/mL, which allowed photography under UV light after electrophoresis to confirm equal sample loading. Hybridizations and washing were essentially according to Church and Gilbert (1984). For quantitative determinations of transcript abundance, DNA gel blots bearing known amounts of specific cloned DNAs, in 10-fold serial dilutions, were included in bags during hybridization of RNA gel blots. Amounts of hybridization to each transcript were quantitated directly by detecting β-decay of ^{32}P with a Betascope 603 blot analyzer (Betagen, Waltham, MA). Counts per minute were converted to transcript mass using the counts per minute values obtained from the DNA standards.

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REFERENCES

Asselin, A., Grenier, J., and Côté, F. (1985). Light-influenced extracellular accumulation of *b* (pathogenesis-related) proteins in *Nicotiana* green tissue induced by various chemicals or prolonged floating on water. Can. J. Bot. **63,** 1276–1283.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (1987). Current Protocols in Molecular Biology. (New York: John Wiley and Sons).

Bell, J.N., Ryder, T.B., Wingate, V.P.M., Bailey, J.A., and Lamb, C.J. (1986). Differential accumulation of plant defense gene transcripts in a compatible and an incompatible plant-pathogen interaction. Mol. Cell. Biol. **6,** 1615–1623.

Bol, J.F., Linthorst, H.J.M., and Cornelissen, B.J.C. (1990). Plant pathogenesis-related proteins induced by virus infection. Annu. Rev. Phytopathol. **28,** 113–138.

Bowles, D.J. (1990). Defense-related proteins in higher plants. Annu. Rev. Biochem. **59,** 873–907.

Carr, J.P., and Klessig, D.F. (1989). The pathogenesis-related proteins of plants. In Genetic Engineering, Vol. 11, J. K. Setlow, ed (New York: Plenum Press), pp. 65–109.

Chester, K.S. (1933). The problem of acquired physiological immunity in plants. Q. Rev. Biol. **8,** 275–324.

Church, G.M., and Gilbert, W. (1984). Genomic sequencing. Proc. Natl. Acad. Sci. USA **81,** 1991–1995.

Cutt, J.R., Harpster, M.H., Dixon, D.C., Carr, J.P., Dunsmuir, P., and Klessig, D.F. (1989). Disease resistance to tobacco mosaic virus in transgenic tobacco plants that constitutively express the pathogenesis-related PR1b gene. Virol. **173,** 89–97.

Dixon, R.A. (1986). The phytoalexin response: Elicitation, signaling, and control of host gene expression. Biol. Rev. Camb. Philos. Soc. **61,** 239–292.

Duchesne, M., Fritig, B., and Hirth, L. (1977). Phenylalanine ammonia-lyase in tobacco mosaic virus-infected hypersensitive tobacco; density-labelling evidence of de novo synthesis. Biochim. Biophys. Acta **485,** 465–481.

El-Basyouni, S.Z., Chen, D., Ibrahim, R.K., Neish, A.C., and Towers, G.H.N. (1964). The biosynthesis of hydroxybenzoic acids in higher plants. Phytochemistry **3,** 485–492.

Fraser, R.S.S. (1982). Are 'pathogenesis-related' proteins involved in acquired systemic resistance of tobacco plants to tobacco mosaic virus? J. Gen. Virol. **58,** 305–313.

Fraser, R.S.S., and Clay, C.M. (1983). Pathogenesis-related proteins and acquired systemic resistance: Causal relationship or separate effects? Neth. J. Plant Pathol. **89,** 283–292.

Friedrich, L., Moyer, M., Ward, E., and Ryals, J. (1991). Pathogenesis-related protein 4 is structurally homologous to the carboxy-terminal domains of hevein, Win-1 and Win-2. Mol. Gen. Genet., in press.

Fritig, B., Gosse, J., Legrand, M., and Hirth, L. (1973). Changes in phenylalanine ammonia-lyase during the hypersensitive reaction of tobacco to TMV. Virology **55,** 371–379.

Gianinazzi, S. (1984). Genetic and molecular aspects of resistance induced by infections or chemicals. In Plant Microbe Interactions—Molecular and Genetic Perspectives, Vol. 1, E. W. Nester and T. Kosuge, eds (New York: Macmillan), pp. 321–342.

Gianinazzi, S., and Kassanis, B. (1974). Virus resistance induced in plants by polyacrylic acid. J. Gen. Virol. **23,** 1–9.

