Wound-inducible expression of a potato inhibitor II–chloramphenicol acetyltransferase gene fusion in transgenic tobacco plants

(tomatoes/proteinase inhibitors/transformation)

ROBERT W. THORNBURG*, GYNHEUNG AN, THOMAS E. CLEVELAND†, RUSSELL JOHNSON, AND CLARENCE A. RYAN‡

Institute of Biological Chemistry and Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164–6340

ABSTRACT   A potato inhibitor II gene (IIK) was isolated from a library of potato genes in λ bacteriophage. An 8-kilobase-pair (kbp) insert was identified using a tomato inhibitor II cDNA as a hybridization probe, and a 2.6-kbp fragment containing the gene was subcloned into the plasmid pUC13 and characterized. The nucleotide sequence of the isolated gene exhibited 87% identity with the wound-inducible tomato inhibitor II cDNA sequence. The amino acid sequence of inhibitor IIK, deduced from the potato gene, exhibited 84% identity with the tomato inhibitor II protein. A 1000-bp restriction fragment from the 5' flanking region of the gene was fused to the open reading frame of the chloramphenicol acetyltransferase (CAT) gene. This fusion was terminated in two ways: (*i*) with a terminator sequence from the potato inhibitor II gene and (*ii*) with a terminator from the *6b* gene of Ti plasmid pTiA6. These chimeric genes were transferred into tobacco cells via a binary Ti vector system, and transgenic plants were regenerated. The CAT gene was expressed in leaves of transformed plants in response to wounding when fused with the inhibitor IIK promoter and terminator regions. The chimeric gene containing the *6b* terminator did not express CAT in response to wounding. The wound-inducible expression of CAT activity was systemic and was induced in tissues distal to the wounded tissues. The time course of wound induction of CAT activity in transgenic tobacco leaves is similar to that found for wound-inducible inhibitor I and II mRNAs in tomato leaves. These results demonstrate that sequences necessary and sufficient for wound inducibility are present within ≈1000 bp of the control regions of the inhibitor IIK genes and that wound-inducible components of tobacco leaf cells can regulate these sequences.

Leaves of plants from the Solanaceae and Leguminosae families accumulate serine proteinase inhibitor proteins when severely damaged by attacking insects or other mechanical agents (1–3). The response is systemic and is triggered by a putative signal or "wound hormone" that is released from the sites of damage (4). Fragments of the plant's cell wall, released by endogenous polygalacturonase activity, have been shown to activate the response in detached leaves and may be part of the signaling process of the plant that induces the expression of the proteinase inhibitor genes (5, 6). The accumulation of proteinase inhibitors is thought to be a defensive response that interferes with the digestive processes of attacking pests (7, 8).

In potato and tomato leaves, two small gene families of serine proteinase inhibitors are wound inducible (9, 10). From wound-inducible mRNAs coding for the two families of inhibitors, cDNAs were isolated (11, 12) and utilized as probes to identify genes from potato and tomato gene

libraries (9, 10). In this report, we describe the isolation of the potato inhibitor IIK gene that has been identified as a wound-inducible gene based on its similarity to a wound-inducible cDNA from tomato leaves and the strong hybridization of its 3' regions with a wound-inducible mRNA from potato leaves. A gene fusion consisting of the chloramphenicol acetyltransferase (CAT) (13) coding region with ≈1 kilobase pair (kbp) each of the inhibitor IIK 5' region and 3' region has been constructed and used to successfully transform tobacco plants with wound-inducible CAT activity.

MATERIALS AND METHODS

DNA modifying enzymes were purchased from Bethesda Research Laboratories or New England Biolabs. ^{32}P-labeled nucleotides, ^{35}S-labeled nucleotides, and [^{14}C]chloramphenicol were purchased from New England Nuclear. *Nicotiana tabacum* cv. Xanthi was maintained on a Murashige and Skoog (14) agar medium as sterile shoot cultures.

