Plant Physiol. (1995) 109: 1191–1197

Induction of Nopaline Synthase Promoter Activity by H_2O_2 Has No Direct Correlation with Salicylic Acid[1]

Ziyu Dai* and Gynheung An

Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164–6340 (Z.D., G.A.); and Department of Biology, Pohang University of Science and Technology, San 31 Hyoja Dong, Pohang, 790–784, Republic of Korea (G.A.)

Transgenic tobacco (*Nicotiana tabacum* L.) plants carrying a fusion between the nopaline synthase (*nos*) promoter and chloramphenicol acetyltransferase (CAT) reporter gene (*cat*) were tested for their response to treatment with H_2O_2. The *nos* promoter-driven CAT activity increased significantly by addition of H_2O_2, reaching the maximum level at 15 mM. Kinetic analysis for CAT activity showed that induction by H_2O_2 was similar to that of methyl jasmonate (MJ), but was much slower than induction by salicylic acid (SA). Time-course experiments for mRNA level also revealed that the response to H_2O_2 treatment was similar to that of MJ. The *nos* promoter displayed a rapid and transient induction of mRNA with SA treatment, with the maximum levels occurring at 3 h, whereas the levels induced by H_2O_2 or MJ treatment increased continuously during the 11-h experimental period. The antioxidants *N*-acetyl-L-cysteine and catechol did not alter the SA effect. The responses of the *nos* promoter to H_2O_2, MJ, and wounding were significantly reduced by deletions of the CAAT box region and the sequence between −112 and −101. However, these deletions did not significantly alter the SA response. This suggests that H_2O_2 may have a different mechanism from that of SA for inducing *nos* promoter activity.

The transferred DNA genes of *Agrobacterium* tumor-inducing plasmid are transcribed in transformed tumor tissues. In nopaline-type tumor tissues, the nopaline synthase gene (*nos*) is one of the most abundant transcripts. Since the *nos* gene was considered to be constitutively active in various plant tissues, the promoter has been used for construction of plant-selective markers (Lichtenstein and Fuller, 1987). Nevertheless, it was later found that the *nos* promoter activity was organ specific and developmentally regulated (An et al., 1988a).

Four positive regulator factors affecting *nos* promoter activity have been previously identified: mechanical wounding, auxin, SA, and MJ (An et al., 1990; Kim et al., 1993). These studies revealed that the *nos* upstream region is essential for the responses to these stimuli. Site-specific mutations and fine deletion analysis of the *nos* promoter upstream region showed that a 20-nucleotide regulatory element located between −131 and −112 is essential for the promoter activity (Kim et al., 1994). The element consists of two hexamer motifs separated by an 8-nucleotide spacer sequence. Deletion of the immediate downstream region of the 20-nucleotide sequence between −112 and −101 significantly reduced the MJ and wound responses compared to the SA response. Similarly, CAAT box (−81 to −63) deletions also resulted in a significant decrease in MJ and wound response (Kim et al., 1993). However, in these mutants SA signaling was not affected as much as that of MJ and wounding. These results suggest that the *nos* promoter upstream region containing the hexamer motif is essential for the SA, MJ, or wound response, whereas the CAAT box region and the sequence immediately downstream from the hexamer motif are required for maximum induction by MJ and wounding.

Jasmonic acid and its methyl ester MJ are naturally occurring plant growth regulators widely found in higher plants (Yamane et al., 1981; Meyer et al., 1984; Sembdner and Parthier, 1993). They were first identified as a senescence-promoting substance and a growth inhibitor in several higher plants (Ueda and Kato, 1980; Dathe et al., 1981; Staswick et al., 1992). Low concentrations of jasmonate and MJ that are found in many plant organs can accumulate rapidly after wounding (Creelman et al., 1992; Koda and Kikuta, 1994) or treatment of plant tissues or plant cell-suspension cultures with fungal elicitors (Gundlach et al., 1992; Mueller et al., 1993). Among the MJ-inducible genes are those coding for proteinase inhibitors of tomato and potato (Farmer and Ryan, 1990; Kim et al., 1992), soybean leaf vegetative storage proteins (Franceschi and Grimes, 1991), and ribosome-inactivating proteins of barley (Reinbothe et al., 1994), which are also wound inducible. It has been proposed that jasmonate and MJ may function as part of a signal transduction system involved in the regulation of plant defense responses to insects and pathogens (Farmer and Ryan, 1992).

