A Mutant Lectin Gene Is Rescued from an Insertion Element That Blocks Its Expression

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The soybean lectin gene Le7 encodes a prevalent seed protein and is highly regulated during the life cycle. The mutant lectin gene allele le7 is not transcribed detectably, contains a 3.5-kb Tgm1 insertion element within its coding region 0.6 kb 3' to the transcription start site, and leads to a lectinless phenotype. To determine whether the Tgm1 element or a secondary mutation was responsible for repressing le7 gene transcription, we eliminated the insertion element by constructing a chimeric lectin gene (le7/Le7) that contained the 5' half of the le7 gene and its promoter region and the 3' half of the wild-type Le7 gene. Transformed tobacco seed containing the le7/Le7 gene produced both lectin mRNA and protein, demonstrating that the mutant lectin gene control region is transcriptionally competent. By contrast, transformed seed containing the le7 gene produced no detectable lectin mRNA. We conclude that the absence of detectable transcription from the le7 gene is due to transcriptional inhibition by the Tgm1 insertion element and that this element acts at a distance to block transcription from an upstream promoter region.

INTRODUCTION

The soybean lectin gene Le7 belongs to a small repertoire of seed protein genes that is expressed at high levels during embryogenesis and is either inactive or active at reduced levels at other developmental periods (Goldberg et al., 1989). The lectin gene is 1 kb in size, lacks introns, and is embedded in a domain containing several closely linked nonseed protein genes that are regulated differently (Goldberg et al., 1983; Vodkin et al., 1983; Okamuro et al., 1986; Goldberg et al., 1989). Transcriptional processes control lectin gene activity with respect to cell type, organ system, and time during the life cycle (Walling et al., 1986; Goldberg, 1988; Goldberg et al., 1989; Lindstrom et al., 1990; R. Yadegari, L. Perez-Grau, and R. B. Goldberg, unpublished data), although post-transcriptional processes also play a role in modulating lectin gene expression (Walling et al., 1986).

Several lectinless soybean lines exist that do not accumulate lectin mRNA or protein during embryogenesis (Pull et al., 1978; Vodkin, 1981; Goldberg et al., 1983). We showed previously that the lectinless soybean line Sooty has a mutant lectin gene allele (le7) with a 3.5-kb insertion element located in the coding region 628 bp downstream from the transcription start site (Goldberg et al., 1983; Vodkin et al., 1983; Rhodes and Vodkin, 1985). Other lectinless lines also have the 3.5-kb insertion element within the Le7 gene (Goldberg et al., 1983; Vodkin et al., 1983). Extensive restriction mapping, heteroduplex, and transcription unit localization studies indicated that the Le7 and le7 gene regions are similar over a distance of 13 kb, except for the presence or absence of the 3.5-kb insertion within the lectin gene (Goldberg et al., 1983). The le7 insertion element, designated as Tgm1, is part of a family of soybean transposon-like elements that have homologies with the Tam1 and En1/Spn mobile element families, although no evidence for Tgm transposition has been observed (Rhodes and Vodkin, 1985, 1988).

Runoff transcription experiments failed to detect transcriptional activity from the mutant le7 lectin gene either 5' or 3' to the Tgm1 insertion (Goldberg et al., 1983). By contrast, the transcriptional activity of nonseed protein genes flanking the le7 allele was indistinguishable from that of the same genes in the wild-type Le7 lectin gene region (Goldberg et al., 1983). This observation suggested that the Tgm1 element within the le7 coding region was exerting a cis-negative effect on transcription initiation in a promoter region more than 600 bp away (Goldberg et al., 1983). Comparison between 1400 bp of Le7 and le7 gene sequences revealed only five base pair differences: two in the promoter, three in the coding sequence, and one in a 3' flanking region (Vodkin et al., 1983). One nucleotide change in the le7 promoter is only 3 bp 5' to the TATA box. The other is in an AT-rich region that interacts with prevalent HMG-like DNA binding proteins (Jofuku et al., 1987; Laux and Goldberg, 1991; Laux et al., 1991). Thus, it was also possible that these mutations, or other unidentified mutations in upstream transcriptional control elements (Goldberg et al., 1989; Lindstrom et al., 1990), were responsible for reducing le7 gene transcription.