Screening of a Library of Potato Genes for Inhibitor II Genes. A Russet Burbank genomic library was a gift of David Anderson (Phytogen, Pasadena, CA). *Escherichia coli* strain K802 (*hsr⁻, hsm⁺, lac⁻, gal⁻, met⁻*) was used as the host. About 3.5×10^5 plaques were screened by using nick-translated tomato inhibitor II cDNA as a probe. Nitrocellulose plaque lifts of the library were prehybridized at 55°C in a solution containing 5× Denhardt's solution, 5× SSPE, 0.1% NaDodSO₄, denatured salmon sperm DNA (100 μg/ml), and poly(A)⁺ RNA (1 μg/ml) (15). For the hybridization step, 0.1 μg of boiled nick-translated inhibitor II cDNA was added to the mixture and incubated at 55°C for 16 hr. Methods for washing, drying, and autoradiography of blots were performed as described (15). Plaques containing DNA that hybridized strongly with the probe were selected, amplified, and screened three additional times to ensure purity of the clone. DNA was isolated from the amplified clones for restriction nuclease digestion, Southern analysis, and subcloning of restriction fragments.

DNA Sequence Determination. Selected restriction fragments obtained from a 2.6-kbp *Taq* I insert were cloned into various sites within M13 bacteriophage. Conditions for cloning, transformation, propagation of M13, isolation of both replicative form and single-stranded DNA, and dideoxy sequencing were by published procedures (16, 17).

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Analyses of restriction fragments were carried out according to the method of Southern (18). After restriction endonuclease digestion, DNA samples were separated on agarose gels, stained with ethidium bromide, denatured, neutralized, and transferred to GeneScreen membranes. Prehybridization solution contains 10% dextran sulfate, 50% deionized formamide, 1% NaDodSO₄, and 1 M NaCl. Hybridization was conducted in this same solution for 16 hr, following addition of boiled nick-translated inhibitor II cDNA (12) and 100 μl of salmon sperm carrier DNA (10 mg/ml). The membranes were twice washed in 2× SSC at room temperature, then in 2× SSC/1% NaDodSO₄ for 30 min at 55°C. Autoradiography was performed for various times using a calcium tungstate intensifier screen.

Transformation of Tobacco Tissues. Plasmids constructed in *E. coli* were transferred into *Agrobacterium tumefaciens* PC2760 containing the helper Ti plasmid pAL4404 (19) by the triparental mating method (20). The structure of the transferred plasmids was analyzed after mating back to *E. coli*. *Agrobacterium* cells carrying both the helper Ti plasmid and the binary vector containing the inhibitor IIK–CAT fusion were cocultured for 2 days with leaf slices from sterile tobacco plants (21). The bacterial cells were washed away and transformed tobacco calli were selected on a Murashige–Skoog agar medium containing 3% sucrose, kanamycin (200 mg/liter), cefotaxime (250 mg/liter), and an appropriate amount of phytohormone (naphthalene acetic acid at 2 mg/liter and benzyladenine at 0.5 mg/liter for callus induction; benzyladenine at 0.5 mg/liter for shoot induction). The cocultivated plant cells were incubated at 28°C under light (3000 lux) for 12 hr/day. Transformed tobacco tissues were visualized within 3–4 weeks after cocultivation. The tissues were transferred to another medium containing exactly the same ingredients and were further incubated as described above. Plants were regenerated on Murashige–Skoog agar medium containing the same concentration of sucrose and antibiotics but lacking the phytohormones. Regenerated plants were transferred to pots and grown in a greenhouse under natural light supplemented with artificial light.

Assays for Wound Inducibility of the CAT Gene. Transformed tobacco plants (20–30 cm tall) were used to assay for the wound-induced expression of CAT activity (22). Extracts from tobacco leaves were prepared by grinding the leaves with a small mortar and pestle and expressing the juice from the debris by pressing with the pestle. The juice was centrifuged for 2 min at 10,000 × *g*, and the clear supernatant was recovered for protein analysis by the method of Bradford (23). A quantity from each extract containing 100 μg of protein was assayed for CAT activity as described, using [¹⁴C]chloramphenicol as substrate (22).

