Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5099–5104, May 1996
Plant Biology

Ozone-induced responses in *Arabidopsis thaliana*: The role of salicylic acid in the accumulation of defense-related transcripts and induced resistance

YOGESH K. SHARMA*, JOSÉ LEÓN†, ILYA RASKIN†, AND KEITH R. DAVIS*‡

*Department of Plant Biology and the Plant Biotechnology Center, The Ohio State University, Columbus, OH 43210-1002; and †AgBiotech, Center for Agricultural Molecular Biology, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903-0231

Communicated by Frederick M. Ausubel, Massachusetts General Hospital, Boston, MA, December 18, 1995 (received for review November 30, 1995)

ABSTRACT **Exposure of *Arabidopsis thaliana* to ozone results in the expression of a number of defense-related genes that are also induced during a hypersensitive response. A potential common link between the activation of defense gene expression during a hypersensitive response and by ozone treatment is the production of active oxygen species and the accumulation of hydrogen peroxide. Here we report that salicylic acid accumulation, which can be induced by hydrogen peroxide and is required for the expression of both a hypersensitive response and systemic acquired resistance, is also required for the induction of some, but not all, ozone-induced mRNAs examined. In addition, we show that ozone exposure triggers induced resistance of *A. thaliana* to infection with virulent phytopathogenic *Pseudomonas syringae* strains. Infection of transgenic plants expressing salicylate hydroxylase, which prevents the accumulation of salicylic acid, or *npr1* mutant plants, which are defective in the expression of systemic acquired resistance at a step downstream of salicylic acid, demonstrated that the signaling pathway activated during ozone-induced resistance overlaps with the systemic acquired resistance activation pathway and is salicylic acid dependent. Interestingly, plants expressing salicylate hydroxylase exhibited increased sensitivity to ozone exposure. These results demonstrate that ozone activates at least two distinct signaling pathways, including a salicylic acid dependent pathway previously shown to be associated with the activation of pathogen defense reactions, and that this latter pathway also induces a protective response to ozone.**

Plants are constantly faced with a complex and changing pattern of environmental signals that must be efficiently integrated to produce a pattern of gene expression that will be most effective at providing an appropriate response for the current conditions. A relatively recent abiotic stress that can have profound deleterious effects on both plant and animals is the accumulation of ozone in the troposphere. Ozone is a major air pollutant found in many industrialized areas throughout the world and has been documented to have harmful effects on human and animal respiration (1) as well as causing extensive damage in natural and cultivated plant populations (2). Recent predictions suggest that tropospheric ozone concentrations and the associated damage to plant populations are likely to increase (3). Plants are quite sensitive to ozone exposure; indeed, ozone pollution has been implicated as causing more damage to vegetation in the United States than any other pollutant (4–6). The effects of ozone exposure of plants can vary greatly, depending on the plant species or cultivar examined, the ozone concentration, and the length of exposure (7–10). Plants experiencing acute short-term exposure to high ozone concentrations [>150 parts per billion (ppb)] usually develop visible damage. In contrast, chronic exposure to lower ozone concentrations generally causes a reduction in growth without visible damage. The types of injury observed in plants subjected to ozone include bleaching or chlorosis of mesophyll cells and the development of necrotic lesions (4, 6, 11, 12). More recent studies have focused on the effects of ozone exposure on patterns of gene expression (13–21). We have previously established that *Arabidopsis thaliana* provides a useful model system for investigating the activation of gene expression by ozone (19, 20). Our studies have demonstrated that there is a significant overlap in the pattern of gene expression observed in ozone-treated *A. thaliana* plants and that observed during a hypersensitive response (HR). These results have recently been confirmed (21). The mechanisms responsible for these ozone effects on gene regulation are not known, and to date, there have not been any reports of the isolation of genes that are specifically and uniquely regulated only by ozone.

Given the fact that ozone enters the mesophyll via stomata where it immediately interacts with water and other cellular components to generate reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide (2, 22–24), it is possible that ozone-induced gene expression is mediated by the production of these active oxygen species. Active oxygen species and lipid peroxidation products resulting from the interaction of active oxygen species with membranes have been implicated as signaling molecules involved in stress-related secondary messenger pathways (reviewed in ref. 25). For example, hydrogen peroxide has been suggested to be an important regulator of disease resistance mechanisms associated with HR and systemic acquired resistance (SAR) (26, 27). In addition to hydrogen peroxide, salicylic acid (SA), a phenolic compound with regulatory properties in both plants and animals, has also been shown to be required for the induction of HR and SAR (28–30). Recent studies (31, 32) indicate that this SA accumulation may be regulated by hydrogen peroxide levels. Based on these results, it would be predicted that if ozone activates changes in gene expression by generating active oxygen species that trigger these defense pathways, this induction should depend in part on the accumulation of SA.