SA has been recognized as an endogenous regulatory signal in plants, especially during plant defense against pathogens (Malamy et al., 1990; Metraux et al., 1990; Raskin, 1992). Several lines of evidence indicate that endogenously produced SA is involved in the signal transduction pathway. Upon TMV infection, SA levels in TMV-

[1] This work was supported in part by National Science Foundation grant No. MCB-9304867.

* Corresponding author; e-mail daizy@wsunix.wsu.edu; fax 1–509–335–3517.

Abbreviations: CAT, chloramphenicol acetyltransferase; MJ, methyl jasmonate; NAC, *N*-acetyl-L-cysteine; PR, pathogenesis related; ROS, reactive oxygen species; SA, salicylic acid; TMV, tobacco mosaic virus.

resistant tobacco (*Nicotiana tabacum* L. xanthi-nc) increase almost 50-fold to 1 μg/g fresh weight in TMV-inoculated leaves, and 10-fold in uninfected leaves of the same plant, but not in susceptible tobacco (Malamy et al., 1990). Induction of PR-1 genes paralleled the rise in SA levels. In tobacco, exogenously applied SA induces the transcription of PR genes that are also induced by TMV infection (Ward et al., 1991; Yalpani et al., 1991). The transgenic tobacco plants, engineered to express a salicylate hydroxylase (*nah*G) gene from *Pseudomonas putida*, are unable to manifest a systemic acquired resistance response, since these plants cannot accumulate SA after pathogen infection (Gaffney et al., 1993). Thus, it has been hypothesized that one of the consequences of pathogen infection is an accumulation of SA in vivo, which induces the expression of a set of proteins that act to further limit infection of the host.

Investigations into the components involved in the SA response have led to the identification of a SA-binding protein that has catalase activity (Chen and Klessig, 1991; Chen et al., 1993). In vitro, the activity of the catalase is inhibited by SA; in vivo, catalase inhibitors including SA inhibit catalase activity and lead to increased H_2O_2 levels and the induction of the PR-1 gene (Chen et al., 1993). Thus, H_2O_2 has been suggested to be a signal molecule acting downstream of SA.

H_2O_2, one of the ROS, plays a crucial role in plant defense responses. It has been suggested that H_2O_2 and other ROS are directly involved in defense-related responses such as antimicrobial agents (Baker et al., 1993; Legendre et al., 1993), lignification (Peng and Kúc, 1992), phytoalexin production (Apostol et al., 1989), lipid peroxidation (Rogers et al., 1988), and oxidative cross-linking of cell-wall structural proteins (Bradley et al., 1992).

In this study, we compared the effects of SA and H_2O_2 on the wild type and various deletion mutants of the *nos* promoter using the *cat* reporter gene. The results showed that the effects of H_2O_2 on the *nos* promoter have a closer correlation with MJ and wound responses than with that of SA.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Transgenic plants were obtained by the co-cultivation method (An et al., 1988b) using tobacco (*Nicotiana tabacum* L. cv Petit Havana SR1) grown aseptically on Murashige and Skoog agar medium supplemented with 3% Suc (Murashige and Skoog, 1962). Kanamycin-resistant transformants were selected and grown in the greenhouse, and seeds were collected after self-fertilization. Seeds were germinated on Murashige and Skoog agar medium containing 50 μg mL^{-1} of kanamycin, and healthy kanamycin-resistant plants were grown in greenhouse conditions.

Stimuli Treatments

All of the experiments were performed with the first or second progenies of transgenic plants. Leaves from 8-week-old plants were used to study the effects of the following chemical stimuli: 1 mM SA (Sigma), 5 mM H_2O_2 (unless otherwise mentioned) (J.T. Baker), 50 μM MJ (Bedoukian Research Inc., Danbury, CT), and antioxidants NAC (50 μM–30 mM, Sigma) and catechol (50 μM–30 mM, Sigma). Stock solutions of SA (200 mM), H_2O_2 (250 mM), NAC (200 mM), and catechol (200 mM) were prepared in distilled water. Stock solution of 5 mM MJ was prepared in 100% ethanol. Leaves were wounded by cutting into 1-cm^2 sections and were floated on Murashige and Skoog liquid medium alone or containing SA, H_2O_2, or MJ. The samples were incubated at room temperature (24°C) under low light (30–40 μmol quanta m^{-2} s^{-1}). The CAT assay was performed using 5 to 100 μg of total protein incubated with [^{14}C]chloramphenicol substrate at 37°C for 30 min, and reaction products were separated by TLC (An et al., 1988b).