RESULTS AND DISCUSSION

Potato inhibitor II genes were obtained from a library of potato genes in Charon 4 phage by plaque hybridization using nick-translated tomato inhibitor II cDNA (12) as a hybridization probe. Approximately 3.5 × 10⁵ plaques were screened at a density of 50,000 plaques per 150-mm Petri plate. Twenty-nine positive plaques that hybridized with the cDNA probe were subsequently rescreened at a lower density.

A clone called inhibitor IIK containing an 8-kbp *Eco*RI insert hybridized most strongly with the wound-induced cDNA probe and was isolated and characterized with restriction enzymes. A 2.6-kbp *Taq* I fragment was subjected to further restriction analysis and sequencing. Fig. 1 shows a restriction map of this insert containing the inhibitor IIK gene. The strategy for sequencing the open reading frame and flanking sequences is also shown.

The nucleotide sequence of the potato inhibitor IIK gene and its coded protein is shown in Fig. 2. The gene is similar to an inhibitor II gene recently isolated from the diploid potato line HH80 12017 (24). The inhibitor II open reading frame is composed of two exons separated by a 117-bp intervening sequence. The intron–exon border sequences are typical of those found in other plants and in animals. The intervening sequence is A+T-rich, the two nucleotides accounting for 98 of the 117 bases (84%). Potato inhibitor IIK protein, like the tomato inhibitor II protein (12), consists of two domains (residues 31–87 and 89–153) that apparently have evolved through gene duplication–elongation events.

In the 5' region of the gene, the putative regulatory sequence TATAA is located 72 bp upstream from the initiation codon and 24 bp upstream from the transcription start, assumed by comparison with tomato inhibitor II cap site (9) determined by primer extension experiments, at nucleotide 258 (heavy arrow, Fig. 2).

Vector Constructions. The isolation and characterization of the proteinase inhibitor IIK gene from potatoes provided the opportunity to fuse the potential regulatory regions of this apparently wound-inducible gene to the open reading frame of the CAT gene to test the capabilities of the promoter and terminator to express CAT under wound-inducible control. An *Sca* I site in the inhibitor II gene (Fig. 1), 18 bases upstream from the translation initiation codon and 30 bases downstream from the putative transcription start site, was
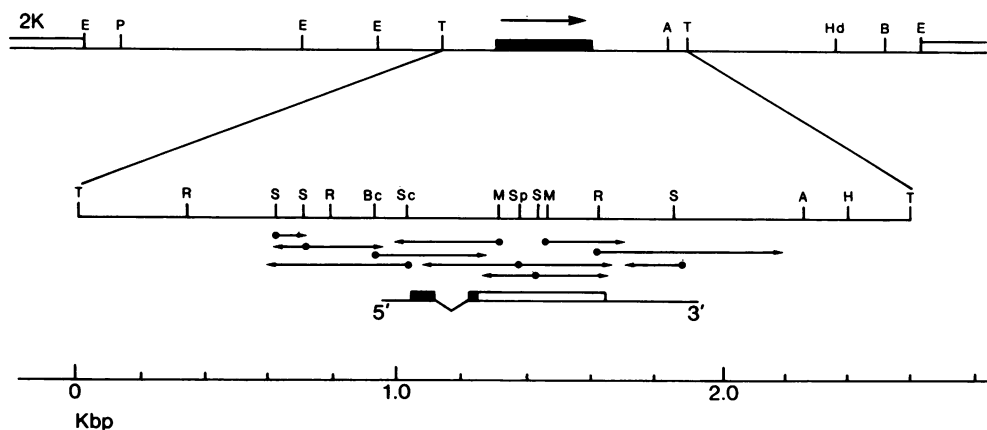
FIG. 1. Restriction map of a 2.6-kbp fragment (pRT8) containing the inhibitor IIK gene, obtained from an 8-kbp insert from a potato genomic library in phage. The sequencing strategy for the structural gene and its flanking regions is shown below. Subclones were prepared in M13 vectors for sequencing, and arrows indicate the direction and the extent of the sequencing. E, *Eco*RI; P, *Pst* I; B, *Bam*HI; Hd, *Hin*dIII; A, *Acc* I; T, *Taq* I; R, *Rsa* I; S, *Sau*3A; Bc, *Bcl* I; Sc, *Sca* I; M, *Msp* I; Sp, *Sph* I; H, *Hae* III.