In this report, we address the question of whether or not ozone exposure of *A. thaliana* activates an effective defense response and whether ozone-induced gene activation requires SA. Our results show that ozone-mediated induction of several defense genes is correlated with the rapid accumulation of SA. Further experiments using transgenic plants expressing salicylate hydroxylase, which prevents the accumulation of signif-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: *Psm*, *Pseudomonas syringae* pv. *maculicola*; GST, glutathione *S*-transferase; HR, hypersensitive response; PAL, phenylalanine ammonia-lyase; PR, pathogenesis related; SA, salicylic acid; SAR, systemic acquired resistance; ppb, parts per billion.

‡To whom reprint requests should be addressed.

icant levels of SA, and the *npr1* mutant, which is unable to develop SAR, indicate that the accumulation of a subset of ozone-induced transcripts requires SA and a functional SAR signal transduction pathway. In addition, it appears that *A. thaliana* has inducible defense mechanisms that provide some protection against ozone exposure and that the induction of this defense requires a SA-dependent signaling pathway.

MATERIALS AND METHODS

Growth of Plants and Ozone Treatments. *A. thaliana*, accession Col-0 (Columbia) was originally obtained from Frederick Ausubel (Massachusetts General Hospital), the NahG transgenic line expressing salicylate hydroxylase (28) was obtained from John Ryals (CIBA–Geigy), and the *npr1* mutant line (33) was obtained from Xinnian Dong (Duke University). Both the NahG and *npr1* mutant lines are in a Col-0 background. Plants were grown in growth chambers using a 12 h photoperiod under fluorescent lights as described (19). Three- to 4-week old plants were transferred to ozone fumigation chambers (Northeastern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Delaware, OH) where they were exposed to 300 ppb ozone for 6 h daily (900–1500) as described (19). Entire plant rosettes were harvested at the indicated times, quick-frozen with liquid nitrogen, and stored at −80°C until processed for SA or nucleic acid isolations.

Bacterial Strains and Plant Inoculations. *Pseudomonas syringae* pv. *maculicola* (*Psm*) KD4326 and *Psm* KD4326 containing *avrB* (*Psm* 4326/*avrB*) were grown in King's B medium containing the appropriate antibiotics as described (34). Leaves of control and ozone-treated plants were inoculated with the virulent *Psm* 4326 or with the avirulent *Psm* 4326/*avrB* using either syringe infiltration or an immersion method (34, 35). *In planta* bacterial multiplication rates were determined by plating serial dilutions of extracts prepared from leaf disks or whole leaves isolated from inoculated plants on selective King's B medium as previously described (34).

Quantitative Measurements of SA. Pooled rosette leaves from three to five plants receiving the same treatment (0.5 g fresh weight) were analyzed for total (free and conjugated forms) and free SA using published procedures (36). Triplicate samples were assayed in all experiments. The data shown have been corrected for extraction efficiencies based on the recovery of an added standard before analysis; extraction efficiencies varied from 71% to 100% in the experiments shown.

RNA Isolation and Analysis. Total RNA was isolated using a standard phenol-SDS/LiCl precipitation procedure and subjected to RNA blot hybridization analysis as described (19). Phenylalanine ammonia-lyase (*PAL*) and pathogenesis-related protein 1 (*PR1*) probes were prepared by random-primer labeling of purified cloned fragments of a genomic *PAL* clone (19) or a *PR1* cDNA (37). A gene-specific glutathione *S*-transferase 1 (*GST1*) probe was generated from the 3′ untranslated region of a cDNA clone provided by Frederick Ausubel (personal communication) by PCR amplification in the presence of ^{32}P-labeled dCTP using the primers GST1-A (5′-GGTTCTTTAAGTGAATCTCAAAC-3′) and GST1-B (5′-CAAGACTCATTATCGAAGATTAC-3′). Hybridization signals were quantitated using a PhosphorImager (Molecular Dynamics) and IMAGE QUANT software. The data shown have been corrected for loading differences using the counts obtained with a control rRNA probe.

RESULTS

Induction of SA Accumulation by Ozone. Treatment of *A. thaliana* plants with 300 ppb ozone caused a rapid and transient accumulation of SA within the first several hours of exposure (Fig. 1). Increases in free SA were observed within the first 3 h after the start of the ozone treatment. Maximum levels 3.5- to 4.5-fold higher than controls were observed 6 h after treatment, after which free SA levels declined to near control values by 24 h. Total SA, which includes both the free and conjugated forms, increased throughout the 24 h following the 6-h ozone treatment with the initial induction kinetics being similar to that observed for the accumulation of free SA. At 24 h after ozone exposure, total SA levels were 3.8- to 4.7-fold higher than the corresponding ambient air treated controls. The induction kinetics for free SA accumulation observed in these experiments was very similar to that previously described for ozone induction of *PAL* and *GST1* mRNAs (19).

