

T7 RNA Polymerase Is Expressed in Plants in a Nicked but Active Form

Miguel A. Caviedes, Sanggyu Park^{1*} and Robert W. Thornburg²

Department of Microbiology & Parasitology, University of Sevilla, Spain

¹*Department of Agricultural Chemistry, Taegu University, Taegu 713-714, Korea*

²*Department of Biochemistry & Biophysics, Iowa State University, U.S.A.*

Abstract : We have prepared several chimeric constructs containing the bacteriophage T7 RNA polymerase gene under control of the wound-inducible potato proteinase inhibitor II (*pin2*) promoter and have transformed *Nicotiana tabacum* plants with these constructs. Southern blot analyses indicate that either one or two copies of the gene constructs are present in the transgenic plants. Northern blot analyses indicate that mRNA encoding T7 RNA polymerase is expressed in a wound-inducible manner. We purified T7 RNA polymerase and prepared antiserum. This antiserum was used for Western blot analyses to demonstrate that a protein which is cross reactive with T7 RNA polymerase is produced. The molecular mass of this protein is 80 kDa, a size which is consistent with the nicked form of the polymerase as is often seen when expressed in *E. coli*. RNA polymerase assays were used to indicate that the nicked form of T7 RNA polymerase is active and capable of incorporating labeled nucleotides into transcripts *in vitro*. Analysis of transgenic plants did indeed show that wound-inducible activation of the T7 RNA polymerase permits the establishment of a genetic system to overexpress genes in plants using T7 RNA polymerase (Received March 20, 1997; accepted May 2, 1997)

Introduction

This work is a study designed to test the feasibility of expressing a novel bacterial regulatory system in plant cells. The bacteriophage T7 RNA polymerase system is becoming the most widely used method of overexpression of genes in *E. coli*.¹⁾ The great utility of this system arises from the fact that the T7 RNA polymerase can be expressed at moderate levels under control of the inducible *lacZ* promoter. The T7 RNA polymerase then recognizes and initiates transcription from a unique 23 nucleotides (nt) promoter. This dual expression system results in high level expression of proteins cloned under the control of 23 nt T7 promoter. The utility of this system for bacterial expression of genes has led to the adoption of this expression method to *S. cerevisiae*²⁾ and mammalian systems^{3,4)}. The studies in *S. cerevisiae* demonstrated the utility of the T7 RNA polymerase system in eukaryotes. In these studies a nuclear localization sequence was incorporated into the T7 RNA polymerase and following transformation, the modified T7 RNA polymerase was shown to localize in the nucleus and could initiate transcription from the T7 promoter of chimeric constructs located either on the chromosome or plasmids. The nuclear localization sequences

also function to direct the T7 RNA polymerase to nucleus of mammalian cells where the polymerase can transcribe stably transformed chimeric genes⁴⁾ or alternatively, the T7 RNA polymerase can also direct transcription in the cytoplasm if chimeric genes are expressed from vaccinia virus vectors which replicate in the cytoplasm.⁵⁾

In addition, bacteriophage T7 RNA polymerase has also been expressed in transgenic tobacco.⁶⁾ In the study the T7 RNA polymerase was shown to be expressed and localized to the nucleus of plant cells. However, these studies did not show whether the T7 RNA polymerase was active. Therefore we have evaluated the expression and activity of the bacteriophage T7 RNA polymerase in transgenic tobacco. We have utilized the wound-inducible potato proteinase inhibitor II promoter⁷⁾ for the expression of T7 RNA polymerase because the level of expression and the mechanism of regulation make this gene ideal for regulating plant transcripts in a defined, inducible manner.

Materials and Methods

Materials

Radiolabeled materials, GeneScreen[®] and GeneScreen

Key words : proteinase inhibitor II (*pin2*), T7 RNA polymerase, wound-inducible expression

*Corresponding author

Plus®, were obtained from Dupont and New England Nuclear. Nitrocellulose was obtained from Micron Separations. Restriction endonucleases were obtained from New England Biolabs, Promega or Stratagene and were used according to the manufacturer's directions. Kits for DNA radiolabeling were from Amersham and those for *in vitro* RNA transcription were from Stratagene.

