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Promoter elements controlling developmental and environmental regulation of a tobacco ribosomal protein gene *L34*

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Abstract

The *rpL34* gene, which encodes a cytoplasmic ribosomal protein with a high homology to the rat 60S r-protein L34, was isolated from a genomic library of tobacco (*Nicotiana tabacum* L. cv. Xanthi-nc). A 1500 bp upstream promoter fragment was fused to the chloramphenicol acetyltransferase (CAT) reporter gene or β-glucuronidase (GUS) reporter gene and transferred into tobacco plants by the *Agrobacterium*-mediated leaf disk transformation method. Analysis of CAT activity in leaf tissues showed that mechanical wounding increased the *rpL34* promoter activity about 5 times as compared to untreated controls and that the promoter activity was further enhanced by plant growth regulators, 2,4-dichlorophenoxyacetic acid and benzyladenine. Histochemical GUS staining patterns of the transgenic plants showed that the *rpL34* promoter activity is high in actively growing tissues, including various meristems, floral organs, and developing fruits. A series of 5′ deletion analyses of the *rpL34* promoter indicated that a 50 bp region located between −179 and −129 is essential for wound, auxin and cytokinin responses. Deletion of this region reduced the promoter activity to an undetectable level. Insertion of the 50 nucleotide sequence into a minimal promoter restored the promoter activity and the promoter strength was proportional to the copy number of the upstream sequence. The role of TATA and CAAT box regions was studied by a series of 3′ deletion analyses. A 3′ deletion up to −28 did not significantly affect the promoter strength. However deletion of the promoter up to 70 bp, which deleted the TATA box region, significantly reduced promoter activity. Further deletion of the promoter up to −104, eliminating the CAAT box region, abolished the promoter activity. These results suggest that the TATA box and CAAT box regions are also important for the *rpL34* promoter activity in addition to the 50 bp upstream region.

Introduction

Protein synthesis by ribosomes is a basic process that occurs in all known organisms. In plant cells, there are three distinct types of ribosomes that are found in the cytosol, mitochondria and plastids. It has been reported that biosynthesis of the different ribosomal proteins (r-protein) is coordinately regulated in many living organisms [31]. In prokaryotes, such as *Escherichia coli*, control of r-protein level is largely dependent upon growth conditions or nutrient availability [28]. In addition, fine control of r-protein synthesis is achieved by autogenous translational feedback of the various r-protein operons [35]. In eukaryotes, cytosolic r-protein gene expression has been extensively studied in animals and yeast. These studies demonstrated coordinated regulation of r-protein gene expression during development. The way in which r-protein synthesis is regulated varies not only from organism to organism but also between different developmental stages of a single organism [37, 46].

In contrast to the wealth of information that has accumulated concerning animal and yeast cytoplasmic r-protein, little is known about the organization and

the regulation of plant cytoplasmic r-protein genes. Over the past few years, an increasing number of plant cytoplasmic r-protein cDNA clones have been isolated. These include S3a [29], S8 [33], S11 [13, 26], S13 [18], S14, S15a [4], S15 [38], S16 [44, 47], S18 [27], S19 [41], L2 [24, 32], L3 [34], L5 [22], L7A [34], L7, L16 [45], L17 [15], L21 [42], L25, L34 [14], L27 [40], and L31 [6].

The expression patterns of some cytoplasmic r-protein genes have been studied. In the small subunit r-protein genes, the relative mRNA steady-state level of maize S11 is about one order of magnitude higher in rapidly growing parts of the plant, such as the roots and shoots of seedlings, than in fully expanded leaf tissue [26]. Similar expression patterns were observed in maize cytoplasmic r-protein S13 [4] and S14 [25] genes. In different organs of mature maize, *Brassica napus*, and *Arabidopsis*, mRNA levels of S13 [18], S15a [4], and S18 [27] are much higher in tissues having mitotic activity, such as root tips, female inflorescence and nodes.

The large subunit r-protein genes L2 [24,32], L7 [41], L17 [30], L25 [14], L27 [40], and L34 [14] also display preferential expression patterns. The tobacco L2, barley L21, and barley L17 genes are strongly expressed in young plant tissues, germinating seeds and actively growing suspension cells. In potato, it has been demonstrated that there is a 15- to 20-fold increase in the steady-state mRNA level of two r-protein genes, S19 and L7, in the early stages of tuberization [41]. A pea cDNA with high homology to r-protein L27 from rat was highly expressed in terminal buds, root apices, and elongating internodes [40]. We have previously reported that the transcript levels of L25 and L34 genes were most abundant in actively growing suspension cells and young tissues [14]. Gantt and Key [11] report that translatable mRNA levels of cytoplasmic r-protein genes are inducible by auxin in soybean hypocotyl. We have demonstrated earlier that mechanical wounding, 2,4-D, and benzyladenine increased the steady-state mRNA amounts of the r-protein genes L25 and L34 [14].