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                                                                                          100
GATCTTTGAAATTAGACAAGTATTATCGGACATCTACTTTTAGTATAGTA AACAAAGTAAAGATCGATAAAGAGAGTAATAAAGAAGAAGCAAGCGTAAG

                                                                                          200
TACCTTGCCAAAATAATTAACTAACAAGCACATCTTTTTTTTTTTATCAAA TATTAATAAAAATAATTTATATTAATATGAAGAAAAAAAAGGTTTTAGTT
                                                                     ↓
                                                                                          300
TGCTATCTTTTTGATCACTCGTTTGCTATAAATAGGTGGAGGAGGACAGA CACTCTTCACCCCAAAATTAAAAGAAAAAGAGGCAGTACTAATTAATTAT

                                                                                          381
CCATC ATG GAT GTT CAC AAG GAA GTT AAT TTC GTT GCT TAC CTA CTA ATT GTT CTT G  GTAAGATTTTCCTTTACTCCTTTT
      Met Asp Val His Lys Glu Val Asn Phe Val Ala Tyr Leu Leu Ile Val Leu G--
                                                                10
                                                                                          479
TTTTTTTTTTAAAAAAAAAAATTCTTGTTTATACATATATATATATATACA CAAGTAGTTTTATATTTTTCCTTTATATTATATTTGTTTGTAG GA TTA
                                                                                             -ly Leu

                                                                                          554
    TTG GTA CTT GTA AGC GCG ATG GAT GTT GAT GCG AAG GCT TGC ATT AGA GAA TGT GGT AAT CTT GGG TTT GGG ATA
    Leu Val Leu Val Ser Ala Met Asp Val Asp Ala Lys Ala Cys Ile Arg Glu Cys Gly Asn Leu Gly Phe Gly Ile
    20                                             30                                40
                                                                                          629
    TGC CCA CGT TCA GAA GGA AGT CCG GAA AAT· CCG ATA TGC ACC AAC TGT TGT GCA GGT TAT AAA GGT TGC AAT TAT
    Cys Pro Arg Ser Glu Gly Ser Pro Glu Asn Pro Ile Cys Thr Asn Cys Cys Ala Gly Tyr Lys Gly Cys Asn Tyr
                             50                                60
                                                                                          704
    TAT AGT GCA AAT GGG GCT TTC ATT TGT GAA GGA CAA TCT GAC CCA AAA AAA CCA AAA GCA TGC CCC CTA AAT TGC
    Tyr Ser Ala Asn Gly Ala Phe Ile Cys Glu Gly Gln Ser Asp Pro Lys Lys Pro Lys Ala Cys Pro Leu Asn Cys
    70                                 80                                      90
                                                                                          779
    GAT CCA CAT ATT GCC TAC TCA AAG TGT CCC CGT TCA GAA GGA AAA TCG CTA ATT TAT CCC ACC GGA TGT ACC ACA
    Asp Pro His Ile Ala Tyr Ser Lys Cys Pro Arg Ser Glu Gly Lys Ser Leu Ile Tyr Pro Thr Gly Cys Thr Thr
                     100                                   110
                                                                                          854
    TGC TGC ACA GGG TAC AAG GGT TGC TAC TAT TTC GGT AAA AAT GGC AAG TTT GTA TGT GAA GGA GAG AGT GAT GAG
    Cys Cys Thr Gly Tyr Lys Gly Cys Tyr Tyr Phe Gly Lys Asn Gly Lys Phe Val Cys Glu Gly Glu Ser Asp Glu
                             130                                      140