To test whether the Tgm1 element was responsible for the decrease in le7 lectin gene transcription, we rescued the le7 promoter region from the insertion sequence by exchanging

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*lel* sequences downstream of nucleotide +521, including the *Tgml* element, with those of the wild-type *Le* gene. The chimeric lectin gene (*lel/Le7*) containing the 5' half of the *lel* gene and the 3' half of the *Le* gene was then transformed into tobacco plants. Our results show that removal of the *Tgml* insertion element from the *lel* coding region restores lectin gene transcription during embryogenesis.

**RESULTS**

**Construction of an Insertionless *lel* Lectin Gene**

The wild-type and mutant lectin gene regions are shown schematically in Figures 1A and 1B, respectively. The *Le* and *lel* genes are present within 17.1- and 16.4-kb EcoRI fragments

(A) Map of the 17.1-kb EcoRI fragment containing the wild-type *Le* seed lectin gene (Goldberg et al., 1983). The 0.7-kb gene represents a nonseed protein gene that is differentially regulated with respect to the lectin gene and is oriented in the same direction (Okamuro et al., 1986). The 0.7-kb gene is approximately 2 kb upstream from the lectin gene transcription start site (Okamuro et al., 1986). The 0.7-kb gene is approximately 29 nucleotides away from the TATA box. (B) Map of the 16.4-kb EcoRI fragment containing the mutant *lel* lectin gene and the 3.5-kb *Tgml* insertion element (Goldberg et al., 1983; Vodkin et al., 1983). The *Tgml* element is inserted at position +521 of the *lel* gene coding sequence (Vodkin et al., 1983). The open region of the *lel* gene is oriented in the same direction (Okamuro et al., 1986). The 0.7-kb gene is approximately 29 nucleotides away from the TATA box. (C) Map of the 13.1-kb *lel/Le* lectin gene fusion containing the 3.7- and 9.4-kb EcoRI/BamHI fragments from the *lel* and *Le* lectin genes, respectively. The open region of the *lel* gene represents *lel* gene sequences, and the closed region represents *Le* gene sequences.

We showed previously that soybean lectin mRNA reaches a maximum level in transgenic tobacco seeds approximately 3 weeks after pollination (Okamuro et al., 1986). This mRNA is localized within storage parenchyma cells of the tobacco embryo and is undetectable in endosperm and other seed tissues (Goldberg, 1988; L. Perez-Grau, R. Yadegari, and R. B. Goldberg, unpublished results). To determine whether the *lel* and *lel/Le* genes were expressed in the transformed plants, polysomal poly(A) mRNAs were isolated from 3-week-old tobacco seeds.

We transferred the 16.4- and 13.1-kb EcoRI fragments containing the *lel* and *lel/Le* genes (Figures 1B and 1C) to tobacco plants using Ti-plasmid-mediated transformation (see Methods). We and others showed previously that the *Le* lectin gene is regulated correctly in transgenic tobacco (Okamuro et al., 1986; Lindstrom et al., 1990). The DNA gel blots presented in Figure 2 for two representative *lel* transformants (T1 and T2) show the presence of the expected 3.7- and 12.7-kb EcoRI/BamHI 5' and 3' *lel* DNA fragments (Figure 1B). These fragments had hybridization intensities similar to those present in the *lel* single copy DNA reconstruction (P1). Figure 2 also shows that two representative *lel/Le* transformants (T3 and T4) contained the expected 3.7-kb *lel* 5' EcoRI/BamHI fragment and 9.4-kb *Le* 3' BamHI/EcoRI fragment (Figure 1C) in amounts equal to those present in the single-copy DNA reconstructions (P1, P2, and P3). DNA gel blot experiments with these and other transformants using Hpal, which does not digest vector DNA and uncovers border fragments (Okamuro et al., 1986), indicated that all transformants investigated had single inserts of the soybean gene regions (data not shown). These data show that we have transferred unarranged single copy *lel* and *lel/Le* regions to the tobacco genome.