Hammerschmidt, R., Nuckles, E.M., and Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium.* Physiol. Plant Pathol. **20,** 73–82.

Kassanis, B., Gianinazzi, S., and White, R.F. (1974). A possible explanation of the resistance of virus-infected tobacco plants to second infection. J. Gen. Virol. **23,** 11–16.

Kauffmann, S., Legrand, M., Geoffroy, P., and Fritig, B. (1987). Biological function of 'pathogenesis-related' proteins: Four PR proteins of tobacco have 1,3-β-glucanase activity. EMBO J. **6,** 3209–3212.

Kauss, H. (1987). Some aspects of calcium-dependent regulation in plant metabolism. Annu. Rev. Plant Physiol. **38,** 47–72.

Keen, N.T. (1990). Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. **24,** 447–463.

Kuc, J. (1982). Induced immunity to plant disease. BioScience **32,** 854–860.

Lagrimini, L.M., and Rothstein, S. (1987). Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. Plant Physiol. **84,** 438–442.

Lagrimini, L.M., Burkhart, W., Moyer, M., and Rothstein, S. (1987). Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: Molecular analysis and tissue-specific expression. Proc. Natl. Acad. Sci. USA **84,** 7542–7546.

Legrand, M., Fritig, B., and Hirth, L. (1976). Enzymes of the phenylpropanoid pathway and the necrotic reaction of hypersensitive tobacco to tobacco mosaic virus. Phytochemistry **15,** 1353–1359.

Legrand, M., Kauffmann, S., Geoffroy, P., and Fritig, B. (1987). Biological function of pathogenesis-related proteins: Four tobacco pathogenesis-related proteins are chitinases. Proc. Natl. Acad. Sci. USA **84,** 6750–6754.

Linthorst, H.J.M., Meuwissen, R.L.J., Kauffmann, S., and Bol, J.F. (1989). Constitutive expression of pathogenesis-related proteins PR-1, GRP, and PR-S in tobacco has no effect on virus infection. Plant Cell **1,** 285–291.

Malamy, J., Carr, J.P., Klessig, D.F., and Raskin, I. (1990). Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. Science **250,** 1002–1004.

Mauch, F., Mauch-Mani, B., and Boller, T. (1988). Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and β-1,3-glucanase. Plant Physiol. **88,** 936–942.

Métraux, J.P., Burkhart, W., Moyer, M., Dincher, S., Middlesteadt, W., Williams, S., Payne, G., Carnes, M., and Ryals, J. (1989). Isolation of a complementary DNA encoding a chitinase with structural homology to a bifunctional lysozyme/chitinase. Proc. Natl. Acad. Sci. USA **86,** 896–900.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., and Inverardi, B. (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science **250,** 1004–1006.

Métraux, J.P., Ahl-Goy, P., Staub, T., Speich, J., Steinemann, A., Ryals, J., and Ward, E. (1991). Induced systemic resistance in cucumber in response to 2,6-dichloro-isonicotinic acid and pathogens. In Advances in Molecular Genetics of Plant-Microbe Interactions, Vol. 1, H. Hennecke and D. P. S. Verma, eds (Dordrecht, The Netherlands: Kluwer), pp. 432–439.

Mohnen, D., Shinshi, H., Felix, G., and Meins, F., Jr. (1985). Hormonal regulation of β-1,3-glucanase messenger RNA levels in cultured tobacco tissues. EMBO J. **4,** 1631–1635.

Payne, G., Middlesteadt, W., Williams, S., Desai, N., Parks, T.D., Dincher, S., Carnes, M., and Ryals, J. (1988a). Isolation and nucleotide sequence of a novel cDNA clone encoding the major form of pathogenesis-related protein R. Plant Mol. Biol. **11,** 223–224.

Payne, G., Parks, T.D., Burkhart, W., Dincher, S., Ahl, P., Métraux, J.P., and Ryals, J. (1988b). Isolation of the genomic clone for pathogenesis-related protein 1a from *Nicotiana tabacum* cv. Xanthi-nc. Plant Mol. Biol. **11,** 89–94.

Payne, G., Middlesteadt, W., Desai, N., Williams, S., Dincher, S., Carnes, M., and Ryals, J. (1989). Isolation and sequence of a genomic clone encoding the basic form of pathogenesis-related protein 1 from *Nicotiana tabacum.* Plant Mol. Biol. **12,** 595–596.