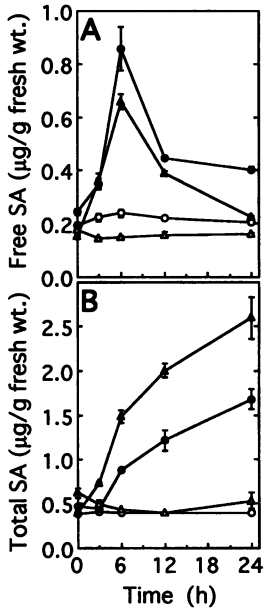

FIG. 1. Ozone-induced accumulation of SA in *A. thaliana*. Plants were exposed to ambient air (open symbols) or 300 ppb ozone (closed symbols) for 6 h. Triplicate samples from different plants were assayed for both total and free SA levels. The data shown are from two independent experiments (○, ●; △, ▲) and represent the mean ± SE.

Ozone Induction of Defense-Related Transcripts Is Modulated by SA. Previous studies (27, 29, 40, 46) have shown that the induction of several defense-related genes during disease resistance responses is associated with the accumulation of SA. The requirement for SA accumulation in the activation of SAR is well documented, and there are some indications that SA is important for HR as well (29, 38). To test whether ozone-induced SA is important for the concomitant accumulation of defense-related transcripts, RNA blot hybridization studies were done to compare the accumulation of three defense-related transcripts, namely *PAL*, *GST1*, and *PR1*, in transgenic plants expressing salicylate hydroxylase via the constitutive expression of a bacterial *nahG* gene (Fig. 2). Expression of salicylate hydroxylase in this line is known to greatly reduce the accumulation of SA and to prevent the expression of SAR and HR in response to pathogen attack (28, 39).

PAL mRNA levels were transiently induced by ozone in both wild-type and NahG plants, with a maximum 4- to 5-fold induction observed 3 h after the initiation of ozone treatment. In ozone-treated wild-type plants, *PAL* mRNA levels decreased to control levels by 6 h, whereas in ozone-treated NahG plants *PAL* mRNA levels remained about 3.5-fold higher than untreated controls, then decreased to control levels by 12 h after treatment. *GST1* transcript levels were also transiently induced in ozone-treated plants. In wild-type plants, *GST1* mRNA accumulated to 12-fold higher levels than controls within 3 h, and remained at a level 8-fold higher for 6 h before returning to near control levels by 12 h. In contrast,

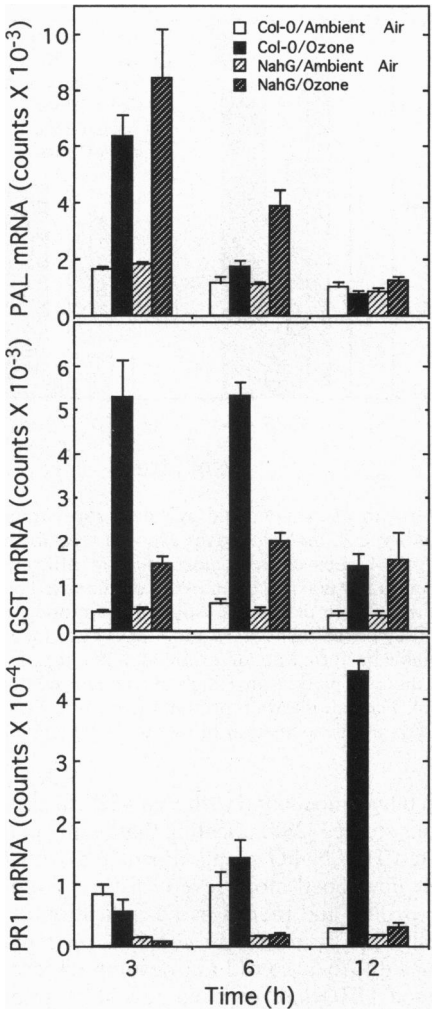

FIG. 2. *PAL*, *GST1*, and *PR1* mRNA levels in wild-type Col-0 and NahG *A. thaliana* leaves exposed to ambient air or 300 ppb ozone. Plants were exposed to ozone for 6 h and leaves collected at the indicated times. Total RNA was prepared and subjected to RNA blot hybridization analysis. The amount of hybridizing radioactivity was quantified using a PhosphorImager; the data shown have been corrected for loading differences by using the counts obtained with a control rRNA probe (19). The data shown represent the mean ± SE of two to four independent experiments in which filters were hybridized together with a specific probe.

ozone-treated NahG plants exhibited a significantly reduced accumulation of *GST1* mRNA; the maximum level of induction was only 4-fold higher than that observed in control plants. Ozone-induced accumulation of *PR1* transcripts was also affected in NahG plants. *PR1* mRNA accumulation in ozone-treated wild-type plants was delayed relative to that observed for *PAL* and *GST1*, with no significant induction detected until 12 h after the initiation of a 6 h ozone exposure, at which time *PR1* transcript levels were 16-fold higher than controls. In stark contrast, *PR1* mRNA levels in ozone treated NahG plants were identical to that observed in untreated controls. Analyses of SA levels showed that ozone did not induce SA accumulation in NahG plants and that the overall levels of SA were 2- to 3-fold lower compared to untreated wild-type plants (data not shown).