                                                                                          947
    CCC AAG GCA AAT ATG TAC CCT GCA ATG TGA CCCTAGACTT GTCCATCTTCTGGATTGGCCAAGTTAATTAATGTATGAAATAAAAGGAT
    Pro Lys Ala Asn Met Tyr Pro Ala Met ***
                     150

                                                                                          1047
GCACACATAGTGACATGCTAATCACTATAATGTGGGCATCAAAGTTGTGT GTTATGTGTAATAACTAATTATCTGAATAAGAGAAAGAGAGATCATCCAT
                                                                                             ↓
                                                                                          1147
ATTTCTTATCCTAAATGAATGACAGTGTCTTTATAATTCTTTGATGAACA GATGCATTTTATTAACCAATTCCATATACATATAAATATTAATCATATAT

                                                                                          1245
AATTAATATCAATTGGTTAGCAAAACCCAAATCTAGTCTAGGTGTGTTTT GCTAATTATGGGGGATAGAGCAAAAAAGAAACTAACGTCTCAAGAATC
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FIG. 2. Nucleotide sequence of a 1.24-kbp fragment of the inhibitor IIK gene and flanking sequences. The TATA box and polyadenylylation signal AATAAG are underlined. Large arrow indicates the site of transcription initiation; small arrow indicates the site of polyadenylylation in the homologous tomato inhibitor II cDNA sequences. The numbering of the amino acids begins at the NH₂ terminus of the transit sequence. The nascent protein is assumed to be processed during or after synthesis between amino acid residues 30 and 31 to produce the mature inhibitor.

used to obtain a 1000-bp fragment of the 5' flanking regions of the gene for the vector construct. An *Rsa* I site 11 bp upstream from the termination codon of the inhibitor IIK gene provided a 3' fragment of ≈1000 bp that could also be used for the construction of the fused gene. The resulting construction, called pRT45 (Fig. 3 *Lower*), eliminated almost all of the open reading frame of inhibitor IIK gene from potential translation, including the signal or transit sequence and the 117-bp intervening sequence. In Fig. 3 (*Upper*) are shown the essential components of the inhibitor IIK–CAT fused gene and its position in the transformation vector pRT45.

The strategy for the construction was as follows: the 2.6-kbp *Taq* I fragment of a plasmid pRT8 (Fig. 1), containing the entire inhibitor IIK gene with the aforementioned 3' and 5' sequences, was purified from low melting agarose, then digested with *Msp* I, and the 1.35-kbp *Taq* I/*Msp* I fragment containing the 5' end of inhibitor IIK was recovered and ligated into the *Acc* I site of pUC13. The insert was removed with *Pst* I (in the multilinker) and *Sca* I and ligated into a *Pst* I/*Hind*II site of pUC13 to give plasmid pRT24. Sequencing of both ends verified that this clone contained the 5' end of the inhibitor IIK gene terminating 18 bp upstream from the ATG start codon. A 782-bp *Bgl* II/*Bam*HI fragment, containing the entire coding region of the CAT gene, was isolated from pGA425 (15) and inserted into the unique *Bam*HI site in the plasmid pRT24 at the downstream terminus of the inhibitor IIK promoter. A 1000-bp *Rsa* I fragment containing the 3' flanking region of the inhibitor IIK gene and 11 bp of the open reading frame with the TAA stop signal was inserted at the 3'

end of the CAT gene to form pRT41. pRT41 was cloned into the *Hind*III site of pGA482 (13), a binary Ti plasmid vector, to produce pRT45 to deliver the chimeric gene to plants.