Thus far, there is a little information on the regulatory elements of these r-protein genes. Fusions of two promoters of *Arabidopsis thaliana* L16 genes to the GUS reporter exhibited preferential activity of the promoter in proliferating tissues, indicating that the promoters carry interesting regulatory elements. However, detailed studies on the promoter elements are lacking. In the present study, we have characterized and identified regulatory elements essential for the promoter activity of the r-protein L34 gene *rpL34* in tobacco.

Materials and methods

Bacterial strains, plant materials

The *E. coli* strains MC1000 and JM 83 (*ara*, *leu*, *lac*, *gal*, *str*) [5] were used as the recipients for routine cloning experiments. *Agrobacterium tumefaciens* LBA4404 containing the Ach5 chromosomal background and a disarmed helper-Ti plasmid pAL4404 [16], was used for transformation of tobacco plants (*Nicotiana tabacum* L. cv. Petit Havana SR1 and *N. tabacum* L. cv. Xanthi).

Genomic DNA cloning

A genomic library of *N. tabacum* cv. xanthi-nc was purchased from ClonTech laboratories (Palo Alto, CA). The DNA used for the library was prepared from 30 day post-emergence seedlings grown under a 16 h light/8 h dark cycle. Genomic DNA was partially digested with *Mbo*I and the fragments were cloned into the *Bam*HI site of EMBL-3 [10]. The average insertion size ranged from 8 to 22 kb. The tobacco genomic library was screened by a plaque hybridization method using the TSC40 clone, which encodes for r-protein L34, as a probe [14]. A plasmid carrying the TSC40 clone was digested with *Eco*RI, the insert was isolated on a low-melting-point agarose gel, and the fragment was labeled with [α-^{32}P]dCTP using the random priming method (T7 QuickPrime kit, Pharmacia LBK Biotechnology, Piscataway, NJ). Phage DNA was prepared by using the method described previously [7].

Construction of deletion mutants

The 1500 bp *Bam*HI-*Hin*dIII fragment carrying the entire promoter region of the *rpL34* gene was cut out from pGA1241-10 and placed in front of the CAT-coding sequence of pGA707 and T7 terminator, forming pGA1241-11. This full-length promoter was used to generate two deletion mutants using unique restriction enzyme sites, *Bgl*II (−438) and *Spe*I (−128), located at the promoter region. These deletions are called pGA1379 and pGA1380, respectively. Three additional deletions were generated using the PCR method. The synthetic oligomers CATGTTGATATAGAC (−343 to −329), CCATGC-

CAAAACC (−228 to −216), and GGGCTAACATG (−179 to −169) were synthesized by using Applied Biosystem DNA synthesizer and used for generation of the 5′ deletion mutants. These deletions were cloned into pGA707 for generating pGA1241-26 (−343), pGA1241-24 (−228) and pGA1241-28 (−179). For stable expression of these constructs, the mutant promoters were subcloned into the binary vector pGA628 via *Hin*dIII and *Kpn*I. The 3′ deletion mutants were generated similarly. The oligonucleotides, GCAAGCTTCAGAAGGGCTAAA (−28 to −40), GCAAGCTTGTGGGACAAGCC (−70 to −81), and GCAAGCTTGATGATAGAATC (−104 to −115) were synthesized and used for construction of the deletions. These plasmids were called pGA1241-38 (−28), pGA1241-39 (−70), and pGA1241-40 (−104), respectively.

Oligonucleotides for mutimers were also prepared using an Applied Biosystem DNA synthesizer. Both sense and anti-sense strands between −184 and −123 bp of the *rpL34* promoter were synthesized with GATC at the 5′ end of each strand. The oligonucleotides were purified by gel electrophoresis. In order to subclone into the non-functional-*rpL34* promoter, a *Sac*II site was created at both ends of the oligonucleotides. Nucleotide extensions were added by self-ligation using T4 DNA ligase. Ligation mixtures containing both monomers and multimers were fused into the *Sac*II site of the *rpL34* promoter 5′ deletion mutant −128. Multimer fragment inserts were confirmed by DNA sequencing. Promoter activity was tested by transient analysis using protoplast electroporation.

Transient and stable transformation analysis of CAT activity

Twenty μg of DNA and 1×10^6 protoplasts prepared from three-day old NT1 suspension-cultured cells were used to perform transient expression assays as described previously [8]. The *rpL34* promoter and its mutant promoter fragments were subcloned into the binary Ti-plasmid vector pGA580 which contains the *cat* reporter gene [2]. *Agrobacterium tumefaciens* strain LBA4404 carrying the binary vector was cocultivated with young leaf segments from *in vitro* grown tobacco plants [1]. A total of 20 to 25 transgenic tobacco plants were obtained for each construct by selecting on an agar medium carrying 50 μg/ml kanamycin. The transgenic plants were maintained in greenhouse conditions. The CAT activity was determined using the crude extracts standardized at 2 to 200 μg of the total soluble protein [1].