Given that ozone-induced accumulation of some defense-related transcripts was altered in NahG plants that do not accumulate SA, we determined whether the expression of these transcripts was altered in a SAR-deficient mutant, *npr1*, which does not accumulate *PR* gene transcripts in response to SA or 2,6-dichloroisonicotinic acid (33). Although there was not a statistically significant difference in the ozone-induced accumulation of *PAL* and *GST1* transcripts in *npr1* and wild-type plants, *GST1* mRNA levels tended to be about 25% lower in *npr1* plants whereas *PAL* mRNA levels were usually elevated in *npr1* compared to the wild-type controls. In contrast, the 8- to 10-fold accumulation of *PR1* mRNA observed in ozone-treated wild-type plants was completely abolished by the *npr1* mutation (Fig. 3).

Ozone Induces Resistance to Bacterial Infection. To determine whether ozone-induced SA accumulation and defense gene expression also triggered an effective defense response, we examined the effects of ozone exposure on the ability of virulent and avirulent *Psm* strains to cause disease symptoms and multiply *in planta*. In wild-type plants inoculated with the virulent strain *Psm* 4326, ozone-treatment resulted in a significant reduction in the severity of disease symptoms. Ozone-treated plants developed fewer and less chlorotic lesions compared to plants maintained in ambient air. The reduction

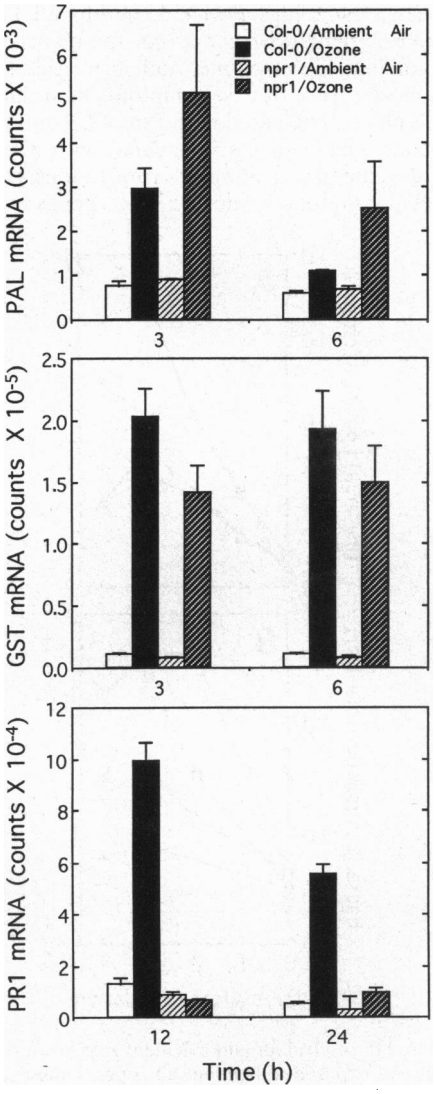

FIG. 3. *PAL*, *GST1*, and *PR1* mRNA levels in wild-type Col-0 and *npr1* mutant *A. thaliana* leaves exposed to ambient air or 300 ppb ozone. Plants were exposed to ozone for 6 h and leaves were collected at the indicated times. Total RNA was prepared and subjected to RNA blot hybridization analysis. The amount of hybridizing radioactivity was quantified using a PhosphorImager; the data shown have been corrected for loading differences using the counts obtained with a control rRNA probe (19). The data shown represent the mean ± SE of two to four independent experiments in which filters were hybridized together with a specific probe.

in symptoms in ozone-treated plants was correlated with reduced *in planta* growth of *Psm* 4326 (Fig. 4). The growth of *Psm* 4326 in ozone-treated plants varied from 10- to 1000-fold and was intermediate between the growth of the virulent and avirulent strains in untreated controls. In some experiments, the growth of the avirulent *Psm* 4326 containing *avrB* was also reduced in ozone-treated plants compared to the untreated controls (Figs. 4*B* and 5). Wild-type plants inoculated with *Psm* 4326/*avrB* exhibited a typical HR and did not develop any disease symptoms when maintained in either ambient air or ozone.

To determine whether the induced resistance caused by ozone treatment was dependent on SA accumulation and the activation of the SAR pathway, we tested whether or not NahG and *npr1* plants exposed to ozone exhibited resistance to *Psm* infection (Fig. 5). In wild-type Col-0 plants left in ambient air, *Psm* 4326 multiplied ≈3 to 4 logs over 3 days and caused severe chlorotic lesions, whereas in ozone-treated plants symptom development was reduced and this virulent strain only multiplied between 1 and 2 logs. *Psm* 4326 multiplied 3 to 4 logs in NahG and *npr1* plants whether or not the plants were maintained in ambient air or ozone, and in all cases the plants developed more severe disease symptoms than that observed in wild-type plants inoculated with *Psm* 4326 and maintained in ambient air. NahG plants inoculated with avirulent *Psm* 4326/*avrB* developed spreading chlorotic lesions, although the overall level of symptom development was not as severe as that