RNA Preparation and Gel Blot Analysis

The fifth through ninth mature leaves were harvested, wounded, and treated as described above. Leaf samples were immediately frozen in liquid nitrogen. RNA was extracted from frozen leaf tissues homogenized using a mortar and pestle. Ice-cold 2 mL g^{-1} GTC buffer (4 M guanidine isothiocyanate, 25 mM sodium acetate, 5 mM EDTA, 0.15 M β-mercaptoethanol) was added to the ground tissue. Extraction mixtures were centrifuged at 10,000 rpm for 15 min at 4°C. Supernatants were transferred into 15-mL centrifuge tubes and CsCl was added to give a final concentration of 0.2 g/mL. Supernatants were then layered slowly over 4.5 mL of 5.7 M baked CsCl solution and ultracentrifuged for 8 h at 55,000 rpm. The RNA pellets were suspended in 0.4 mL of RNase-free H_2O, phenol:chloroform extracted, precipitated with ethanol, and resuspended in 100 to 150 mL of RNase-free H_2O. Total RNA concentration was quantified spectrophotometrically. Integrity of the RNA samples was checked by agarose (1.3%, w/v):formaldehyde (2%, v/v) gel electrophoresis followed by ethidium bromide staining.

Thirty- to 50-μg total RNA samples size-fractionated by gel electrophoresis were transferred to Hybond-N^+ nylon membrane (Amersham) by capillary blotting for approximately 15 h and then baked at 80°C for 2 h. Hybridizations were performed at 60°C in 0.25 M Na_2PO_4 (pH 7.2), 7% SDS, and 10 mg mL^{-1} BSA (Church and Gilbert, 1984) to radioactive probes synthesized by randomly priming a *Sma*I fragment containing the entire *cat* coding region using α-[^{32}P]dCTP and T7 DNA polymerase (T7 QuickPrime Kit, Pharmacia). The blots were first washed in a solution of 0.2× SSPE (30.2 mM NaCl, 2 mM Na_2HPO_4, 2 mM EDTA, pH 7.4) and 0.1% SDS at 40°C for 5 min followed by a second wash at 60°C for 10 min and a final wash at 60°C for 0.5 to 1 min. The blots were exposed to x-ray film at −70°C with intensifying screens and the film was analyzed using an LKB (Bromma, Sweden) 2202 ultrascan laser densitometer to quantitate the relative expression levels of mRNA.

RESULTS

H_2O_2 Dose Response

Recently, it was proposed that SA inhibits catalase activity and thereby enhances the generation of H_2O_2, which

induces expression of defense-related genes associated with systemic acquired resistance (Chen et al., 1993). Therefore, we have examined the effect of H_2O_2 on *nos* promoter activity. Transgenic tobacco leaves carrying the fusion molecule between the *nos* promoter and the *cat* reporter gene were treated with different concentrations of H_2O_2 for 22 h. The results in Figure 1 show that maximum concentration of H_2O_2 for *nos* promoter activity was 15 mM. Twenty millimolar H_2O_2 resulted in some inhibition of *nos* promoter activity that coincided with decreased total protein content, probably due to leaf tissue damage. However, 5 mM H_2O_2 induced *nos* promoter activity more than 75% of the maximum induction and did not exhibit any apparent damage on the leaf tissues. Thereafter, 5 mM H_2O_2, a level used for PR gene induction (Chen et al., 1993), was used for all other experiments in this study. In the following experiments the induction by 5 mM H_2O_2 is compared with that of 1 mM SA or 50 μM MJ. Previous studies showed that these levels of SA and MJ dramatically induced *nos* promoter activity (Kim et al., 1993).

Induction Kinetics

We have examined whether there is any correlation between H_2O_2 and SA effects on *nos* promoter activity. To define the possible relationship between these two chemicals, we first compared the induction kinetics of each chemical individually. Figure 2 shows that CAT activity was most rapidly induced by SA treatment. By comparison, H_2O_2 treatment induced CAT activity more slowly during the first 8 h, followed by a dramatic increase. This induction pattern is similar to that of wounding and MJ treatments. Upon normalizing CAT activity for each treatment at 22 h as 100%, the induction kinetics of the H_2O_2 treatment was similar to that of wounding and MJ (Fig. 2B). During the first 8 h of incubation, CAT activity induced by H_2O_2, MJ, or wounding increased to about 15% of the maximal activity, whereas SA treatment enhanced CAT activity up to 52%. After 11 h of incubation, CAT activity induced by