Kanamycin, hygromycin, rifampicin, tetracycline, carbenicillin and ampicillin were obtained from Sigma. Cefotaxime was obtained from a local veterinary clinic. All other materials were of the highest purity available and were obtained from local sources.

The *Nicotiana tabacum* cv Xanthi plants used for all transformations were a clonal line established in 1983 at Washington State University. Seeds from transgenic

plants were surface sterilized in a 10% (v/v) solution of commercial bleach and rinsed in numerous changes of sterile water for about 15 minutes. Seeds were then placed on MS agar⁸⁾ containing kanamycin (200 mg/l) in a growth chamber until the plants were approximately 10 cm tall. The plants were then transferred to soil and grown in the greenhouse.

DNA Manipulations and Transformation

Procedures for DNA manipulations were standard methods^{9,10)} except for the use of a 5'/3' *Bgl*III/*Pst*I adaptor used in the construction of pRT99 (See Fig. 1). This oligonucleotide, 5'-GATCTCGA-3', was synthesized at the Iowa State University Nucleic Acid Facility. This adaptor is complementary to both the 5' overhang of *Bgl*III and the 3' overhang of *Pst*I and per-

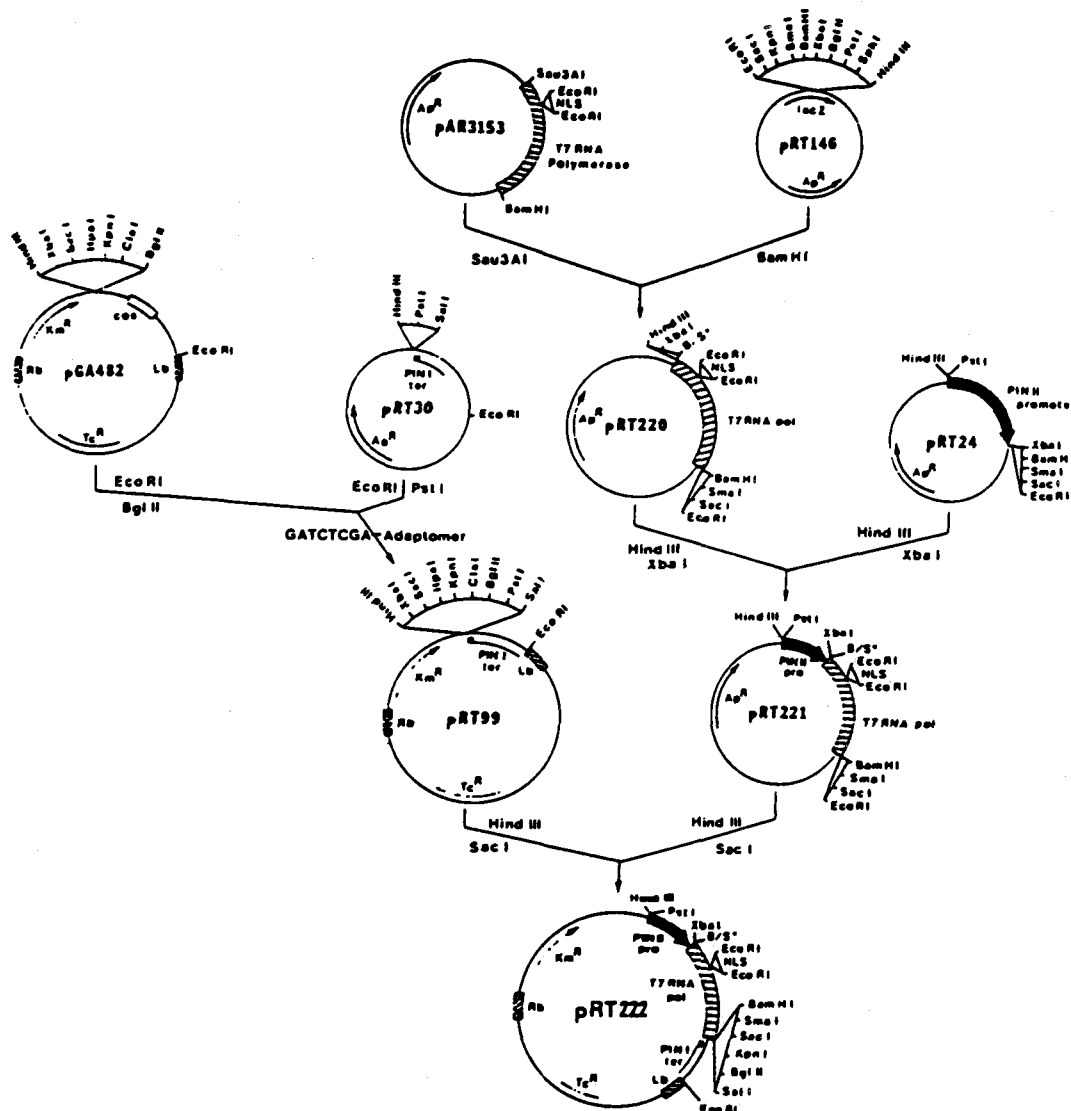


Fig. 1. Preparation of the *pin2*-T7 RNA polymerase construct. PINII pro, promoter of the potato proteinase inhibitor II (*pin2*) gene; PIN I ter, terminator of the potato proteinase inhibitor I (*pin1*) gene; T7 RNA pol, bacteriophage T7 RNA polymerase gene; NLS, nuclear localization signal from SV40 Large T antigen; Rb and Lb, right border and left border of the *Agrobacterium* T-DNA region, respectively; *Ap^R* and *Tc^R* denote bacterial selectable markers for ampicillin and tetracycline resistance, respectively; *Km^R* denotes a nopaline synthase-NPTII chimeric construction that is functional as a kanamycin selectable marker in plants.