**Transfer of the *lel* and *lel/Le* Genes to Transgenic Tobacco Plants**

We showed previously that soybean lectin mRNA reaches a maximum level in transgenic tobacco seeds approximately 3 weeks after pollination (Okamuro et al., 1986). This mRNA is localized within storage parenchyma cells of the tobacco embryo and is undetectable in endosperm and other seed tissues (Goldberg, 1988; L. Perez-Grau, R. Yadegari, and R. B. Goldberg, unpublished results). To determine whether the *lel* and *lel/Le* genes were expressed in the transformed plants, polysomal poly(A) mRNAs were isolated from 3-week-old tobacco seeds,
these experiments that the \( Ie1 \) gene contains a functional promoter and that removal of the \( Tgm1 \) element restores the ability of the \( le1 \) promoter to direct the synthesis of lectin mRNA during seed development.

**The \( le1/Le1 \) Lectin Gene Is Expressed at the Protein Level**

We used gel blots containing mature tobacco seed proteins to determine whether the \( le1/Le1 \) gene was expressed at the protein level. Figure 4 shows that lectin antisera reacted with 29- and 31-kD proteins in seeds from representative plants containing the \( le1/Le1 \) gene (T3 and T4) and the \( Le1 \) gene (T5). These proteins were the same as those produced by the antisera with purified soybean seed lectin (soybean lectin lanes).

Seven of the nine transformants studied containing the \( le1/Le1 \) gene produced detectable levels of lectin protein (data not shown). By contrast, seeds containing the \( Le1 \) gene (T1 and T2) did not contain detectable 29- and 31-kD lectin proteins and produced background signals with the lectin antisera similar to those produced with untransformed seed proteins (lane C). These results indicate that restoring the \( le1 \) reading frame permits a lectin protein to be produced and that removal of the \( Tgm1 \) insertion element from the \( le1 \) coding region enables the rescued \( le1 \) gene to be expressed at both the mRNA and protein levels during seed development.

**Figure 2. Representation of Lectin Gene Sequences in Transformed Tobacco Plants.**

DNA (5 \( \mu \)g) from \( le1 \) plants (T1 and T2), \( le1/Le1 \) plants (T3 and T4), and untransformed plants (C) were digested with EcoRI and BamHI, fractionated by electrophoresis on an agarose gel, transferred to nitrocellulose, and hybridized with the 17.1-kb \( Le1 \) EcoRI fragment (Figure 1A) as indicated in Methods. The 3.7- and 12.7-kb EcoRI/BamHI DNA fragments represent the 5' and 3' ends of the \( le1 \) gene, respectively. The 7.7- and 9.4-kb EcoRI/BamHI DNA fragments represent the 5' and 3' ends of the \( Le1 \) gene, respectively. EcoRI- and BamHI-digested pS-5 DNA (P1), \( pl/e1 \) DNA (P2), and pL9-4 DNA (P3) were included as size markers and single-copy DNA controls.

![Figure 2](image)

**Figure 3. Lectin mRNA in Transformed Tobacco Seeds.**

Tobacco seed and soybean embryo polysomal poly(A) mRNAs were fractionated by electrophoresis on denaturing agarose gels, transferred to nitrocellulose, and hybridized with either a labeled lectin L9 cDNA plasmid (Goldberg et al., 1983) or a labeled pLVGneo2103 plasmid containing the \( nptII \) and \( nos \) genes (Zambryski et al., 1983; Hain et al., 1985) as indicated in Methods.