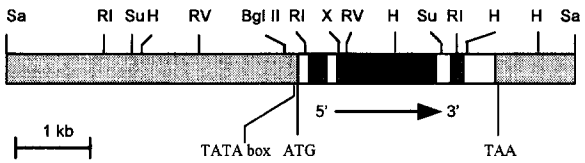
Histochemical GUS assays

The full-length promoter −1500 fragment or 5′ deletion mutant −438 were used for the construction of fusions to the *GUS* reporter gene [17] in pBI103.1. The T2 tobacco lines were generated by self-pollination of the primary transformants. Histochemical assay of GUS expression was performed on intact seedlings or excised organs from mature plants as modified from Jefferson [17]. Materials were pretreated with 90% acetone for 1 to 5 h at −20 °C and rinsed twice with 50 mM sodium phosphate buffer (pH 7.0) [39]. Tissues were incubated in 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucoronide, 1 mM EDTA, 0.05% (v/v) Triton X-100, 0.1 mM potassium ferrocyanide and 0.1 mM potassium ferricyanide. The samples were briefly vacuum-infiltrated and then incubated at 37 °C in the dark of 0.3 to 15 h, as required. Reactions were stopped and tissues were cleared through a sequential treatment with 70% and 95% ethanol at 45 °C for 1 to 5 h. Some tissues, such as floral organs, were further treated with 10% bleach for 20 min to 2 h. The tissues were stored in 75% ethanol.

Results

Isolation of rpL34 *promoter*

The genomic clone λ4022 was isolated from a tobacco genomic library using a cDNA clone encoding r-protein L34 as a probe. The insert was excised with *Sal*I and subcloned into the *Sal*I site of pBluescript SK(−) to form pGA1241-1. The size of the genomic fragment was 7.5 kb. A restriction map of the clone was constructed with the following enzymes: *Hin*dIII, *Xba*I, *Stu*I, *Eco*RI, and *Eco*RV (Fig. 1A). The coding region was located by hybridization of the restriction fragments with the *rpL40* cDNA clone and by sequencing the DNA fragments. It was found that the coding region was split by three introns. The 135 bp first intron is located at the 27th codon that encodes glycine. The longest is the 1.3 kb second intron which is located between the 53rd (glycine) and the 54th (isoleucine) codons. The last intron is 77 bp long and located at the 90th codon (arginine). The DNA sequence of the promoter region between the *Bgl*II site and the transcrip-



(a)

-438		AGATCTCT (Bgl II)	CTTTGTATTC	TTATTGATGT	ACTGGTTTGA
-400	AGATGAATAA	AATCTTTCAT	TCCACCAAAA	AAAGAATGAA	AATAAAATTT
-350	TAATATACAT (-343)	GTTGATATAG	ACAAAGAAGA	AAAAAAAAGT	TGTGATTACA
-300	TTTATTGACT	ATTTGATGCC	AATATCTATA	ACTAGAGCTA	TTTTCTATCA
-250	ATTATATGGG	TATGTTGTTA	TACCATGCCA (-228)	AAACCTCAAT	TCATAATGTG
-200	CTTGTTTAAA	CCCAGTTTAA	TGGGCTAACA (-179)	TGTTGATGGG	CTTATAGGCC
-150	CGTCTGATTT	CCTTGCCAGA	CACTAGTAAG (Spe I(-128))	TAAATGATTC	TATCATCCAA (-104)
-100	TATCAACCGT	GGGATCTAGG	GCTTGTCCCA (-70)	CTTATATACA	CTATATATAT
-50	TTAACTTTCC	TTTAGCCCTT	CTGCTTCAGC (-28)	CCCCAAAACA	AAGAAAGAAG
+1	CTACAGAGAG	AATAGCAGCG	CCGCCGTGAA	AA ATG-3'	

(b)

Figure 1. Restriction map of the *rpL34* gene and the DNA sequence of the promoter region. A (top). Restriction map of the 7.5 kb *Sal*I fragment carrying the tobacco *rpL34* gene. Introns are shown as dark shadow bars. The coding regions are shown in the open bars. Letters represent restriction enzyme sites: B, *Bgl*II; H, *Hin*dIII; RI, *Eco*RI; RV, *Eco*RV; SA, *Sal*I; Su, *Stu*I. The arrow indicates direction of transcription. B (bottom). The nucleotide sequence of the 5'-upstream region of the *rpL34* promoter from −438 to the translation initiation codon (+35). The position +1 corresponds to the start site of the rpL34 cDNA clone [14]. Deletion end points are underlined. The CAAT box and TATA box sequences are italicized and dotted-underlined. The ATG start codon is double underlined.

tion initiation site ATG is shown in Fig. 1B. Putative CAAT box (CCAATATC) and TATA box (CATATATA) sequence elements are present at positions −104/−97 and −57/−50, respectively.