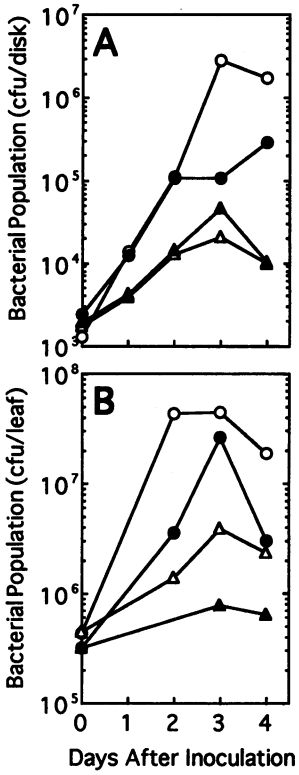

FIG. 4. Growth of virulent and avirulent *Psm* strains in wild-type *A. thaliana* leaves exposed to ambient air (open symbols) or 300 ppb ozone (closed symbols) for 6 h. Leaves of Col-0 plants were inoculated with either the virulent *Psm* 4326 (○, ●) or the avirulent derivative containing *avrB* (△, ▲) by infiltration with 10^6 cfu ml^{-1} (*A*) or by immersion in 2×10^8 cfu ml^{-1} (*B*). At the indicated times, 0.6-cm diameter disks were isolated from infiltrated leaves or whole leaves were isolated from immersion-inoculated plants. Disks or leaves from three different plants receiving the same treatment were pooled and homogenized in 10 mM $MgCl_2$, and serial dilutions of the homogenates were plated on King's B medium containing 50 μg ml^{-1} rifampin. Similar results were obtained in two additional independent experiments. cfu, Colony-forming unit.

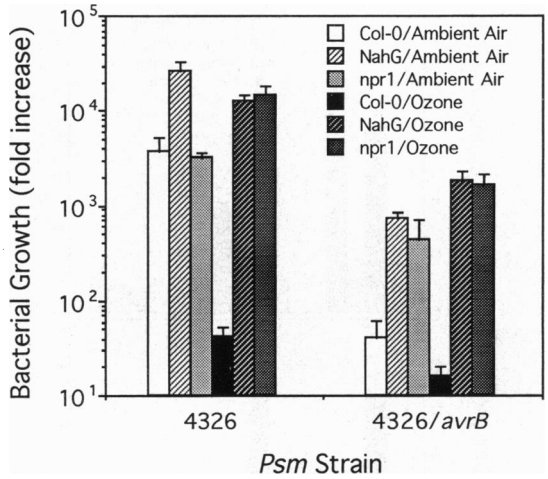

FIG. 5. Growth of virulent and avirulent *Psm* strains in NahG, *npr1*, and wild-type *A. thaliana* leaves exposed to ambient air or 300 ppb ozone for 6 h. Leaves were inoculated by infiltration with 10^6 colony-forming units per ml^{-1} Psm 4326 or the avirulent derivative containing *avrB*. Three disks were isolated from independent plants receiving the same treatment at T_0 and 3 days after inoculation. The number of viable bacteria were determined in homogenates of pooled samples via dilution plating on King's B medium containing 50 μg ml^{-1} rifampin. The data shown represent the mean ± SE fold increase observed 3 days after inoculation in two to four independent experiments.

observed in plants inoculated with *Psm* 4326. This is consistent with previous studies (28) indicating that NahG plants express an attenuated HR. NahG plants inoculated with *Psm* 4326/*avrB* usually developed more severe disease symptoms when exposed to ozone, and there was no indication of an ozone-induced resistance response. In contrast, *npr1* plants inoculated with *Psm* 4326/*avrB* did not develop disease symptoms and expressed HR when exposed to either ambient air or ozone. The *in planta* growth of *Psm* 4326/*avrB* was significantly higher in NahG and *npr1* plants compared to that observed in wild-type Col-0; however, there were no significant differences in bacterial growth between NahG and *npr1* plants maintained in ambient air or ozone.

DISCUSSION

The precise mechanisms that mediate the activation of defense-related genes by ozone and other environmental stresses that impose oxidative stress on plants have not been previously defined. Studies have shown that there is a significant overlap in the patterns of gene expression observed in ozone-treated plants and plants exhibiting a disease resistance response, suggesting that there may be some commonalty between the signal transduction pathways for disease resistance and ozone (16, 17, 19, 20). An initial study has shown that tobacco plants treated with either ozone or UV light accumulated SA and exhibited increased resistance to infection with tobacco mosaic virus (40). Since SA has recently been shown to be a critical factor in the induction of SAR and may also modulate HR, it is possible that SA is a common link between these various stress-activated pathways. To directly address how much overlap exists between the regulation of ozone-induced responses and disease resistance, and whether SA is a component of the signal transduction pathway triggered by ozone, we have used the established model system, *A. thaliana*. Numerous studies have been published that define the response of *A. thaliana* to pathogen infection (reviewed in refs. 41–43) and several recent studies have documented that *A. thaliana* is also an appropriate model for ozone studies (19–21, 44).