Payne, G., Ahl, P., Moyer, M., Harper, A., Beck, J., Meins, F., Jr., and Ryals, J. (1990a). Isolation of complementary DNA clones encoding pathogenesis-related proteins P and Q, two acidic chitinases from tobacco. Proc. Natl. Acad. Sci. USA **87,** 98–102.

Payne, G., Ward, E., Gaffney, T., Ahl-Goy, P., Moyer, M., Harper, A., Meins, F., Jr., and Ryals, J. (1990b). Evidence for a third structural class of β-1,3-glucanase in tobacco. Plant Mol. Biol. **15,** 797–808.

Richardson, M. (1991). Seed storage proteins: The enzyme inhibitors. Methods Plant Biochem. **5,** 259–305.

Richardson, M., Valdes-Rodriquez, S., and Blanco-Labra, A. (1987). A possible function for thaumatin and a TMV-induced protein suggested by homology to a maize inhibitor. Nature **327,** 432–434.

Roberts, W.K., and Selitrennikoff, C.P. (1988). Plant and bacterial chitinases differ in antifungal activity. J. Gen. Microbiol. **134,** 169–176.

Ross, A.F. (1961). Systemic acquired resistance induced by localized virus infections in plants. Virology **14,** 340–358.

Ross, A.F. (1966). Systemic effects of local lesion formation. In Viruses of Plants, A.B.R. Beemster and J. Dijkstra, eds (Amsterdam: North-Holland), pp. 127–150.

Ryals, J., Ward, E., and Métraux, J.-P. (1991). Systemic acquired resistance: An inducible defense mechanism in plants. In The Biochemistry and Molecular Biology of Inducible Enzymes and Proteins in Higher Plants, J.L. Wray, ed (Cambridge: Cambridge University Press), in press.

Shinshi, H., Mohnen, D., and Meins, F., Jr. (1987). Regulation of a plant pathogenesis-related enzyme: Inhibition of chitinase and chitinase mRNA accumulation in cultured tobacco tissues by auxin and cytokinin. Proc. Natl. Acad. Sci. USA **84,** 89–93.

Shinshi, H., Wenzler, H., Neuhaus, J.-M., Felix, G., Hofsteenge, J., and Meins, F., Jr. (1988). Evidence for N- and C-terminal processing of a plant defense-related enzyme: Primary structure of tobacco prepro-β-1,3-glucanase. Proc. Natl. Acad. Sci. USA **85,** 5541–5545.

Slusarenko, A.J., Croft, K.P., and Voisey, C.R. (1991). Biochemical and molecular events in the hypersensitive response of bean to *Pseudomonas syringae* pv. *phaseolicola*. In Biochemistry and Molecular Biology of Host-Pathogen Interactions, C.J. Smith, ed (Oxford: Oxford University Press), in press.

Tuzun, S., Rao, M.N., Vögeli, U., Schardl, C.L., and Kuc, J. (1989). Induced systemic resistance to blue mold: Early induction and accumulation of β-1,3-glucanases, chitinases, and other pathogenesis-related proteins (b-proteins) in immunized tobacco. Phytopathology **79,** 979–983.

Vance, C.P., Kirk, T.K., and Sherwood, R.T. (1980). Lignification as a mechanism of disease resistance. Annu. Rev. Phytopathol. **18,** 259–288.

Van Loon, L.C. (1975). Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. 'Samsun' and 'Samsun NN' IV. Similarity of qualitative changes of specific proteins after infection with different viruses and their relationship to acquired resistance. Virology **67,** 566–575.

Van Loon, L.C., and Antoniw, J.F. (1982). Comparison of the effects of salicylic acid and ethephon with virus-induced hypersensitivity and acquired resistance in tobacco. Neth. J. Plant Pathol. **88,** 237–256.

Ward, E.R., Payne, G.P., Moyer, M.B., Williams, S.C., Dincher, S.S., Sharkey, K.C., Beck, J.J., Taylor, H.T., Ahl-Goy, P., Meins, F., Jr., and Ryals, J.A. (1991). Differential regulation of β-1,3-glucanase messenger RNAs in response to pathogen infection. Plant Physiol. **96,** 390–397.

White, R.F. (1979). Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology **99,** 410–412.

Woloshuk, C.P., Meulenhoff, J.S., Sela-Buurlage, M., van den Elzen, P.J.M., and Cornelissen, B.J.C. (1991). Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. Plant Cell **3,** 619–628.