(A) **Lectin Probe**

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<th>Tobacco Seed mRNA</th>
<th>Soybean Embryo mRNA</th>
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(B) **NOS/NPTII Probe**

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<th>Tobacco Seed mRNA</th>
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<td>( le1/Le1 ) C Le1</td>
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**Figure 4. Lectin Antiserum Reacts with Tobacco Seed Proteins.**

Control hybridization of the \( le1/Le1 \) and \( Le1 \) seed mRNAs with the \( nptII \) probe produced 1.3-kb \( nptII \) transcripts with similar signal intensities (Figure 3B, lanes \( le1/Le1 \) and \( Le1 \)). Because only one \( Le1 \) transformant was used as a positive control in this experiment (Okamuro et al., 1986), we do not know whether the difference in \( le1/Le1 \) and \( Le1 \) lectin mRNA levels was due to position effects or to minor changes in the \( le1 \) gene region (Figure 1) that influence transcriptional and/or posttranscriptional processes. Nevertheless, we conclude from these experiments that the \( le1 \) gene contains a functional promoter and that removal of the \( Tgm1 \) element restores the ability of the \( le1 \) promoter to direct the synthesis of lectin mRNA during seed development.
Our results show that removal of the \textit{Tgml} element from the mutant \textit{le1} seed lectin gene restores the ability of this gene to be transcribed and expressed during seed development. This supports our original hypothesis that the insertion of the \textit{Tgml} element into the lectin gene coding region causes a cis-dominant repression of transcriptional initiation in the promoter region >0.6 kb upstream from the insertion site (Goldberg et al., 1983). Because we exchanged \textit{Lei} 3' sequences for those in the \textit{le1} gene containing the \textit{Tgml} element (Figure 1), we cannot formally exclude the possibility that unidentified nucleotide changes in the \textit{le1} gene region downstream of the \textit{Tgml} element are responsible for the transcriptional block on the \textit{le1} promoter. We consider this highly unlikely because transcriptional control elements are not present in the \textit{le1} gene region (R. Yadegari and R. B. Goldberg, unpublished results) and because there is a one-to-one correlation between the presence of the \textit{Tgml} element in the lectin gene and the lectinless phenotype in all soybean lines investigated (Goldberg et al., 1983; Vodkin et al., 1983). Thus, the simplest explanation for our results is that the functional \textit{le1} promoter is inhibited by the \textit{Tgml} element.

The silencing effect of the \textit{Tgml} element is exerted only on the lectin gene itself and does not influence the transcription of several nonseed protein genes that are situated 2 kb upstream and 6 kb downstream from the \textit{le1} gene (Goldberg et al., 1983; Okamuro et al., 1986). Thus, \textit{Tgml} appears to act locally on transcriptional control elements that are present in the lectin gene promoter and that are scattered over a 1.7-kb domain 5' to the transcription start site (Goldberg et al., 1989; Lindstrom et al., 1990; R. Yadegari and R. B. Goldberg, unpublished results). In this respect, \textit{Tgml} differs significantly from the yeast \textit{HMRE} silencer element that acts bi-directionally from its site within the genome to block the transcription of contiguous promoters at long distances (Brand et al., 1985).

Transposons and insertion elements are powerful mutagens and have been shown to exert a variety of effects on target gene activity (Gridley et al., 1987; Vodkin, 1989; Weil and Wessler, 1990). In general, insertions in exons result in null alleles due to mRNA and/or protein truncation, unless alternative splice sites within the transposon are used to excise element sequences post-transcriptionally from read-through transcripts (Weil and Wessler, 1990). Insertions within the promoter region can either abolish the ability of the gene to be transcribed and lead to a null allele, or they can alter the transcriptional specificity of the gene during development by interrupting cis-control elements (Gridley et al., 1987; Vodkin, 1989; Weil and Wessler, 1990). Only in rare instances has it been shown that insertions within the gene itself have a negative effect on upstream transcription initiation, and, in those cases that have been studied, the insertions are introns (Gridley et al., 1987; Kratochwil et al., 1989; Vodkin, 1989; Weil and Wessler, 1990). For example, a maize \textit{Mu} element in the first intron of the \textit{Adh1} gene reduces transcription by twofold to threefold compared to the wild-type allele (Rowland and Strommer, 1985). More strikingly, a retroviral insert in the first intron of the mouse c1(1) collagen gene blocks transcription in a tissue-specific manner by inactivating putative cis-control elements within the intron as well as by altering the methylation state and chromatin conformation of the promoter region (Kratochwil et al., 1989). The \textit{Tgml} insertion within the lectin gene appears to be unique in that it exerts its effect from within coding sequences that are neither spliced (Goldberg et al., 1983; Vodkin et al., 1983) nor play a role in the transcriptional regulation of the lectin gene during development (R. Yadegari and R. B. Goldberg, unpublished results).