Characterization of the ribosomal protein L34 promoter

A 5-kb *Eco*RV fragment carrying the promoter region was subcloned into pBluescript SK(−) to form pGA1241-9. One *Eco*RV site is located ca. 1.5 kb upstream of the ATG start codon and the other is located within the second intron. The promoter region was isolated from pGA1241-9 by PCR amplification of the 1.5 kb region using a primer starting immediately upstream of the ATG sequence. This fragment was connected to the *cat* reporter gene and the chimaeric fusion was transferred to tobacco plants using the binary pGA628 [2]. Transgenic tobacco plants were generated and CAT activity was measured after various treatments to fully expanded leaves. Previously, we

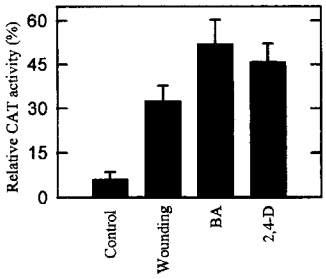


Figure 2. Responses of the *rpL34* promoter/CAT to mechanical wounding, BA, and 2,4-D. Leaf segments of transgenic plants carrying the *rpL34* promoter/CAT fusion were incubated for 22 h in MS medium with wounding or with 5 μM BA, of 1 μM 2,4-D. A control sample was harvested before incubation. The CAT activity was measured using 5 μg of total soluble protein. Values are the average of 10 independent transgenic plants ±SD.

reported that the tobacco *rpL34* mRNA level was inducible by wounding and phytohormones [14]. Similarly, CAT activity was increased about five-fold by wounding and further enhanced by 5 μM BA and 1 μM 2,4-D treatments (Fig. 2). This result indicates that the 1.5 kb fragment contains the regulatory elements controlling the promoter.

The expression pattern of the rpL34 *promoter*

The expression patterns of the *rpL34* promoter were studied using the *GUS* reporter gene. The full-length promoter (1500 bp) was placed upstream of the GUS-coding region using the binary vector pBI101.3. This construct was transformed into tobacco plants and the transgenic plants were selected on kanamycin-containing medium. Ten transgenic plants that showed GUS activity were selfed and the T2 offspring were studied. All of the offspring displayed a similar expression pattern. The GUS staining patterns of seeds and various vegetative tissues are shown in Fig. 3. There was no detectable GUS activity in dried seed (Fig. 3A). However, in 2-day old seedling, a strong GUS activity was present in root apex (Fig. 3B). Four-day old (Fig. 3C) and 16-day old (Fig. 3D) seedlings showed higher GUS activity in the root apex, shoot apex, and cotyledons. Higher magnification of the roots of the 16-day old seedlings exhibited strong GUS activity in primary and lateral root apexes (Fig. 3E and 3F). In older plants, strong GUS activity were detected in parenchyma tissues (Fig. 3G), leaf primordia, and apical meristem (Fig. 3H). In mature leaves, GUS activity was low. However, mechanical wounding induced