The sequence of the chimeric gene at the 5' fusion (data not shown) demonstrated that the inhibitor IIK open reading frame plus 18 bp of the 5' untranslated region had been replaced with the CAT coding region and 42 bp of its own 5' untranslated region. This construction brought the translation initiation codon of the CAT gene to a position 80 bp downstream from the transcription initiation site as compared to 47 bp in the intact inhibitor IIK gene. The presence of the termination region of the inhibitor II gene in pRT41 was shown by Southern hybridization.

A second construction was identical to the pRT45 except that the 3' region of the inhibitor II gene in the construct was replaced with the terminator sequence of the 6b gene of pTiA6. In this construct, the plasmid pRT24 containing the 5' region of inhibitor II was inserted in pGA492 (13), which is a binary transformation vector containing the open reading coding region of CAT with the 6b terminator from the Ti plasmid. This vector has a polylinker site at the 5' terminus of the CAT coding region for cloning in selected promoter sequences. The 1000-bp inhibitor II 5' region was cloned into a *Bgl* II site of pGA492 to provide the identical fusion between the inhibitor II promoter and the CAT gene as found in pRT45. Therefore, the only difference in gene fusions between pRT45 and pRT50 was in the terminator region.

The two constructs pRT45 and pRT50 (Fig. 3) provided opportunity to directly compare the effects of the two
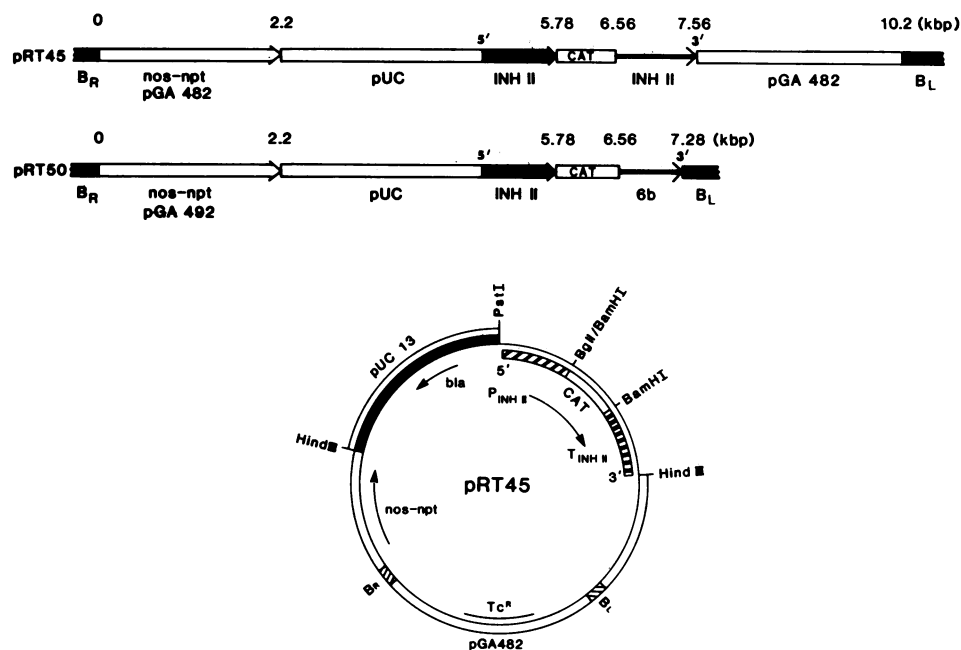
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FIG. 3. (*Upper*) Constructs in the binary Ti vector containing 1000 bp of inhibitor IIK (INH II) 5' region beginning 18 bp upstream from the translation initiation codon and 1000 bp of the inhibitor IIK 3' beginning 11 bp upstream from the translation termination codon, fused with the open reading frame of the CAT gene (pRT45), and a similar construct containing the 5' region of the inhibitor IIK gene and the CAT open reading frame but with the *6b* terminator of the Ti plasmid A6 in place of the inhibitor IIK terminator (pRK50). (*Lower*) A circular map of pRT45. Hatched regions are from the inhibitor IIK gene and solid region is from pUC13. B$_L$, T-DNA left border; B$_R$, T-DNA right border; nos-npt, a chimeric *nos–npt* fusion for a plant selectable marker; bla, β-lactamase gene; Tc^R, tetracycline resistance; T$_{INH II}$, inhibitor IIK terminator; P$_{INH II}$, inhibitor IIK promoter.

terminators on the levels of expression of CAT regulated by the inhibitor IIK 5' region.