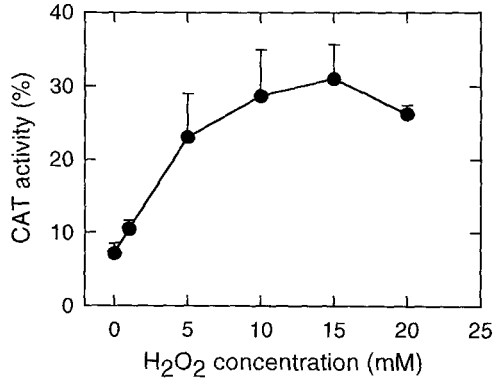

Figure 1. The response of *nos* promoter activity to varying H_2O_2 concentrations. The *nos* promoter activity was determined by measurement of CAT activity using 5 μg of total soluble protein. Each point is the mean of three independent transgenic tobacco plants ± SD.

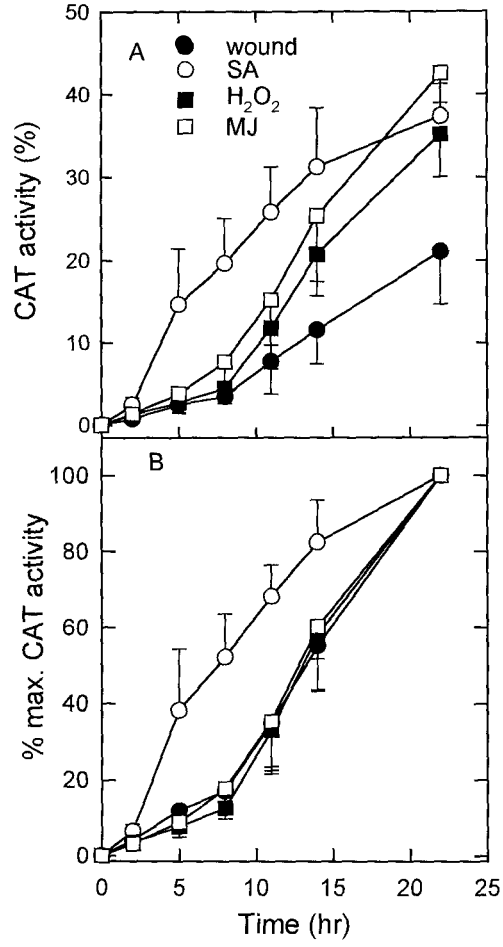

Figure 2. A, Induction kinetics of the *nos* promoter by wounding, 1 mM SA, 5 mM H_2O_2, and 50 μM MJ. The CAT activity was measured using 5 μg of total soluble protein. B, These results are shown as a percentage of the maximum value of CAT activity for each treatment. Each point is the mean of three independent transgenic tobacco plants ± SD.

H_2O_2, MJ, or wounding increased rapidly. These results suggest that the induction mechanism of H_2O_2 may differ from that of SA.

To confirm these observations, the effects of various stimuli were studied by quantifying mRNA levels (Fig. 3). As was found with the CAT activity, the mRNA accumulation pattern of H_2O_2 treatment was very different from that of treatment with SA, and much more similar to that of treatment with MJ. The transcript level of the *cat* reporter gene induced by SA increased very early, reached a peak within 3 h, and then decreased to low levels during the extended incubation period. For H_2O_2 or MJ treatments, however, the level of mRNA of the *cat* reporter gene continued to increase during 11 h of incubation.

Antioxidant Effects

Since antioxidants inhibit the generation of ROS, examination of responses of SA with or without antioxidants

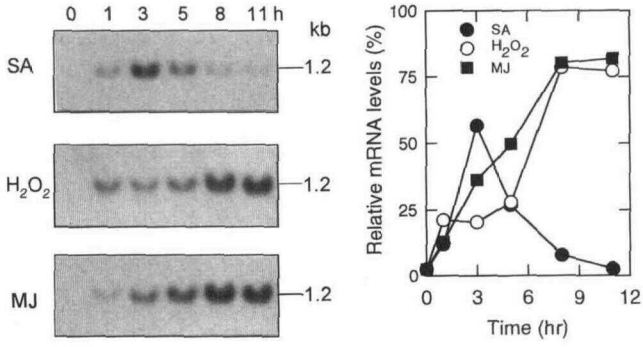

Figure 3. Induction kinetics of *cat* mRNA with 1 mM SA, 5 mM H_2O_2, or 50 μM MJ treatment. Thirty micrograms of total RNA were hybridized with the radioactively labeled probe prepared from the *cat*-coding region. Autoradiographs of the RNA blotting are shown on the left. Relative RNA levels are plotted on the right.