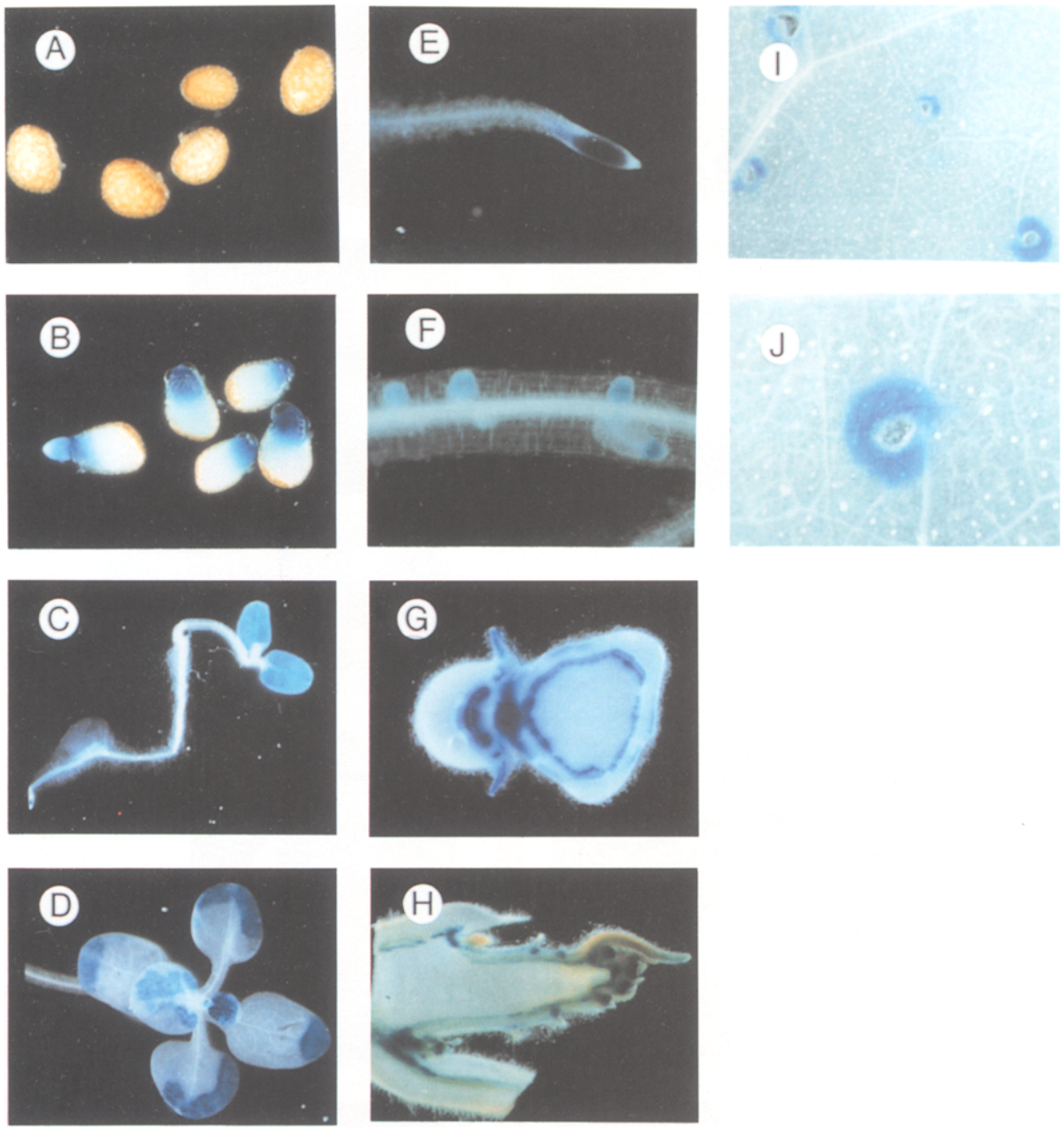


Figure 3. The *rpL34* promoter-driven *GUS* expression pattern. A, dried seeds; B, 2-day old seedlings; C, 4-day old seedlings; D, 16-day old seedlings; E, root apex; F, lateral roots of older plants; G, cross section of a stem; H, shoot apex; I, wounded leaf tissues; J, close-up view of one of wounded sites of leaf tissue.

GUS expression in the tissues adjacent to the wounded sites (Fig. 3I and 3J).

The GUS activity was also observed in proliferating tissues of various floral organs (Fig. 4). In young flowers, strong activity was detected in almost all of the floral organs. In developing anther, GUS activity was found in pollen grains (Fig. 4D and F). When the flower became mature, GUS staining was localized to the ovary and stigma (Fig. 4B). A cross-section of the ovary showed strong expression in seeds and vascular bundles (Fig. 4H). After fertilization, GUS activity was