mits the two ends to be joined. Routinely, 4 ng of adaptomer is added to a 25 or 50 ml ligation reaction. We have successfully used such adaptomers on several occasions for ligating 3' and 5' digested ends.

DNA constructs were mated into *Agrobacterium tumefaciens* LBA4404 as described.¹¹⁾ These *Agrobacterium* strains were used to transform *Nicotiana tabacum* to kanamycin resistance was performed as previously described.^{7,12)} Selection was performed on 200 mg/l kanamycin.

Blotting

DNA was isolated from transgenic plants by the methods of Dellaporta *et al.*¹³⁾ Southern blots were carried out using GeneScreen® Plus and hybridization was done with labeled DNA probes according to the manufacturer's directions.

RNA was isolated by the method of Wadsworth *et al.*¹⁴⁾ Northern blots were carried out¹⁰⁾ using GeneScreen® and hybridization was done with radiolabeled RNA transcripts.¹⁵⁾

Protein extracts were prepared from the foliage by grinding the foliage in a plastic bag with a hand-held tissue homogenizer. Total protein was quantitated by the method of Bradford.¹⁶⁾ Western blots were conducted as previously described¹⁷⁾ using 12% SDS PAGE and nitrocellulose as a support. After transfer the membranes were blocked with milk protein and probed with rabbit antiserum raised against purified T7 RNA polymerase protein. After washing away excess antiserum, the blot was probed with [¹²⁵I]-labeled protein A and exposed to a film.