provides a further test of the possible relationship between SA and H_2O_2 on the *nos* promoter. Leaf discs were treated with either 1 mM SA, different concentrations of the antioxidants NAC or catechol, or 1 mM SA with different concentrations (0–30 mM) of the antioxidants NAC or catechol for 11 h. Figure 4 shows that 1 mM NAC or catechol treatments did not affect wound or SA responses. Concentrations of the chemicals ranging from 50 μM to 30 mM all showed similar results (data not shown).

Deletion Analysis

Previous studies showed that the full-length *nos* promoter (5′−263) and the 5′ deletion mutant −155 were induced to similar levels of activity by SA or MJ (Kim et al., 1993). In the internal deletion mutant −112/−101 and the CAAT box deletion mutant −81/−63, however, the MJ response was significantly reduced compared to that of SA. If the induction by H_2O_2 shares the same signaling pathway with SA, these two deletion mutants should not affect the H_2O_2 response. Therefore, the responses to SA, H_2O_2, and MJ were examined in transgenic tobacco plants carrying the deletion mutants (Ha and An, 1989). At least four transgenic plants for each deletion mutant that previously showed positive expression of the *cat* reporter gene were measured for CAT activity.

The results shown in Figure 5 demonstrated that transgenic plants carrying the full-length *nos* promoter or deletion mutant 5′−155 were induced to similar levels by SA, H_2O_2, and MJ. In the deletion mutants −112/−101 and −81/−63, the induction by H_2O_2 and MJ was dramatically decreased, whereas the induction by SA was maintained at relatively high levels.

Figure 6 shows the levels of mRNA accumulated after 5 h of wounding, SA, H_2O_2, or MJ treatments of the full-length *nos* promoter and deletion mutant −81/−63. In the full-length promoter (Fig. 6A), substantial amounts of the *cat* mRNA accumulated as a result of H_2O_2, SA, or MJ treatments, whereas in the deletion mutant −81/−63 (Fig. 6B), only SA treatment resulted in a detectable level of mRNA accumulation.

DISCUSSION

Previous studies have shown that the *nos* promoter is induced by wounding, auxin, SA, and MJ (An et al., 1990; Kim et al., 1993, 1994). The present study shows that H_2O_2 also induces *nos* promoter activity. H_2O_2 is hypothesized to be involved in the signal transduction pathway for PR genes during induction by SA. However, there are some discrepancies in the effects of H_2O_2 on PR genes. Chen et al. (1993) found that increasing H_2O_2 levels, either directly or by elevating SA, caused an increase in PR-1 accumulation. However, induction of acidic-type PR-1 accumulation by SA was unaffected by the antioxidants NAC and pyrrolidine dithiocarbamate, which should destroy H_2O_2 (Green and Fluhr, 1995). Further evidence for the existence of non-ROS-requiring pathways for SA comes from studies of systemic induction of PR gene expression in tobacco. It has been shown that even very slight increases in SA levels in systemic tissue can induce PR gene expression by means of an undefined pathway that was suggested not to include H_2O_2 (Vernooij et al., 1994).

Much of the information on the relationship between SA and H_2O_2 was obtained from studying the effects of these chemicals on PR genes. We have studied the relationship of the two chemical signals in controlling a different promoter to investigate whether these effects can be generally applicable to other genes. In this study, we have observed that the induction pattern of the *nos* promoter by H_2O_2 was significantly different from that of SA. It is interesting that the H_2O_2 induction kinetics were very similar to those caused by wounding or MJ treatments. The induction of

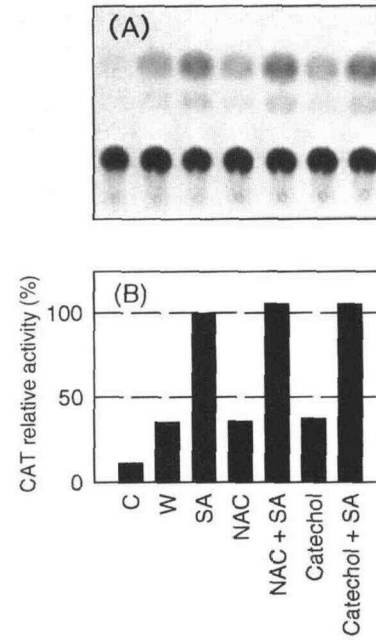

Figure 4. The effects of antioxidants on SA responses. A, The TLC autoradiograph shows representative samples treated with various stimuli for 11 h. B, Relative CAT activities for control (C), wound (W), and the antioxidants NAC and catechol with or without SA treatments normalized to the 100% base level induced by SA. The amount of total soluble protein used for CAT assay was 5 μg.