Transformation and Wound Induction of CAT Expression. Tobacco leaf tissue was transformed with the plasmid vector by the cocultivation method described by An *et al.* (21). Transformed callus tissue, selected using kanamycin resistance, did not exhibit any detectable expression of the CAT gene. Over 80 plantlets were regenerated from the transformed callus cells and grown in sterile culture. All of the plantlets tested exhibited a very low CAT expression. The regenerated plants were grown in a greenhouse to a height of 20–30 cm and tested for wound inducibility of the CAT gene.

In Fig. 4 is shown the wound induction of CAT expression in transformed tobacco plants. In each experiment, the leaf at the position designated in the drawing was wounded by crushing the tissue between a flat file and a rubber stopper. Twenty-four hours later, the wounded leaf was assayed, as were unwounded leaves just above and below the wounded leaf. Although the response varied among transformants, it is clear that the CAT gene is expressed in response to injury. In some severely wounded plants, a response was noted in the adjacent upper unwounded leaf, indicating that a systemic wound signal was activating CAT expression several centimeters away from the wound site in the same manner that the proteinase inhibitors are induced in tomato, potato, and alfalfa plants (1–4). As expected, little or no CAT expression was found in leaves below the wounded leaf, since the wound signal PIIF (proteinase inhibitor inducing factor) is transported primarily upward (25).

To further examine the direction and extent of the signal regulating CAT expression, leaves of another transformed plant were wounded at the middle of a leaf laterally to the main leaf vein. Twenty-four hours after wounding, leaf sections from the tip, middle (wounded), and basal regions were assayed for CAT activity. Sections of the adjacent upper and lower leaf were also assayed. CAT activity was induced most strongly near the wound site and was systemically induced more toward the tip of the leaf than toward the

base. No response was found in the adjacent upper or lower leaves of the plant; but the wounding in this experiment was
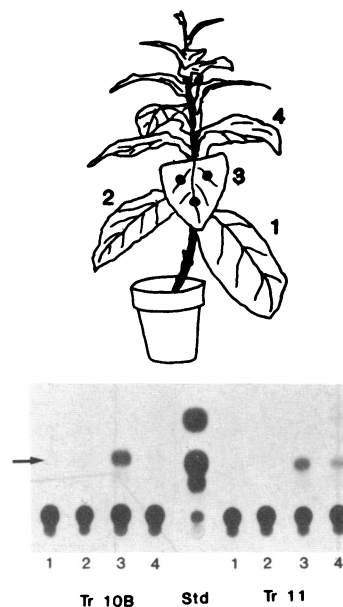

FIG. 4. Wound-induced expression of the CAT gene in transgenic tobacco plants transformed with pRT45. Regenerated plants were 20–30 cm tall when assayed for wound-inducible CAT activity. Two independently selected transformed tobacco calli were regenerated into plants (Tr 10B and Tr 11) and assayed. Numbers indicate leaf positions on the plants as shown. Leaf 1 was assayed for CAT prior to wounding. Leaves 2, 3, and 4 were assayed 24 hr after wounding leaf 3 by crushing leaf tissue on several locations between a rubber stopper and a flat file. Assays show autoradiography of the conversion of [^{14}C]chloramphenicol to [^{14}C]chloramphenicol acetates by 100 μl of total leaf extract. Arrow points to 3-acetylchloramphenicol, the major product of the CAT enzyme. *E. coli* CAT protein was used as a standard (Std).