Induction of *pin2* promoter

Induction of *pin2* promoter constructs in the foliage of the transgenic plants was performed in one of two ways, either with a pair of surgical hemostats as previously described¹⁸⁾ or by sucrose induction as described.^{19,20)}

T7 RNA polymerase assays

T7 RNA polymerase was assayed by a modification of the method of Ikeda and Richardson.²¹⁾ Briefly, plant extracts containing 100 mg of total protein were incubated in a reaction (total volume 25 ml) containing 15 mM Tris-HCl, pH 8.0, 3 mM MgCl₂, 0.7 mM spermidine, 18 mM NaCl, 300 mg of yeast tRNA, 1 unit of RNase Block (Stratagene), 300 mM GTP, 300 mM ATP, 300 mM CTP and 1 mCi [α -³²P]-UTP (800 mCi/mmol). After incubation at 37°C for 30 min, the entire reaction mixture was passed through a 1 ml Sephadex G-50 column equilibrated in 10 mM Tris-HCl, pH 8.0, 50 mM EDTA. The columns were eluted with 200 μ l aliquots of 10 mM Tris-HCl, pH 8.0, 50 mM EDTA. The radiolabeled nucleotides eluted in fraction number three.

Aliquots of this fraction were then spotted on silica gel thin layer chromatography plates and exposed to X-ray film for visualization.

Results and Discussion

The usefulness of adapting a bacterial gene expression system to function in plants was investigated. T7 RNA polymerase strategy might provide a unique method for the overproduction of transgenes in plants, as well as for the coordinate regulation of multiple genes in response to a single event. To evaluate the utility of T7 RNA polymerase system in plants, a series of chimeric constructions using the T7 RNA polymerase gene have been prepared under the control of the wound-inducible potato proteinase inhibitor II (*pin2*).

The construction of the plant transformation vector pRT222 is illustrated in Fig. 1. The plasmid pAR3153 was kindly provided by Dr. F. William Studier of Brookhaven National Laboratories. The 2.2 kb *Sau3AI* fragment bearing the T7 RNA polymerase gene containing a nuclear localization sequence was moved into the *BamHI* site of pRT146, a pUC19 derivative to make the vector pRT220. Next, the wound-inducible potato proteinase inhibitor II (*pin2*) promoter was isolated as a 0.9 kb *HindIII/XbaI* fragment and inserted into the *HindIII/XbaI* sites upstream of the T7 RNA polymerase gene to make the plasmid pRT221. The 3.1 kb *pin2*-T7 RNA polymerase gene was isolated as a *HindIII/SacI* fragment and inserted into the *HindIII/SacI* sites of the plant transformation vector pRT99 to make pRT222. The plasmid, pRT99, was synthesized from pGA482¹¹⁾ by inserting an *EcoRI/PstI* fragment of pRT30 [containing 2.0 kb terminator region of the potato proteinase inhibitor I (*pin1*) gene²²⁾] into the *BglIII/EcoRI* sites of pGA482 using an 8 bp adaptomer (5'-GATCTC-GA-3') to join the 5' overhang from the *PstI* site with the 3' overhang from the *BglIII* site.

The construct pRT222 was transferred into *Agrobacterium tumefaciens* LBA4404 and subsequently transformed *Nicotiana tabacum* to kanamycin resistance.

To demonstrate that the resulting plants were transgenic, these plants were characterized by Southern blot analysis. These analyses (Fig. 2) show that the gene constructs are present in the genome of the transformed plant lines. In addition, plant lines containing both one and two copies of the *pin2*-T7 RNA polymerase gene containing the nuclear localization sequence were produced. Because the *EcoRI* restriction enzyme cuts outside of the T-DNA, the number of bands present in these *EcoRI* digests provides direct evidence of the number of copies in the genome of that plant. According to the quantification analysis on the autoradiograph using a laser densitometer, the plant Tr