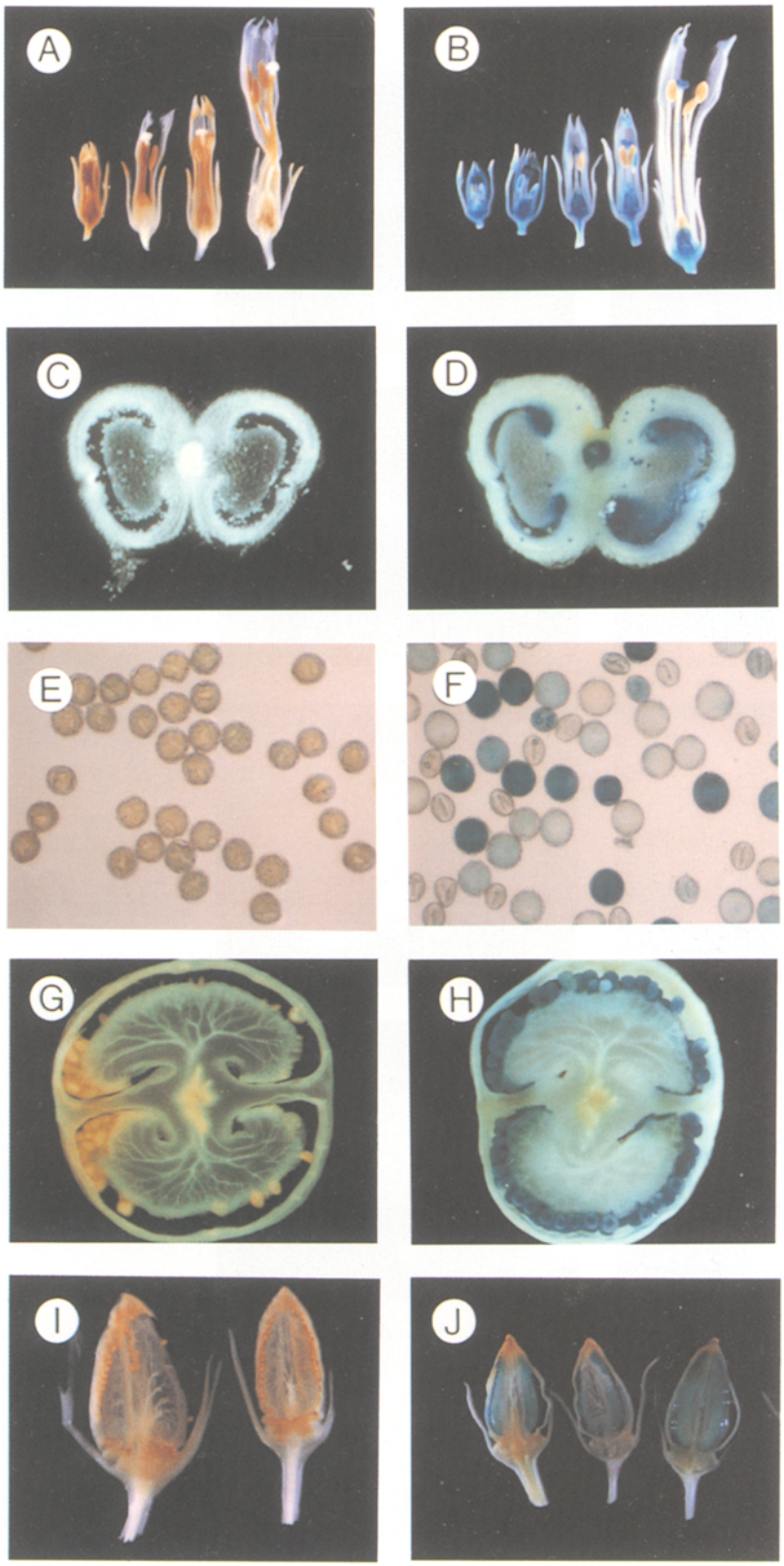


Figure 4. Histochemical analysis of temporal and spatial expression patterns of *rpL34*-GUS during tobacco floral organ development: longitudinal sections of wild-type (A) and a transgenic (B) flowers at different developmental stages; cross-section of wild-type (C) and transgenic (D) anthers; wild-type (E) and transgenic (F) pollen grains; cross-section of wild-type (G) and transgenic (H) ovaries; longitudinal sections of ovaries of wild-type (I) and transgenic (J) flowers.

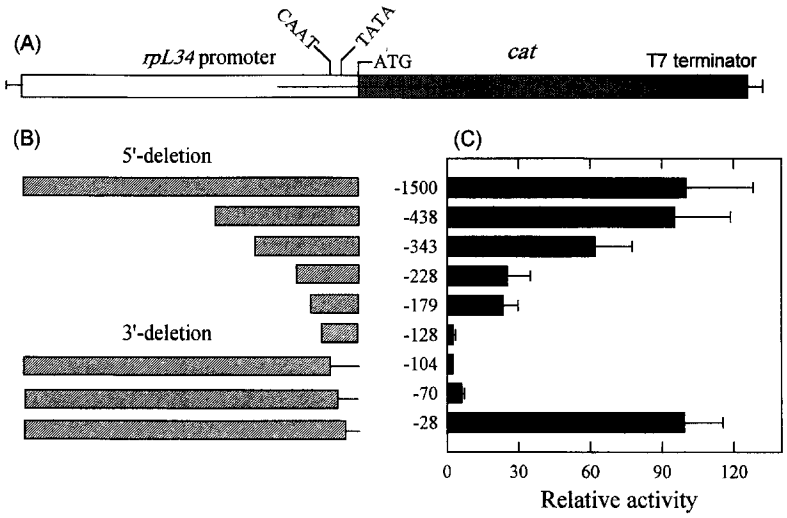


Figure 5. Deletion analyses of the *rpL34* promoter by transient assays. A. Diagram of the *rpL34/cat* gene fusion. B. Various lengths of promoter fragments connected with the *cat* reporter gene are indicated with the last 5′ of 3′ nucleotide position listed to the right of the figure. C. Relative CAT activity as compared with the activity of the full-length (1500 bp) promoter, with corresponding bp deletions listed to the left of the figure. Data are the means of 5 to 8 independent replications ±SD.

detected primarily in the developing seeds (Fig. 4H and J).

Transient analysis of the promoter elements

The promoter fragment of the *rpL34* gene was characterized by two sets of deletion analyses. In the first set, five 5′ deletions were generated. The 5′ −438 and 5′ −129 deletions were obtained by removing the DNA sequence upstream of the restriction enzyme sites *Bgl*II and *Spe*I, respectively. Three additional deletions, 5′ −343, −228, and −179, were generated by the PCR approach. A set of 3′ deletions, 3′ −102, −69, and −27, of the promoter region were also produced by the PCR approach. These deletions were connected to the *cat* reporter gene and the chimeric fusions were introduced into tobacco protoplasts by electroporation.

Transient activity of the mutant promoters was studied by measuring CAT activity of the tobacco cells 40 h after expression of the fusion genes. The results in Fig. 5 are the average of four independent experiments. Level of CAT activity was compared to the full-length (5′ −1500) promoter. Deletion of ca. 1 kb region upstream of −438 did not alter the promoter strength. However, removal of the 95 bp DNA sequence between −438 and −343 reduced the promoter activity by 40%. Deletion of an additional 115 bp between −343 and −228 further reduced the promoter activity to 30% of the full strength. These results indicate that the sequence between −438 and −228 contains at least two elements which regulate the promoter. Strength of the 5′ −179 promoter was similar to the 5′ −228. However, removal of the 50 bp fragment between −179 and −129 dramatically decreased promoter activity. Therefore, it appears that this 50 bp upstream region is essential for strong *rpL34* promoter activity.