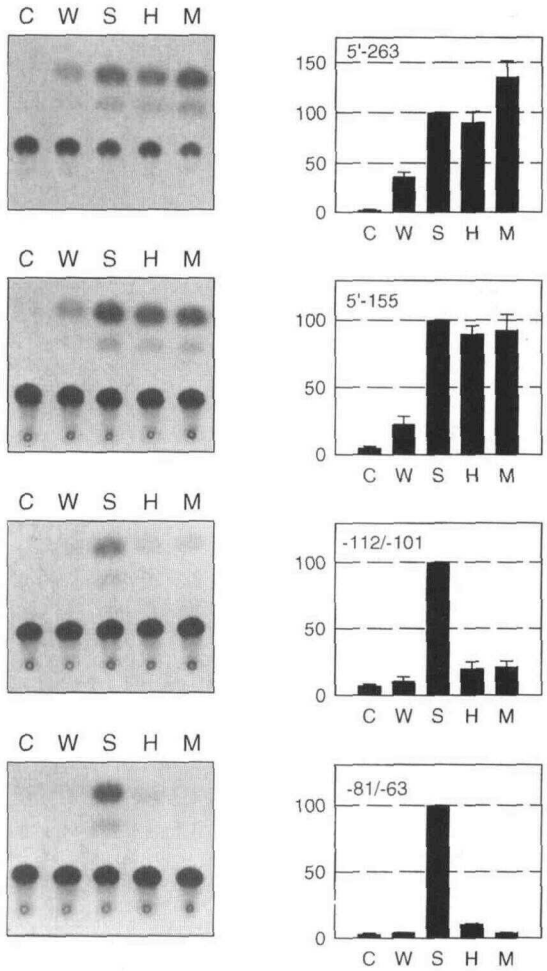

Figure 5. Deletion analysis of the H_2O_2 and SA response elements of the *nos* promoter. Results from a representative sample are shown in the TLC autoradiograph on the left. Relative CAT activities of control (C), wound (W), H_2O_2 (H), and MJ (M) treatments normalized to the 100% base level induced by SA (S) are shown in bar graphs on the right. Each point is the mean of three independent transgenic tobacco plants ± SD. The amount of total soluble protein used for CAT assay was 5 μg for 5′−263 and 5′−155, and 100 μg for −112/−101 and −81/−63.

the *nos* promoter by SA was very rapid compared with MJ and H_2O_2 responses and reached a steady-state level by 5 h. At that stage the SA-induced CAT activity was about 5 times greater than that resulting from H_2O_2 and MJ treatments. Although the relative rate of movement of SA, H_2O_2, and MJ to their receptor sites is not known, there is no reason to expect that H_2O_2 and MJ would diffuse more slowly to the receptor sites than SA. These results suggest that the action of SA on the *nos* promoter has a different mechanism from that of H_2O_2, which may share a similar induction pathway with that of wounding or MJ. If the induction of the *nos* promoter by SA is mediated by H_2O_2, as hypothesized for the SA-induced signal transduction of PR genes, then the *nos* promoter should respond to H_2O_2 faster than to SA. H_2O_2 is known to freely cross cell membranes, serving as a substrate for many important reactions in cell-wall reinforcement (Bowler et al., 1992).

The function of antioxidants in living cells is to prevent the accumulation of ROS such as H_2O_2, superoxide, and hydroxyl radicals. NAC and catechol are two nontoxic chemicals used for in vivo and in vitro studies as antioxidants. The NAC can directly scavenge oxidants and increase intracellular glutathione levels (Roederer et al., 1990). If SA response is related to H_2O_2, the SA effect should be altered in the presence of antioxidants. However, in this study we found that NAC and catechol did not affect SA response.

The mRNA accumulation kinetics also provided further evidence of the different induction mechanisms between SA and H_2O_2. Induction of mRNA level by SA rapidly reached a maximal level by 3 h. The rapid and transient induction of the 35S promoter by SA treatment has also been reported (Qin et al., 1994). We found that, in contrast to SA, treatment with H_2O_2 or MJ slowly increased the mRNA level. These results further indicate that the H_2O_2 response may have a different mechanism from that of SA.

