hypokalemic periodic paralysis, we have found a recombinant between this disorder and the sodium channel gene (22), suggesting that separate loci may exist.

The ultimate proof of a defect in the sodium channel gene in HYPP will require definition of the molecular lesion. This is the next step toward understanding of the structure and function of the sodium channel, establishment of molecular diagnostic procedures in HYPP, and realization of an animal model of the disorder.

**REFERENCES AND NOTES**

16. PCR primers were generated with GC clamps and restriction enzyme sites according to the general design previously described (T. S. Khurana, E. P. Hoffman, L. M. Kunkel, *J. Biol. Chem.*, in press). The primer sequences are as follows: Na2 forward, 5'-ggggGGATCCgatgtgctatgagaccctg-3'; Na2 reverse, 5'-gggAAGCTTcacctcaggagagta-3'; Na3 forward, 5'-ggggGGATCCggagatgaacaacctacagatt-3'; Na3 reverse, 5'-gggAAGCTTcatgaagacaatgaaggtctc-3'.
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**Salicylic Acid: A Likely Endogenous Signal in the Resistance Response of Tobacco to Viral Infection**

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Some cultivars of tobacco are resistant to tobacco mosaic virus (TMV) and synthesize pathogenesis-related (PR) proteins upon infection. In search for the signal or signals that induce resistance or PR genes, it was found that the endogenous salicylic acid levels are in resistant, but not susceptible, cultivars increased at least 20-fold in infected leaves and 5-fold in uninfected leaves after TMV inoculation. Induction of PR1 genes paralleled the rise in salicylic acid levels. Since earlier work has demonstrated that treatment with exogenous salicylic acid induces PR genes and resistance, these findings suggest that salicylic acid functions as the natural transduction signal.

Living organisms have evolved a complex array of biochemical pathways that enable them to recognize and respond to signals from the environment. These pathways include receptor organs, hormones, second messengers, and enzymatic modifications. At present, little is known about the signal transduction pathways that are activated during a plant's response to attack by a pathogen, although this knowledge is central to our understanding of disease susceptibility and resistance. A common form of plant resistance is the restriction of pathogen proliferation to a small zone surrounding the site of infection. In many cases, this restriction is accompanied by localized death (necrosis) of host tissues. Together, pathogen restriction and local tissue necrosis characterize the hypersensitive response (HR). In addition to local defense responses, many plants respond to infection by activating defenses in uninfected parts of the plant. As a result, the entire plant is more resistant to a secondary infection. This systemic acquired resistance (SAR) can persist for several weeks or more (1) and often confers cross-resistance to unrelated pathogens (2).

The interaction between tobacco (*Nicotiana tabacum* Linn.) and TMV has been used extensively as a model for the study of plant disease and resistance. In general, infection of tobacco leaves with TMV results in one of two distinct responses. TMV-infected tobacco cultivars that have the dominant N gene are resistant and display both HR and SAR (1). In contrast, tobacco cultivars that lack the N gene (nn genotype) are susceptible to TMV. The virus replicates and spreads rapidly throughout these plants, causing stunting and the appearance of mosaic patterns of chlorosis on the youngest leaves.

Resistant (NN genotype), but not susceptible (nn), cultivars produce several new proteins in response to TMV infection, including five distinct families of proteins referred to as pathogenesis-related (PR) proteins (PR1 through PR5) (3, 4). PR proteins are found in many plants, and different subsets can be induced by various viral, viroid, fungal, and bacterial pathogens and by certain environmental and chemical stresses (5). The defense-related enzymatic activities of some of these PR proteins (6, 7) and the correlation between PR gene expression and resistance suggest that these...
proteins are involved in SAR and HR (8). However, the recent demonstration that transgenic tobacco plants constitutively expressing a single introduced PR gene did not exhibit enhanced resistance implies that multiple factors may be necessary for resistance (9, 10).

Application of exogenous salicylic acid or its derivative acetyl salicylic acid is particularly effective in inducing PR genes in many plants, including tobacco (11, 12). Application of salicylic acid also results in increased resistance of the treated areas to TMV and to some other viruses (13) and fungi (14).

To determine whether endogenous salicylic acid is involved in activation of PR genes, the closely related tobacco cultivars Xanthi (nn) and Xanthi nc (NN) [in which the N gene has been crossed to Xanthi (nn) from Nicotiana glutinosa] were mock- or TMV-inoculated, and the endogenous levels of salicylic acid and PR1 gene expression were monitored. Salicylic acid did not exceed the basal levels (~0.01 μg per gram of fresh weight) in mock-inoculated Xanthi nc (NN) (Fig. 1A) or in TMV- or mock-inoculated Xanthi (nn) tobacco, which is susceptible to this virus. In Xanthi nc (NN), little, if any, salicylic acid was detected in TMV-infected leaves at 0, 6, 12, or 18 hours after inoculation. Salicylic acid levels began to rise at 24 to 36 hours and increased 20-fold or more over basal levels by 42 to 48 hours. Salicylic acid levels remained high for the remainder of the experiment (Fig. 1A).

Induction of PR1 gene expression paralleled increased salicylic acid levels. Little PR1 mRNA was seen in mock- or TMV-inoculated Xanthi (nn) plants or in the mock-inoculated leaves of resistant Xanthi nc (NN) tobacco (8). In contrast, in TMV-infected leaves of the resistant Xanthi nc (NN) plants, PR1 mRNA levels were detectable at 24 to 36 hours and were maintained at high levels throughout the remainder of the experiment (Fig. 1B). Thus, not only does treatment with exogenous salicylic acid induce PR1 gene expression (Fig. 1C), but also the endogenous levels of salicylic acid and expression of PR1 genes rise in parallel in TMV-resistant plants undergoing the HR.