How does the \textit{Tgml} element affect lectin gene transcription? First, sequences within the element could compete with cis-control elements in the \textit{le1} promoter for a limited number of factors necessary for lectin gene transcription. Because the \textit{Tgml} element does not interfere with the transcription of other genes during soybean embryogenesis and tobacco seed development, these factors would have to be lectin-gene-specific and/or be present in a lectin transcription complex acted upon locally by the insertion element. Second, transcription originating from within the \textit{Tgml} element could interfere with transcription of the \textit{le1} promoter, as has been shown for duplicated human \textit{a}-globin genes (Proudfoot, 1986). Run-off transcription studies demonstrated that the \textit{Tgml} element contains sequences that are transcribed during embryogenesis in mutant \textit{le1} soybean plants (Goldberg et al., 1983). In addition, three low prevalence mRNAs homologous to sequences within the \textit{Tgml} element were found to be present in \textit{le1} embryo poly(A) mRNA (J. K. Okamuro and R. B. Goldberg, unpublished results). Assuming that these transcripts originate from within the \textit{Tgml} element and not a related \textit{Tgml} family member (Rhodes and Vodkin, 1988), the potential exists for interference and/or competition between the \textit{le1} and \textit{Tgml} transcription initiation events. Finally, the \textit{Tgml} element could alter the chromatin conformation and/or methylation state of the \textit{le1} element.
gene analogous to the effects of the mov13 retroviral insert within the mouse collagen α1(1) gene intron (Kratochwil et al., 1989). How the Tgml insertion element exerts its unique silencing effect on the lectin gene promoter and what sequences are involved remain to be determined.

**METHODS**

**Growth of Plants**

Tobacco plants (Nicotiana tabacum cv SR1) and soybean plants (Glycine max cv Dare) were grown in the greenhouse as described previously (Goldberg et al., 1983; Okamuro et al., 1986). Tobacco seeds were harvested from capsules either at maturity or 3 weeks after hand pollination (Okamuro et al., 1986). Weights of tobacco seeds and capsules at different developmental stages were presented elsewhere (Barker et al., 1988).

**Isolation of Lectin Genes**

The pL9-4 and pS-5 plasmids containing the Le7 and le7 lectin genes and the L9 lectin cDNA plasmid were described previously (Goldberg et al., 1983; Okamuro et al., 1986).

**DNA Isolation and Labeling**

Leaf nuclear DNAs and plasmid DNAs were isolated as outlined in Jofuku and Goldberg (1988). Plasmid DNAs were labeled by nick-translation (Goldberg et al., 1983; Okamuro et al., 1986).

**Gel Blot Studies**

DNA and RNA gel blots were carried out according to previously published procedures (Goldberg et al., 1983; Okamuro et al., 1986; Jofuku and Goldberg, 1988). Protein gel blots were carried out according to Johnson et al. (1984).

**Polysomal mRNA Isolation**

Tobacco and soybean polysomal poly(A) mRNAs were isolated as outlined previously (Goldberg et al., 1983; Okamuro et al., 1986).

**Seed Protein Isolation**

Tobacco seed proteins were isolated according to the procedure of Sano and Kawashima (1983).

**Transformation of Tobacco Plants**

The le7 and le7/Le7 lectin gene regions (Figure 1) were recloned into the pLGVneo2103 plasmid (Hain et al., 1985), transferred to the disarmed Agrobacterium pGV3850 Ti-plasmid vector (Zambryski et al., 1983) by the procedure of Van Haute et al. (1983), and introduced into tobacco leaf discs according to Horsch et al. (1985) using kanamycin selection. Generation of transgenic tobacco plants containing the Le7 gene was described previously (Okamuro et al., 1986).

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**REFERENCES**


A mutant lectin gene is rescued from an insertion element that blocks its expression.
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