We have now shown that ozone treatment of *A. thaliana* causes induced resistance to a bacterial pathogen concomitant with the induction of defense-related genes that are also activated during HR and/or SAR. The induction of disease resistance and defense transcript accumulation was correlated with the accumulation of SA. Ozone treatment caused a rapid accumulation of both free and glycosylated forms of SA, although the induction of free SA was transient, reaching maximum levels ≈6 h after the initiation of a 6-h ozone treatment. Interestingly, the kinetics of the accumulation of the defense-related *PAL* and *GST1* mRNAs were similar to that of free SA. The ≈4-fold induction of free SA levels to 0.7–1.0 μg/g fresh weight in *A. thaliana* leaves by ozone is very similar to that observed in ozone-treated tobacco leaves (40). This level of induction is also comparable to that observed in *A. thaliana* leaves expressing a HR after infection with turnip crinkle virus (45) and tobacco leaves infected with tobacco mosaic virus (46, 47). The correlation between the accumulation of SA with the activation of defense gene expression and increased resistance to bacterial infection suggests that ozone can trigger the signal transduction pathways that are activated during SAR and HR.

To further define the role of SA in mediating ozone-induced responses, we took advantage of the availability of two *A. thaliana* lines that are altered in the expression of SAR. One line, developed by Ryals and coworkers (28), is a transgenic line that expresses salicylate hydroxylase (NahG), which converts SA into catechol, thus preventing the accumulation of SA. This line has been shown to be unable to mount a successful SAR response and is also attenuated with respect to HR (28, 39). Our results clearly show that NahG plants are also affected in their response to ozone. NahG plants are deficient in ozone-induced disease resistance and do not exhibit the induction of *PR1* transcripts observed in ozone-treated wild-type plants. These results indicate that ozone does indeed activate an SAR-like response that depends on SA accumulation. Interestingly, ozone-induced *PAL* mRNA accumulation was unaffected in NahG plants, demonstrating that the ozone-induced accumulation of *PAL* transcripts does not depend on SA accumulation. Previous studies have shown that *PAL* expression can be induced by hydrogen peroxide and it has been suggested that this induction is mediated by jasmonic acid (refs. 48 and 49 and references therein). A possible role of jasmonic acid in ozone-induced *PAL* expression is consistent with our results. Since SA seems to inhibit the expression of jasmonic acid induced gene expression (50), it would be expected that *PAL* transcript levels might be higher in NahG plants due to the extremely low levels of SA present in these plants. We observed that *PAL* transcript levels were indeed higher in both control and ozone-treated NahG plants compared to wild-type controls.

In definite contrast to *PAL* mRNA induction, *PR1* mRNA induction by ozone was abolished, and *GST1* mRNA accumulation was clearly attenuated in NahG plants. These results demonstrate that induction of *PR1* mRNA is totally dependent on SA accumulation and is consistent with results from *PR1* induction in pathogen-infected *A. thaliana* (28, 37). The fact that ozone-induced *GST1* transcript accumulation was only partially reduced in NahG plants indicates that *GST1* levels are controlled by at least two independent regulatory pathways, one of which is dependent on SA accumulation. The presence of a SA-dependent pathway for *GST1* induction is consistent with the observation that *GST1* can be induced by exogenous application of SA (G. Yu, X. Dong, and F. Ausubel, personal communication).

Further comparisons of disease resistance and defense-related transcript accumulation were done using the SAR-deficient *npr1* mutant (33). *npr1* plants do not accumulate PR proteins in response to SA or 2,6-dichloroisonicotinic acid, suggesting that NPR1 functions in the SAR signal transduction pathway at a step downstream of SA. Consistent with this interpretation, we found that ozone-induced *PAL* transcript accumulation, which was normal in NahG plants, was also unaffected in *npr1* plants. However, in contrast to the results observed in NahG plants, ozone-induced *GST1* mRNA accumulation was only slightly reduced compared to that observed in wild-type plants. This suggest that the SA-dependent component of *GST1* regulation does not require NPR1. As was the case for NahG plants, *PR1* mRNA levels remained very low in ozone-treated *npr1* plants, which was correlated with the loss of ozone-induced resistance to *Psm* infection. The fact that ozone-induced disease resistance was disrupted by the *npr1* mutation, and that this mutation does not appear to interfere with the expression of HR (33), indicates that the ozone-induced resistance is mediated primarily by the SAR pathway.

NahG plants were also found to be more susceptible to treatment with 300 ppb ozone compared to untransformed control plants. Ozone-treated NahG plants developed necrotic lesions and became slightly chlorotic, whereas such lesions were never observed in untransformed controls. This increased susceptibility was somewhat variable in younger plants but was consistently observed in plants after the onset of flowering (data not shown). This observation indicates that ozone actually activates a protective antioxidant response that depends in part on SA accumulation. *npr1* mutant plants were not obviously more susceptible to ozone treatment, suggesting that the antioxidant response does not require NPR1. The basis of this protective response is likely to be related to the induction of various antioxidant enzymes (19, 21).