To confirm the above conclusions, deletion mutants of the *nos* promoter were used. It was shown previously that the *nos* promoter was induced to a similar level by 1 mM SA and 50 μM MJ and that deletions of either the −112/−101 or −81/−63 region affected the MJ response much more strongly than the SA response (Kim et al., 1993). In this study, we have demonstrated that deletion of the −112/−101 or −81/−63 region significantly reduced the H_2O_2 response compared to that of SA. The results illustrate that these regions are important for the effects of H_2O_2 as well as for the MJ response.

Our observations suggest that SA may affect the induction of plant genes by at least two different pathways. As proposed from the studies of PR proteins, SA interacts with catalase, thereby increasing the level of H_2O_2, which triggers various cellular responses. This process is slow (oc-

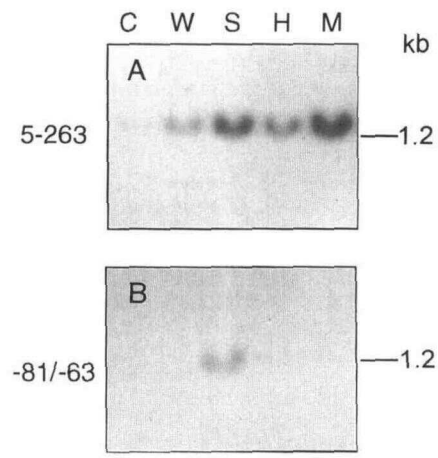

Figure 6. RNA gel-blot analysis for transgenic tobacco carrying the *nos* promoter of 5′−263 (A) and internal deletion mutant −81/−63 (B). Thirty micrograms of total RNA for 5′−263 and 90 μg of total RNA for −81/−63 were isolated from leaf samples treated with various stimuli for 5 h and hybridized with the radioactively labeled probe prepared from the *cat*-coding region.

curring over several hours) compared to the response found in the *nos* promoter, which is induced within 1 h of SA treatment. It appears that the SA response of the *nos* promoter occurs by a different pathway, which is unrelated to H_2O_2.

ACKNOWLEDGMENTS

We thank Dr. Daniel F. Klessig for valuable suggestions on antioxidant experiments; Michael A. Costa and Dr. Gerald E. Edwards for critically reading the manuscript; Mrs. Kyungsook An for maintaining transgenic tobacco; and Michael A. Costa, Hong-Gyu Kang, Yong-Yoon Chung, and Jianwei Gao for technical assistance.

Received May 18, 1995; accepted August 31, 1995.
Copyright Clearance Center: 0032-0889/95/109/1191/07.

LITERATURE CITED

An G, Costa MA, Ha S-B (1990) Nopaline synthase promoter is wound inducible and auxin inducible. Plant Cell **2:** 225–233

An G, Costa MA, Mitra A, Ha S-B, Marton L (1988a) Organ-specific and developmental regulation of the nopaline synthase promoter in transgenic tobacco plants. Plant Physiol **88:** 547–552

An G, Ebert PR, Ha S-B (1988b) Binary vectors. *In* SB Gelvin, RA Schilperoort, eds, Plant Molecular Biology Manual. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–19

Apostol I, Heinstein PF, Low PS (1989) Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. Plant Physiol **90:** 109–116

Baker CJ, Orlandi EW, Mock NM (1993) Harpin, an elicitor of the hypersensitive response in tobacco caused by *Erwinia amylovora*, elicits active oxygen production in suspension cells. Plant Physiol **102:** 1341–1344

Bowler C, Montagu MV, Inze D (1992) Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol **43:** 83–116

Bradley DJ, Kjellbom P, Lamb CJ (1992) Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell **70:** 21–30

Chen Z, Klessig DF (1991) Identification of a soluble salicylic acid-binding protein that may function in signal transduction in the plant disease-resistance response. Proc Natl Acad Sci USA **88:** 8179–8183

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science **262:** 1883–1886

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

Creelman RA, Tierney ML, Mullet JE (1992) Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. Proc Natl Acad Sci USA **89:** 4938–4941

Dathe W, Ronsch H, Preiss A, Schade W, Sembdner G, Schreiber K (1981) Endogenous plant hormones of the broad bean, *Vicia faba* L. (−)-Jasmonic acid, a plant growth inhibitor in pericarp. Planta **153:** 530–535

Farmer EE, Ryan CA (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci USA **87:** 7713–7716