not as severe as the experiment shown in Fig. 4 and may have limited the magnitude of the wound signal. The levels of CAT activity in all of the experiments were <10% of that expressed when it is under constitutive regulation by the nopaline synthase (*nos*) promoter (26). This indicates that the efficiency of the wound induction may be limited by some factors that we do not yet understand. We have now assayed >50 transgenic plants derived from 10 independent calli transformed with pRT45 that have averaged a 7.85-fold increase in CAT activity over noninduced plants. The plants all exhibited a low level of CAT activity without wounding, indicating that a low constitutive expression of the gene is occurring in the transformed tobacco.

In contrast to the wound inducibility of CAT using the pRT45 constructs, the pRT50 constructs containing the 6b terminator were not wound inducible. Assays of 70 plants from 15 different selections of calli transformed with pRT50 indicated that little or no wound inducibility was present. A few of the plants showed a low level of wound induction, but overall the activity was negligible. The plants did, however, exhibit a low level of constitutive expression even though the wound inducibility was absent.

A time course of CAT activity following wound induction is shown in Fig. 5 for selected pRT45 and pRT50 transformants. Several wounds were inflicted on three different transformed plants and CAT activity was assayed in the wounded leaves at various intervals for 24 hr. The time course for induction of CAT is very similar to the wound induction of proteinase inhibitors and its mRNA in severely wounded tomato leaves (27), increasing for 12–16 hr and ceasing or declining thereafter. Again, in the case of pRT50 transformants, CAT was not significantly induced.

Several conclusions can be deduced from the experiments reported here. (*i*) The nucleotide sequences necessary and sufficient for wound induction are present in the pRT45 constructs; (*ii*) cellular components are present in tobacco leaves that can regulate wound induction both locally and systemically with a time course of induction of CAT activity that resembles the induction of proteinase inhibitor induction in tomato leaves; (*iii*) the 3' region of inhibitor II appears to contain sequences or structures necessary for the wound inducibility of CAT activity, (*iv*) the level of wound-inducible CAT expression by the potato inhibitor II control regions is significantly lower (<10%) than that by the *nos* promoter. This low activity may reflect the inefficiency of the potato inhibitor II control regions in tobacco; it may indicate a requirement for components of the potato inhibitor structural

gene open reading frame for maximal wound induction; or it may reflect the stability of the chimeric gene products. Transformation of tomato with the intact gene may provide information concerning this question.

As yet, the only sequence similarity found within 3' regions among wound-inducible inhibitor I and II genes from tomato and potato has been a short palindromic sequence CAT-TATAATG 30–40 bases upstream from the polyadenylylation signal. This similarity was first noted by Graham *et al.* (12) in the wound-inducible cDNAs isolated from tomato leaves. The sequence is not present in the 6b terminator and therefore is not present in the pRT50 construct, which is not wound inducible.

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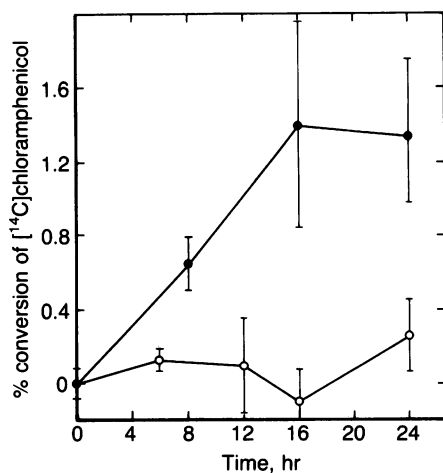27. Graham, J., Hall, G., Pearce, G. & Ryan, C. A. (1986) *Planta*, in press.

FIG. 5. Time course of the increase in CAT activity in leaves of transgenic tobacco plants transformed with constructs pRT45 (●) or pRT50 (○) after wounding at time 0. Results are shown as mean ± SEM.