The overlap in the induction of defense-related responses by ozone and pathogens is most likely due to the fact that both stresses initiate the production of active oxygen species. The initial interaction of avirulent pathogens with plants results in an "oxidative burst," which is thought to trigger subsequent signaling events that ultimately result in disease resistance (26, 49, 51, 52). The interaction of ozone with plants also results in the production of active oxygen species that could mimic an oxidative burst and thus activate the HR and SAR pathways (2, 24, 25). This raises the possibility that the necrotic lesions often observed in ozone-treated plants are not simply due to toxic effects of ozone, but may be a consequence of ozone activating the programmed cell death component of HR (53, 54). Other studies have shown that UV-irradiation also activates stress-related gene expression patterns that overlap with those in ozone-exposed plants. This UV response may also involve the accumulation of SA (40, 55), suggesting that a number of distinct stresses may be detected through their ability to generate active oxygen species. Thus, further mechanistic information obtained from additional genetic and molecular studies of the effects of ozone on *A. thaliana* are likely to have a direct bearing on the activation of defense genes in response to a number of other biotic and abiotic stresses.

We thank J. Ryals, X. Dong, and F. Ausubel for providing us with seed stocks, clones, and unpublished data. We also thank J. Rebbeck and A. Scherzer for their help with the ozone treatments and P. Leicht for growing plants. This article is based on work supported by the U.S. Department of Agriculture, Cooperative State Research Service, under Agreement 94-37100-0758.

1. Mustafa, M. G. (1990) *Free Radical Biol. Med.* **9,** 245–265.
2. Mehlhorn, H. & Wellburn, A. R. (1994) in *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants*, eds. Foyer, C. H. & Mullineaux, P. M. (CRC, Boca Raton, FL), pp. 155–175.
3. Chameides, W. L., Kasibhatla, P. S., Yienger, J. & Levy, H., II (1994) *Science* **264,** 74–77.
4. Krupa, S. V. & Manning, W. J. (1988) *Environ. Pollut.* **50,** 101–137.
5. Heagle, A. S. (1989) *Annu. Rev. Phytopathol.* **27,** 397–423.