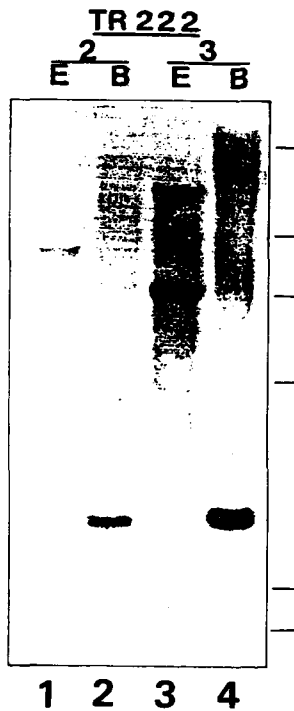


Fig. 2. Southern blot analysis of transformed *N. tabacum* lines containing the *pin2*-T7 RNA polymerase constructs. Ten micrograms of DNA were electrophoresed on a 1% agarose gel, the DNA was transferred to Genescreen[®] Plus and probed with ³²P-labeled T7 RNA polymerase gene. Lane 1 and 2 contain DNA from transformant 222 plant #2 (Tr222#2), lanes 3 and 4 contain DNA from transformant 222 plant #3 (Tr222#3). In lanes 1 and 3 the DNA was digested with *EcoRI*; in lanes 2 and 4 the DNA was digested with *BamHI*. *HindIII* cut lambda molecular markers were run with each of these gels. The lines to the right of each gel indicate where these markers run. Marker sizes are 23, 9.4, 6.6, 4.4, 2.3, and 2.0 kbp.

Tr222#2 contains one copy of the construct and the plant Tr222#3 contains two copies of the construct.

To evaluate whether the chimeric constructs are expressed in transgenic plants, the expression of the T7 RNA polymerase in these plants was examined by Northern blot analysis. As shown in Fig. 3, a mRNA band that hybridizes to radiolabeled T7 RNA polymerase was observed in the wound-induced leaves of Tr222#2 and Tr222#3 but not the uninduced leaves of these plants. The smaller sized band migrating just below the T7 RNA polymerase band shows up in all plant samples indicating this species might be expressed regardless of induction. The identity of this RNA is in question at present.

Because the Northern blots demonstrated that the T7 RNA polymerase mRNA is expressed in plants, the expression of the protein was observed in the Tr222 transgenic plants. Purified T7 RNA polymerase was used to prepare antiserum in rabbits²³⁾ and the resulting antiserum was used in Western blot analysis of these plants.

As shown in Fig. 4, an immunoreactive protein was

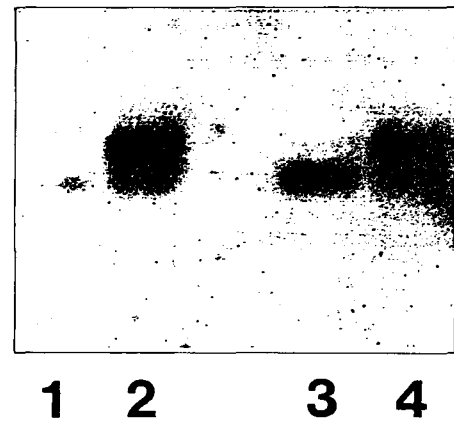


Fig. 3. Northern blot analysis of transformed *N. tabacum* lines Tr222#2 (lanes 1 and 2) and Tr222#3 (lanes 3 and 4). Lanes 1 and 3 were from uninduced leaves. Lanes 2 and 4 were from induced leaves by wounding with a pair of surgical hemostats. The leaf RNAs were isolated after overnight induction.

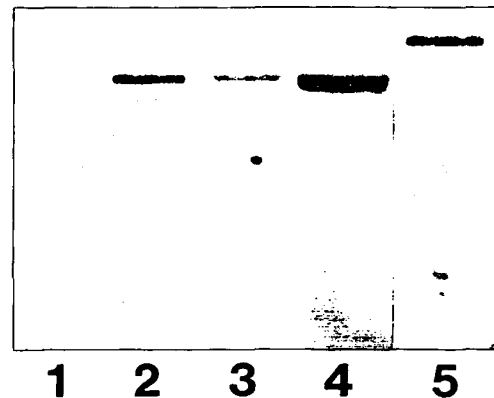


Fig. 4. Western blot analysis of transformed *N. tabacum* plants containing the T7 RNA polymerase gene. Leaves of transgenic plants, either Tr222#2 (lanes 1 and 2) or Tr222#3 (lanes 3 and 4), were removed from the plant and induced as described in the Fig. 3. In lanes 1 and 3 the leaves were homogenized prior to induction and in lanes 2 and 4, the leaves were homogenized after overnight induction. Lane 5 was T7 RNA polymerase expressed in *E. coli* BL21::DE3.

identified in extracts of these plants. Further, this band was induced to high levels following wounding or induction of the leaf tissues with sucrose (compare lanes 1 and 2, Tr222#2; and lanes 3 and 4, Tr222#3). Comparison of lanes 2 and 4 indicate that doubling the copy number of the wound-inducible *pin2*-T7 RNA polymerase gene in the plants, increase the level of expression of the T7 RNA polymerase.