In the 3′-end deletions, activity of the 3′ −28 promoter remained almost at the same level as the full-length promoter. However, the 3′ −70 promoter exhibited very low level of the expression and the 3′ −104 promoter showed almost no detectable activity. The DNA sequence between −28 and −104 contains the conserved CAAT and TATA box sequences, suggesting the importance of these elements.

Roles of the 50 bp fragment between −179 and −129

The roles of the 50 bp DNA sequence between −179 and −129 were further studied by gain-of-function assay. The 50 bp region was synthesized and multimerized by self-ligation. These multimers were inserted in front of the non-functional promoter 5′ −128, which was fused to the *cat* reporter gene. Activity of these promoters were measured by transient assay using tobacco cells. The results showed that insertion of one copy of the region restored CAT activity to the level of 5′ −179 (Fig. 6). Insertion of two copies increased the CAT activity by ca. 3-fold. Insertion of three copies further

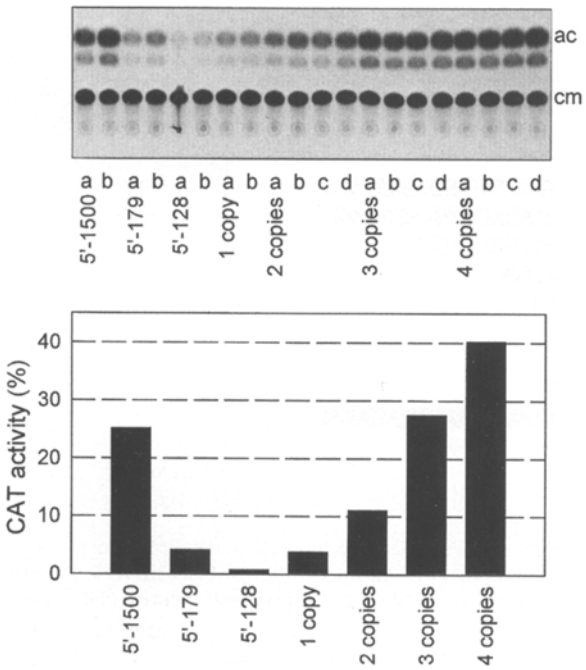


Figure 6. Transient analysis of multimerization (1 to 4 copies) of the 50 bp region between −179 and −129. The 50 nucleotide fragment was self-ligated and inserted into the non-functional *rpL34* promoter 5′ −129. The upper panel is the TLC autoradiograph showing the CAT activity of replicate samples indicated by a, b, c, d; cm, chloramphenicol; ac, acetylchloramphenicol. The lower panel is the corresponding relative CAT activity. Data are averages of 4 to 6 replications.

enhanced CAT activity by 6-fold which is the level of the full-length promoter. Four copy insertion resulted in CAT activity greater than the full-length promoter activity.

Stable transformation analysis of the rpL34 *promoter*

Effects of the 5′ deletions on the promoter characteristics were studied by stable transformation. Fusions between the mutated promoter fragments and CAT-coding region were inserted into the binary vector pGA628. Transgenic tobacco plants containing these chimaeric genes were obtained by the *Agrobacterium*-mediated co-cultivation method. Fifteen to 20 independent transgenic plants were analyzed for each construct. Leaves from 50-day old tobacco plants were wounded by cutting into small pieces and incubating for 20 h in MS medium. Effects of phytohormones on the *rpL34* promoter activity were also studied by adding 1 μM 2,4-D or 4.5 μM BA into the culture medium.

Characteristics of the 5′ −438 promoter were similar to the full-length promoter (Fig. 7). The CAT activity was increased ca. 5-fold by wounding. Activity was further enhanced by treatment with 2,4-D of BA. Similar induction patterns were also observed with the 5′ −343 and 5′ −228 promoters, though the degree of the induction was less. Activity of the 5′ −179 promoter was extremely low and only a low level of induction by BA was detectable. However, CAT activity was not detectable in transgenic plants carrying the 5′ deletion −128 mutant. These results confirm the data from the transient assay that the sequence between −179 and −128 is important for the *rpL34* promoter activity.