6. Runeckles, V. C. & Chevone, B. I. (1992) in *Surface Level Ozone Exposures and their Effects on Vegetation*, ed. Lefohn, A. S. (Lewis, Chelsea, U.K.), pp. 189–252.
7. Guderian, R., Tingey, D. T. & Rabe, R. (1985) in *Air Pollution by Photochemical Oxidants*, ed. Guderian, R. (Springer, Berlin), pp. 129–334.
8. Heck, W. W. (1989) in *Air Pollutions's Toll on Forests and Crops*, eds. Meckenzie, J. J. & El-Ashry, M. T. (Yale Univ. Press, New Haven, CT), pp. 235–315.
9. Sanders, G. E., Colls, J. J. & Clark, A. G. (1992) *Ann. Bot.* **69,** 123–133.
10. Foy, C. D., Lee, E. H., Rowland, R. A. & Devine, T. E. (1995) *J. Plant Nutr.* **18,** 649–667.
11. Reiling, K. & Davison, A. W. (1992) *New Phytol.* **120,** 29–37.
12. Tanaka, K. (1994) in *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants*, eds. Foyer, C. H. & Mullineaux, P. M. (CRC, Boca Raton, FL), pp. 365–378.
13. Reddy, G. N., Dai, Y.-R., Negm, F. B., Flores, H. E., Arteca, R. N. & Pell, E. J. (1991) in *Active Oxygen/Oxidative Stress and Plant Metabolism*, eds. Pell, E. J. & Steffen, K. L. (Am. Soc. of Plant Physiologists, Rockville, MD), pp. 262–267.
14. Bowler, C., Van Montagu, M. & Inze, D. (1992) *Annu. Rev. Plant Physiol. Mol. Biol.* **43,** 83–116.
15. Schraudner, M., Ernst, D., Langebartels, C. & Sandermann, H. (1992) *Plant Physiol.* **99,** 1321–1328.
16. Ernst, D., Schraudner, M., Langebartels, C. & Sandermann, H. (1992) *Plant Mol. Biol.* **20,** 673–682.
17. Eckey-Kaltenbach, H., Ernst, D., Heller, W. & Sandermann, H. J. (1994) *Plant Physiol.* **104,** 67–74.
18. Schlagnhaufer, C. D., Glick, R. E., Arteca, R. N. & Pell, E. J. (1995) *Plant Mol. Biol.* **28,** 93–103.
19. Sharma, Y. K. & Davis, K. R. (1994) *Plant Physiol.* **105,** 1089–1096.
20. Sharma, Y. K. & Davis, K. R. (1995) *Plant Mol. Biol.* **29,** 91–98.
21. Conklin, P. L. & Last, R. L. (1995) *Plant Physiol.* **109,** 203–212.
22. Grimes, H. D., Perkins, L. K. & Boss, W. F. (1983) *Plant Physiol.* **72,** 1016–1020.
23. Kanofsky, J. R. & Sima, P. (1991) *J. Biol. Chem.* **266,** 9039–9042.
24. Kanofsky, J. R. & Sima, P. D. (1995) *J. Biol. Chem.* **270,** 7850–7852.
25. Kangasjärvi, J., Talvinen, J., Utriainen, M. & Karjalainen, R. (1994) *Plant Cell Environ.* **17,** 783–794.
26. Levine, A., Tenhaken, R., Dixon, R. & Lamb, C. (1994) *Cell* **79,** 583–593.
27. Chen, Z., Malamy, J., Henning, J., Conrath, U., Sánchez, C. P., Silva, H., Ricigliano, J. & Klessig, D. F. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4134–4137.
28. Delaney, T. P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut, R. M., Kessmann, H., Ward, E. & Ryals, J. (1994) *Science* **266,** 1247–1250.
29. Klessig, D. F. & Malamy, J. (1994) *Plant Mol. Biol.* **26,** 1439–1458.
30. Shulaev, V., León, J. & Raskin, I. (1995) *Plant Cell* **7,** 1691–1701.
31. León, J., Lawton, M. A. & Raskin, I. (1995) *Plant Physiol.* **108,** 1673–1678.
32. Neuenschwander, U., Vernooij, B., Friedrich, L., Uknes, S., Kessmann, H. & Ryals, J. (1995) *Plant J.* **8,** 227–233.
33. Cao, H., Bowling, S. A., Gordon, A. S. & Dong, X. (1994) *Plant Cell* **6,** 1583–1592.
34. Wanner, L. A., Mittal, S. & Davis, K. R. (1993) *Mol. Plant-Microbe Interact.* **6,** 582–591.
35. Whalen, M. C., Innes, R. W., Bent, A. F. & Staskawicz, B. J. (1991) *Plant Cell* **3,** 49–59.
36. Yalpani, N., León, J., Lawton, M. A. & Raskin, I. (1993) *Plant Physiol.* **103,** 315–321.
37. Uknes, S., Mauchmani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E. & Ryals, J. (1992) *Plant Cell* **4,** 645–656.
38. Ryals, J., Lawton, K. A., Delaney, T. P., Friedrich, L., Kessmann, H., Neuenschwander, U., Uknes, S., Vernooij, B. & Weymann, K. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4202–4205.
39. Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. & Ryals, J. (1993) *Science* **261,** 754–756.
40. Yalpani, N., Enyedi, A. J., León, J. & Raskin, I. (1994) *Planta* **193,** 373–376.
41. Davis, K. R. (1992) in *Molecular Signals in Plant-Microbe Communication*, ed. Verma, D. P. S. (CRC, Boca Raton, FL), pp. 393–406.
42. Dangl, J. L. (1993) *Int. Rev. Cytol.* **144,** 53–83.
43. Mauch-Mani, B. & Slusarenko, A. J. (1993) *Trends Microbiol.* **1,** 265–270.
44. Ormrod, D. P., Landry, L. G. & Conklin, P. L. (1995) *Physiol. Plant.* **93,** 602–610.
45. Uknes, S., Winter, A. M., Delaney, T., Vernooij, B., Morse, A., Friedrich, L., Nye, G., Potter, S., Ward, E. & Ryals, J. (1993) *Mol. Plant-Microbe Interact.* **6,** 692–698.
46. Malamy, J., Carr, J. P., Klessig, D. F. & Raskin, I. (1990) *Science* **250,** 1002–1004.
47. Yalpani, N., Silverman, P., Wilson, T. M. A., Kleier, D. A. & Raskin, I. (1991) *Plant Cell* **3,** 809–818.
48. Gundlach, H., Muller, M. J., Kutchan, T. M. & Zenk, M. H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2389–2393.
49. Mehdy, M. C. (1994) *Plant Physiol.* **105,** 467–472.
50. Peña-Cortés, H., Albrecht, T., Prat, S., Weiler, E. W. & Willmitzer, L. (1993) *Planta* **191,** 123–128.
51. Doke, N., Miura, Y., Sanchez, L. M. & Kawakita, K. (1994) in *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants*, eds. Foyer, C. H. & Mullineaux, P. M. (CRC, Boca Raton, FL), pp. 177–197.
52. Chandra, S. & Low, P. S. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4120–4123.
53. Greenberg, J. T., Guo, A., Klessig, D. F. & Ausubel, F. M. (1994) *Cell* **77,** 551–563.
54. Dietrich, R. A., Delaney, T. P., Uknes, S. J., Ward, E. R., Ryals, J. A. & Dangl, J. L. (1994) *Cell* **77,** 565–577.
55. Willekens, H., Van Camp, W., Van Montagu, M., Inzé, D., Langebartels, C. & Sandermann, H. (1994) *Plant Physiol.* **106,** 1007–1014