Our initial observations indicated that the molecular mass of this protein was only 80 kDa instead of the expected 98 kDa. This result indicates that the pRT222 construct might have inadvertently acquired a translation stop codon during the subcloning process. To evaluate this possibility, the polymerase gene from the *pin2*-T7 RNA polymerase construct, pRT222, was subcloned into pBluescript(SK+) and expressed in *E. coli* BL21::DE3. As shown in lane 5 of Fig. 4, the molecular

mass of the resultant protein is the expected 98 kDa indicating that the T7 RNA polymerase gene had not acquired a stop codon in the sequence during the cloning process. Thus, the smaller sized protein produced in the plants must arise from some other mechanism.

One clue to elucidate this problem was found in the work of Ikeda and Richardson²¹⁾ which indicates that the T7 RNA polymerase has a site 172 amino acids from the C-terminus that is readily cleaved by proteases. However, the resulting two subunits do not disassociate under normal conditions and the nicked enzyme remains active. This nicked form of the polymerase shows bands on SDS PAGE of 80 kDa and 23 kDa. The size of the large fragment is identical to the size we observed for the protein produced T7 RNA polymerase and thus, the T7 RNA polymerase produced in tobacco is a nicked form of the enzyme. Apparently, the smaller fragment runs off our gels, or is not immuno-cross reactive with our anti-T7 RNA polymerase antibody.

Thus Western blot analysis demonstrates that the T7 RNA polymerase is expressed in nicked form in a wound-inducible manner. As with other *pin2* gene constructs,^{19,24)} these constructs are also induced by sucrose.

Because the plants produced the nicked form of the T7 RNA polymerase they were examined whether they produced enzyme containing RNA polymerase activity. As shown in Fig. 5, labeled UTP can indeed be incorporated into new transcripts driven from the T7 promoter. Moreover, this incorporation of radioactivity into transcripts is inducible (compare lanes 3 and 4 for Tr222#2 plants and lanes 5 and 6 for Tr222#3 plants). Thus, the T7 RNA polymerase was produced in an inducible manner, however, the size of the protein indicated that the protein is proteolytically nicked but does indeed retain enzyme activity. The nicked form of T7 RNA polymerase has also been observed in *E. coli*.²¹⁾ Because the nicked form of polymerase retains some activity in *E. coli*, we therefore, verified that the transgenic plants produced inducible RNA polymerase activity. The verification that transgenic plants did indeed show wound-inducible activation of the T7 RNA polymerase permits the establishment of a genetic system to overexpress genes in plants using the T7 RNA polymerase.

We are presently exploring the use of reporter constructs harboring β -glucuronidase (GUS) and chloramphenicol acetyl transferase (CAT) genes under the control of a bacteriophage T7 promoter. We anticipate that a system which utilizes the T7 RNA polymerase to drive expression of reporter genes will have widespread use in the expression of genes in plants. This system could be used for expressing transgenes at high levels or alternatively expressing multiple transgenes under the coordinate control of a single promoter, in our case the potato proteinase inhibitor II promoter. Our experimental