Discussion

In this study, we have isolated the genomic clone of the r-protein gene L34 from tobacco and elucidated the regulatory elements controlling the promoter activity using transient and stable transformation analyses. The 1500 bp region upstream from the coding region was fused to either CAT or GUS reporter and the promoter characteristics were studied in transgenic tobacco plants. The results indicate that the *rpL34* promoter activity appears closely associated with cell proliferation. This correlation is most apparent in meristemic tissues. A strong correlation between r-protein gene expression and cell proliferation was similarly observed in other r-protein genes [4, 11, 18, 24, 26, 27, 32, 40, 41]. Recently, the expression pattern of other r-protein genes, *rpS18A* and *rpL16B*, has been determined through promoter/GUS fusion [27, 45]. The promoter was most active in the proliferating tissues and comparable to the *rpL34* promoter activity observed in this study. We have also observed that the 1500 bp upstream region contains the regulatory elements involved in the promoter induction by wounding and phytohormones, confirming our previous observation by measuring the *rpL34* transcript levels [14]. Auxin inducibility of the *rpL16A* promoter/GUS fusion was also reported [45] and is known to regulate r-protein synthesis. It was earlier reported that auxin induces an increase in mRNA level of cytosolic r-proteins in plants [11, 12]. There is some evidence that auxin may exert a translational control of the r-proteins by regulating the phosphorylated status of ribosomal proteins [36]. However, detailed mechanisms of how auxin regulates the r-protein synthesis are not known. Our studies demonstrate that increase in

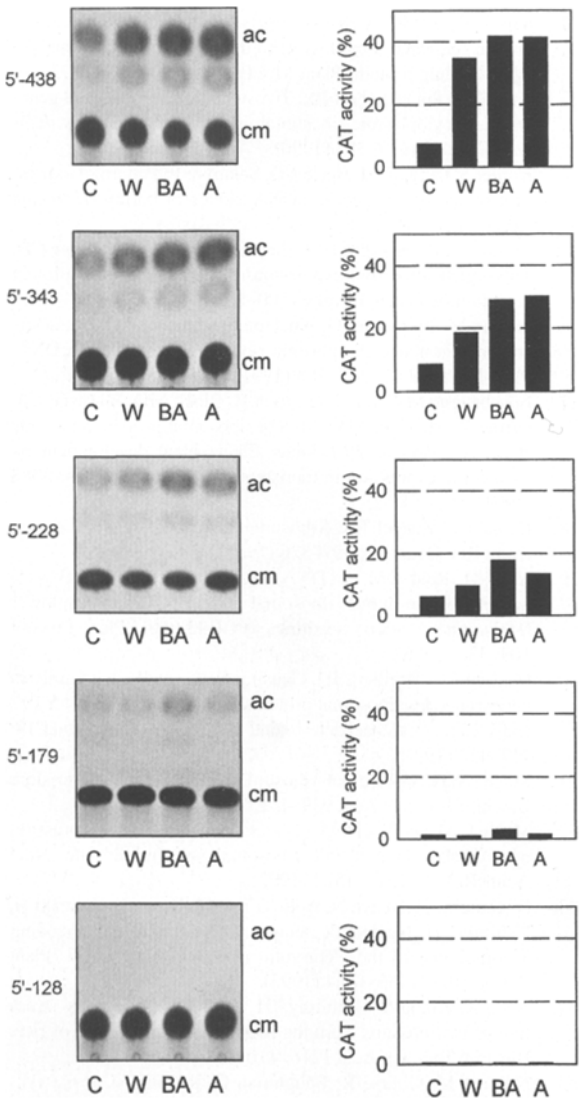


Figure 7. Stable transformation analysis of the 5′ deletion mutants. Transgenic plants carrying various lengths of the 5′ deletion mutant promoter/*cat* gene fusion were analyzed for the inducibility by wounding (W), BA, or 2,4-D (A) treatment. Control samples (C) were harvested before treatments. The TLC autoradiograph from a representative sample is shown on the left, cm, chloramphenicol; ac, acetylchloramphenicol. Relative CAT activities are shown on the right. The amount of total soluble protein used for the assay was 5 μg for 5′ −438, 5′ −343, and 5′ −228, 100 μg for 5′ −179, and 200 μg for 5′ −128. Results are averages of 5 independent transgenic tobacco plants.

r-protein synthesis by phytohormones is controlled in part by enhancing the promoter activity.

We have elucidated the regulatory region of the *rpL34* promoter in order to eventually identify elements controlling the promoter activity. Transient analysis showed that the sequence between −438 and −228 contains at least two elements which are involved in the promoter strength. Stable transformants lacking the region between −438 and −228 still exhibited wound and phytohormone inducibility, indicating that this upstream region contains general enhancers. Both transient and stable analyses revealed that the 50 bp fragment between −179 and −129 is essential for the *rpL34* promoter activity. Multiplication of this region caused a proportional increase in promoter strength. However, it is unclear which sequence elements within this upstream region play an important role. The TGTCTC element which appears to be an auxin responsive element of soybean SAUR genes is not present in the *rpL34* promoter upstream region [43]. The region also does not contain the TGACGT element which is present in a variety of promoters including the octopine synthase gene, nopaline synthase gene, and cauliflower mosaic virus 35S transcript [3, 9, 20]. The sequence has been identified as a regulatory element induced by various stimuli including auxins and salicylic acid. The G-box sequence CACGTG, which was identified as a wound inducible element of the potato proteinase inhibitor II gene, is not found in the upstream region [21]. Therefore, it seems that the *rpL34* promoter contains a new class of regulatory elements that control wound and phytohormone inducibilities. Further elucidation of the *rpL34* promoter will identify these regulatory elements.

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