Farmer EE, Ryan CA (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell **4:** 129–134

Franceschi VR, Grimes HD (1991) Induction of soybean vegetative storage proteins and anthocyanins by low-level atmospheric methyl jasmonate. Proc Natl Acad Sci USA **88:** 6745–6749

Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H, Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. Science **261:** 754–756

Green R, Fluhr R (1995) UV-B-induced PR-1 accumulation is mediated by active oxygen species. Plant Cell **7:** 203–212

Gundlach H, Müller MJ, Kutchan TM, Zenk MH (1992) Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc Natl Acad Sci USA **89:** 2389–2393

Ha S-B, An G (1989) Cis-acting regulatory elements controlling temporal and organ-specific activity of nopaline synthase promoter. Nucleic Acids Res **17:** 215–223

Kim S-R, Choi J-L, Costa MA, An G (1992) Identification of G-box sequence as an essential element for methyl jasmonate response of potato proteinase inhibitor II promoter. Plant Physiol **99:** 627–631

Kim S-R, Kim Y, An G (1993) Identification of methyl jasmonate and salicylic acid response elements from the nopaline synthase (*nos*) promoter. Plant Physiol **103:** 97–103

Kim Y, Buckley K, Costa MA, An G (1994) A 20 nucleotide upstream element is essential for the nopaline synthase (*nos*) promoter activity. Plant Mol Biol **24:** 105–117

Koda Y, Kikuta Y (1994) Wound-induced accumulation of jasmonic acid in tissues of potato tubers. Plant Cell Physiol **35:** 751–756

Legendre L, Rueter S, Heinstein PF, Low PS (1993) Characterization of the oligogalacturonide-induced oxidative burst in cultured soybean (*Glycine max*) cells. Plant Physiol **102:** 233–240

Lichtenstein CP, Fuller SL (1987) Vectors for the genetic engineering of plants. *In* PWJ Rigby, ed, Genetic Engineering, Vol 6. Academic Press, Orlando, FL, pp 103–183

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

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

Meyer A, Miersch O, Buttner C, Dathe W, Sembdner G (1984) Occurrence of the plant growth regulator jasmonic acid in plants. J Plant Growth Regul **3:** 1–8

Mueller MJ, Brodschelm W, Spannagl E, Zenk M (1993) Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. Proc Natl Acad Sci USA **90:** 7490–7494

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497

Peng M, Kúc J (1992) Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. Phytopathology **82:** 696–699

Qin X-F, Holuigu L, Horvath DM, Chua N-H (1994) Immediate early transcription activation by salicylic acid via the cauliflower mosaic virus *as-1* element. Plant Cell **6:** 863–874

Raskin I (1992) Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol **43:** 439–463

Reinbothe S, Reinbothe C, Leintzen C, Becker W, Apel K, Parthier B (1994) JIP60, a methyl jasmonate-induced ribosome-inactivating protein involved in plant stress reactions. Proc Natl Acad Sci USA **91:** 7012–7016

Roederer M, Staal FJT, Raju PA, Ela SW, Herzenberg LA, Herzenberg LA (1990) Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. Proc Natl Acad Sci USA **87:** 4884–4888

Rogers KR, Albert F, Anderson AJ (1988) Lipid peroxidation is a consequence of elicitor activity. Plant Physiol **86:** 547–553

Sembdner G, Parthier B (1993) The biochemistry and the physiological and molecular actions of jasmonates. Annu Rev Plant Physiol Mol Biol **44:** 569–589

Staswick PE, Su W, Howell SH (1992) Methyl jasmonate inhibition of root growth and induction of a leaf protein factor. Proc Natl Acad Sci USA **89:** 6837–6840

Ueda J, Kato J (1980) Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). Plant Physiol **66:** 246–249

Vernooij B, Friedrich L, Morse A, Reist R, Kolditz-jawhar R, Ward E, Uknes S, Kessmann H, Ryals J (1994) Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. Plant Cell **6:** 959–965

Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Metraux J-P, Ryals JA (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell **3:** 1085–1094

Yalpani N, Silverman P, Wilson TMA, Kleier DA, Raskin I (1991) Salicylic acid is a systemic signal and inducer of pathogenesis-related proteins in virus-infected tobacco. Plant Cell **3:** 809–818

Yamane H, Takagi H, Abe T, Yokota T, Takahashi N (1981) Identification of jasmonic acid in three species of higher plants and its biological activities. Plant Cell Physiol **22:** 689–697