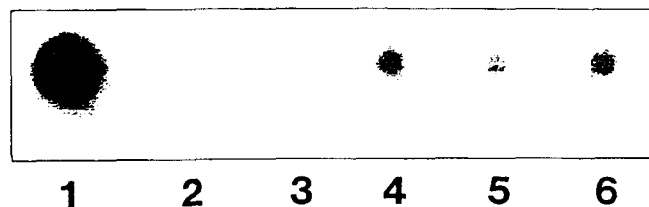


Fig. 5. T7 RNA polymerase assays from Tr222 plant extracts. Assays were performed by a published method using 1 mCi α -[³²P]-UTP (800 mCi/mmol). Lanes 1 and 2 are control lanes which contain 1 unit of added T7 RNA polymerase and no addition, respectively. Leaves of transgenic plants, either Tr222#2 (lanes 3 and 4) or Tr222#3 (lanes 5 and 6), were removed from the plant and induced with 3% sucrose. Lanes 3 and 5 are from uninduced leaves and lanes 4 and 6 are from induced leaves. Leaves were homogenized after overnight induction.

design is to develop secondary reporter genes under the control of the bacteriophage T7 promoter which utilize a second transformation marker (hygromycin). Thus, individual transformant can be prepared and genetic crosses can be to combine the transgenes

Acknowledgments

The authors would like to thank Drs. Basil Nikolau and Alan Myers for informative discussions and Mrs. Mahmoudian, H., Ward, M., McDermott, J., and Minion, F. C. for technical assistance. Mr. Eberson Calvo from Dr. Randy Shoemakers's laboratory provided the purified T7 RNA polymerase. The authors would like to thank the American Soybean Association and NATO for support. Dr. Miguel Caviedes gratefully acknowledges Research Fellowships received from Junta de Andalucia, Ministerio de Educacion y Ciencia and NATO.

References

1. Rosenberg, A. H., Lade, B. N., Chui, D.-S., Lin, S.-W., Dunn, J. J. and Studier, F. W. (1987) Vectors for selective expression of cloned DNAs by T7 RNA polymerase., *Gene*, **56**, 125-135.
2. Benton, B. M., Eng, W.-K., Dunn, J. J., Studier, F. W., Sternglanz, R. and Fisher, P. A. (1990) Single-mediated import of bacteriophage T7 RNA polymerase into the *Saccharomyces cerevisiae* nucleus and specific transcription of target genes., *Mol. Cell. Biol.* **10**, 353-360.
3. Dunn, J. J., Dripll, B., Bernstein, K. E., Westphal, H. and Studier, F. W. (1988) Targeting bacteriophage T7 RNA polymerase to the mammalian cell nucleus., *Gene*, **68**, 259-266.
4. Lieber, A., Kiessling, U. and Strauss, M. (1989) High level gene expression in mammalian cells by a nuclear T7 phage RNA polymerase., *Nucl. Acids Res.*, **17**, 8485-8493.
5. Fuerst, T. R., Niles, E. G., Studier, W. and Moss, B. (1986) Eukaryotic transient-expression system based on

- recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase., *Proc. Natl. Acad. Sci. USA.*, **83**, 8122-8126.
6. Lassner, M. W., Jones, A., Daubert, S. and Comai, L. (1991) Targeting of T7 RNA polymerase to tobacco nuclei mediated by an SV40 nuclear location signal., *Plant Mol. Biol.*, **17**, 229-234.
 7. Thornburg, R. W., An, G., Cleveland, T. E., Johnson, R., and Ryan, C. A. (1987) Wound-inducible expression of a potato proteinase inhibitor II-chloramphenicol acetyltransferase gene fusion in transgenic tobacco plants. *Proc. Natl. Acad. Sci. USA.* **84**, 744-748.
 8. Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures., *Physiol. Plant.*, **15**, 473-497.
 9. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
 10. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Smith, J. A., Seidman, J. G. and Struhl, K. (1987) in 'Current Protocols in Molecular Biology'. John Wiley & Sons, New York
 11. An, G. (1987) Binary vectors, *Methods in Enzymol.*, **153**, 292-305.
 12. Waldron, C., Murphey, E. B., Roberts, J. L., Gufstafson, G. D., Armor, S. L. and Malcolm, S. K. (1985) resistance to hygromycin B: A new marker for plant transformation studies., *Plant Mol. Biol.*, **5**, 103-108.
 13. Dellaporta, S. L., Wood, J. J. and Hicks, J. B. (1983) A plant DNA miniprep: version II., *Plant Mol. Biol. Rep.*, **1**, 19-21.
 14. Wadsworth, G. J., Redinbaugh, M. G. and Scandalios, J. G. (1988) A procedure for the small-scale isolation of plant RNA suitable for RNA blot analysis., *Anal. Biochem.*, **172**, 279-283.
 15. Davanloo, P., Rosenberg, A. H., Dunn, J. J. and Studier, F. W. (1984) Cloning and expression of the gene for bacteriophage T7 RNA polymerase., *Proc. Natl. Acad. Sci. USA.*, **81**, 2035-2039.
 16. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding., *Anal. biochem.*, **72**, 248-254.
 17. Thornburg, R. W. and Baseman, J. B. (1983) Comparison of major protein antigen and protein profiles of *Treponema pallidum* and *Treponema pertenuis*., *Infect. Immun.*, **42**, 623-627.
 18. Graham, J. S., Hall, G., Pearce, G. and Ryan, C. A. (1986) Regulation of synthesis of proteinase inhibitors I and II mRNAs in leaves of wounded tomato plants., *Planta*, **169**, 399-405.
 19. Johnson, R. and Ryan, C. A. (1990) Wound-inducible potato inhibitor II genes: Enhancement of expression by sucrose., *Plant Mol. Biol.*, **14**, 527-536.
 20. Kernan, A. and Thornburg, R. W. (1989) Auxin levels regulate the expression of a wound-inducible proteinase inhibitor II-chloramphenicol acetyl transferase gene fusion *in vitro* and *in vivo*., *Plant Physiol.*, **91**, 73-78.
 21. Ikeda, R. A. and Richardson, C. C. (1987) Enzymatic properties of proteolytically nicked RNA polymerase of bacteriophage T7., *J. Biol. Chem.*, **262**, 3790-3799.
 22. Cleveland, T., Thornburg, R. and Ryan, C. (1987) Molecular characterization of a wound-inducible inhibitor I gene from potato and the processing of its mRNA and protein., *Plant Mol. Biol.*, **8**, 199-207.
 23. Spielman, H., Erikson, R. P. and Epstein, C. J. (1974) The procedure of antibodies against mammalian LDH-1., *Anal. Biochem.*, **59**, 462-467.
 24. Thornburg, R. W., Kernan, A. and Molin, L. (1990) CAT protein is expressed in transgenic tobacco in field tests following attack by insects., *Plant Physiol.*, **92**, 500-505.

T7 RNA polymerase 유전자의 담배식물에서의 발현

Miguel A. Caviedes, 박상규^{1*}, Robert W. Thornburg² (스페인 세빌라 대학교 미생물학 및 기생충학과, ¹대구대학교 농화학, ²아이오와 주립대학교 생화학 및 생물리학과)

초록 : 박테리오파지 T7 RNA polymerase 유전자를 식물체내에서 이용할 수 있을지 알아보기 위하여 상처유발인 감자 단백질 분해효소 억제제 유전자의 프로모터에 박테리오파지 T7 RNA polymerase 유전자를 연결시킨 후 담배에 도입시켰다. 형질전환 식물체의 DNA에 대한 Southern hybridization에 의하면 T7 RNA polymerase 유전자가 식물체내에 1~2 copy가 존재하며, Northern hybridization에 의하면 T7 RNA polymerase의 RNA가 상처에 따라 생성되는 것을 확인하였다. 또한 Western hybridization에 의하면 식물체내 T7 RNA polymerase 단백질이 생성되는데 그 크기는 대장균에서 생성되는 단백질 크기와 유사한 80 kDa 이었으며 시험관내에서 전사체에 뉴클레오타이드를 결합시키는 능력이 있음도 확인하였다. 따라서 T7 RNA polymerase 유전자를 이용하여 식물체내에서 원하는 유전자의 발현을 증대시킬 수 있을 것으로 사료된다.

찾는 말 : proteinase inhibitor II (*pin2*), T7 RNA polymerase, wound-inducible expression

*연락처