In resistant plants, there is tissue damage associated with lesion formation during the HR. However, no endogenous salicylic acid was detected when leaves of Xanthi nc (NN) plants were severely abraded, sliced with a razor blade, or injured with Dry Ice chips. These observations indicate that the salicylic acid induction that follows TMV infection is not a generalized response to wounding or tissue death.

If salicylic acid is involved in the activation of systemic as well as local plant defenses, the amount of endogenous salicylic acid in uninfected leaves of TMV-inoculated Xanthi nc (NN) tobacco should increase in parallel or before induction of PR gene expression and SAR. Indeed, salicylic acid concentrations in these leaves rose above basal levels by 48 hours, plateaued at 72 hours at five- to tenfold over basal levels, and remained relatively constant through at least 7 days after inoculation (Fig. 2A). The systemic increase in salicylic acid was followed by the appearance of PR1 mRNAs, which were first detected at 72 hours and increased in quantity thereafter (Fig. 2B). The 24-hour delay in PR1 gene expression relative to the rise in salicylic acid levels, observed in only the uninfected leaves of inoculated plants, might be due to the fact that the salicylic acid content in these leaves is lower than that in the infected leaves. The small amount of salicylic acid might also explain the relatively low steady-state quan-
tities of PR1 mRNAs in uninfected leaves as compared to infected leaves (Figs. 1B and 2C). No PR1 gene expression or salicylic acid increase was seen in uninfected leaves of TMV-inoculated Xanthi (NN) plants or in mock-inoculated plants of either genotype (Fig. 2A).

In 1983 Van Loon (15) postulated that salicylic acid acts by mimicking an endogenous pholic signal that triggers PR gene expression and disease resistance. Our results suggest that the signal is salicylic acid itself. Susceptible Xanthi (NN) plants carry the PR genes, and these genes can be activated by treatment with exogenous salicylic acid (Fig. 1C) but not with TMV. Therefore, it is likely that infection of susceptible plants fails to trigger the signal transduction pathway that leads to salicylic acid production, resistance, and PR gene expression.

Métrax et al. (16) present independent evidence suggesting that salicylic acid plays a role in the induction of SAR in cucumber after pathogen attack. In addition, Raskin and co-workers recently demonstrated that salicylic acid is an endogenous regulator of heat and odor production in the inflorescences of some thermogenic lilies (17-19). These three studies suggest that salicylic acid plays a broad and important role in signal transduction in plants.

**REFERENCES AND NOTES**

20. Using a more sensitive assay, we have since found that basal levels range from <0.005 to 0.02 µg per gram of fresh weight, as indicated in the text.
21. Salicylic acid was extracted from 1 g, fresh weight, of leaf tissue and analyzed by high-performance liquid chromatography and spectrofluorescence as described previously (18). The presence of salicylic acid was confirmed by gas chromatography-mass spectrometry. Salicylic acid recovery was 55%. The data shown were not corrected for this factor. Total leaf RNA was prepared as previously described [J. O. Berry, B. J. Nikolau, J. P. Carr, D. F. Klessig, *Mol. Cell. Biol.* 5, 2238 (1985)] and 20 µg of each preparation was analyzed by Northern (RNA) blot and hybridization to 32P-labeled PR1 cloned cDNA [J. P. Carr, D. C. Dixon, J. P. Carr, D. F. Klessig, *Nucleic Acids Res.* 16, 9856 (1988)].
23. Supported in part by the Du Pont Company (Wilmington, DE), where some of the experiments were performed, and in part by the National Science Foundation.

**Increase in Salicylic Acid at the Onset of Systemic Acquired Resistance in Cucumber**


In an effort to identify the signal compound that mediates systemic acquired resistance (SAR), changes in the content of phloem sap were monitored in cucumber plants inoculated with either tobacco necrosis virus or the fungal pathogen *Colletotrichum lagenarium*. The concentration of a fluorescent metabolite was observed to increase transiently after inoculation, with a peak reached before SAR was detected. The compound was purified and identified by gas chromatography-mass spectrometry as salicylic acid, a known exogenous inducer of resistance. The data suggest that salicylic acid could function as the endogenous signal in the transmission of SAR in cucumber.

PLANTS INOCULATED WITH NEROTROPHIC PATHOGENS such as fungi, bacteria, or viruses react by inducing a transient resistance against subsequent fungal, bacterial, or viral infections (1-4). This induced resistance can be restricted to areas of the first inoculation (3) but may spread to other parts of the plant to establish SAR (1). It has been proposed that SAR is mediated by an endogenous signal that is produced in the infected leaf and translocated in the phloem to other plant parts where it activates resistance mechanisms (1, 2, 5).

Cucurbitaceae have the unique property of releasing phloem sap from cut stem or petiole surfaces (6). We used a high-performance liquid chromatograph (HPLC) system, originally devised for the detection of hydroxylated polyamines (7), to analyze the fluorescent peak appeared sooner after infection of the infected leaf and translocated in the phloem to other plant parts where it activates resistance mechanisms (1, 2, 5).

Further analysis of the metabolite indicated that it consisted of several ultraviolet-absorbing components (Fig. 2). Only one of these was fluorescent and separated at the position of the initial metabolite. A preparation of this fluorescent fraction was derivatized to convert the pionic compounds to their volatile trimethylsilyl derivatives (8), and this was used for analysis by capillary gas chromatography-mass spectrometry (GC-MS). The chemical ionization mass spectra and chromatographic retention data showed that the trimethylsilyl ester of...