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Cellular localization of soybean storage protein mRNA in transformed tobacco seeds

(transformation/*in situ* hybridization/gene regulation)

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ABSTRACT **We transformed tobacco plants with a soybean β-conglycinin gene that encodes the 1.7-kilobase β-subunit mRNA. We showed that the β-conglycinin mRNA accumulates and decays during tobacco seed development and that β-conglycinin mRNA is undetectable in the tobacco leaf. We utilized *in situ* hybridization to localize β-conglycinin mRNA within the tobacco seed. β-Conglycinin mRNA is not detectable within the endosperm but is localized within specific embryonic cell types. The highest concentration of β-conglycinin mRNA is found in cotyledon storage parenchyma cells. We conclude that sequences required for embryo expression, temporal control, and cell specificity are linked to the β-conglycinin gene, and that factors regulating β-conglycinin gene expression are compartmentalized within analogous soybean and tobacco seed regions.**

β-Conglycinin is a storage protein that accumulates to high levels in the soybean seed (1, 2). The major β-conglycinin polypeptides, designated α′, α, and β (3, 4), are encoded by 2.5-kilobase (kb) (α′/α) and 1.7-kb (β) mRNAs, respectively (5, 6). β-Conglycinin mRNAs are highly homologous, differ primarily by the presence or absence of a 0.56-kb insertion sequence, and are transcribed from different genes (refs. 7 and 8; also unpublished data). The β-conglycinin gene family contains at least 15 members that are clustered in small domains on at least two different chromosomes (ref. 9; J.J.H. and R.B.G., unpublished results). Most β-conglycinin genes either encode or are preferentially homologous with the 1.7-kb β-subunit mRNA (J.J.H. and R.B.G., unpublished results).

β-Conglycinin gene expression is highly regulated during the soybean life cycle. β-Conglycinin mRNA accumulates at precise embryonic stages, is less prevalent in the axis than the cotyledons, and is undetectable in mature plant organ systems (5, 8, 10, 11). In addition, the 2.5-kb α′/α mRNA accumulates prior to the 1.7-kb β mRNA during embryogenesis (refs. 8 and 10; J.J.H. and R.B.G., unpublished results). Transcriptional control processes are responsible for restricting β-conglycinin gene expression to the embryonic phase of the life cycle (5, 11). By contrast, posttranscriptional events regulate β-conglycinin mRNA differential accumulation (unpublished data). Neither selective gene amplification nor detectable differences in methylation patterns accompany the developmental changes in β-conglycinin gene expression (5, 11).

The DNA sequences and cellular factors responsible for controlling β-conglycinin gene expression have not yet been identified. To begin to identify cis control regions, Beachy *et al.* (12) and Chen *et al.* (13) demonstrated that an α′-subunit gene is expressed during petunia seed development and that a 0.09-kb region located within 0.26 kb of the 5′ gene end is required for high seed expression levels. It has not yet been established, however, whether β-conglycinin mRNA or any other seed protein mRNA is spatially distributed to the correct cell types in transformed seeds. Nor has it been established whether or not β-conglycinin mRNA accumulates and decays during seed development in transformed plants as occurs in soybean.

Here, we introduced a β-conglycinin β-subunit gene into tobacco plants. Using *in situ* hybridization, we demonstrate that β-conglycinin mRNA is detectable only within the tobacco embryo and is localized preferentially within cotyledon and upper axis cells. In addition, we show that β-conglycinin mRNA is not detectable in the tobacco leaf, and that β-conglycinin mRNA appears and disappears at precise stages of tobacco seed development. We conclude that sequences required for both temporal control and cell specificity are linked to the β-subunit gene and that regulatory molecules that control β-conglycinin gene expression are spatially distributed within analogous tobacco and soybean seed regions.

MATERIALS AND METHODS

Isolation of a β-Conglycinin Plasmid. The characteristics of the β-conglycinin cDNA plasmid A-16 have been described (5).

DNA Isolation and Labeling. DNAs were isolated as described by Fischer and Goldberg (14) and were labeled by nick-translation (15).

Polysomal mRNA Isolation. Soybean and tobacco polysomal mRNAs were isolated as described (16, 17).

Filter Hybridization Experiments. DNA gel-blot and RNA dot-blot experiments were carried out according to published procedures (18–20). RNA gel-blot studies were performed as described (5) except that nitrocellulose filters were used.

***In Situ* Hybridization Experiments.** *In situ* hybridization studies were carried out as described by Cox *et al.* (21) with modification. In brief, seeds 19 days after pollination (DAP) were fixed in 10% formalin/5% acetic acid/50% ethanol (22), embedded in paraffin, and sliced into 10-μm sections. Single-stranded β-conglycinin ^{35}S-labeled RNA probes were synthesized by using the pGEM transcription system (Promega Biotec). The ^{35}S-labeled RNA probes were hydrolyzed to approximately 0.2 kb in size and then were hybridized with slides containing fixed seed sections for 14 hr at selection criteria of 42°C, 0.3 M Na^+, and 50% formamide (21). A ^{35}S-labeled anti-mRNA probe was used to localize β-conglycinin mRNA within the tobacco seed while a ^{35}S-labeled mRNA probe monitored nonspecific hybridization within the fixed seed section. Following hybridization, the fixed seed sections were incubated with RNase A at 50

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Abbreviation: DAP, days after pollination.

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μg/ml, washed at criteria of 57°C and 0.02 M Na^+ and overlaid with film emulsion (21). Seed sections were stained with 0.5% toluidine blue, and both bright-field and dark-field microscopy were used to visualize cell types and autoradiographic grains.

R-Loop Formation. R loops were formed as described by Fischer and Goldberg (14).

Nuclease S1 Mapping. Nuclease S1 protection experiments were carried out by published procedures (23, 24).

Transformation of Tobacco Plants. Tobacco (*Nicotiana tabacum* cv. Wisconsin 38) leaf disks (25) were transformed with the β-conglycinin gene as described by Okamuro *et al.* (17).

RESULTS

Characterization of a β-Conglycinin Gene Region. We utilized the β-conglycinin cDNA clone A-16 (5) to select β-conglycinin genomic clones from a soybean genomic DNA library. A-16 represents the 2.5-kb α/α′-subunit mRNA but hybridizes with the 1.7-kb β-subunit mRNA as well (5). Fig. 1 shows the β-conglycinin genomic clone, designated λA16-B3, that contains the gene region that was utilized in the transformation studies. Two β-conglycinin genes, designated *CG-4* and *CG-8*, and a nonseed protein gene (NSP) are localized within this 17.3-kb genomic segment (Fig. 1*A*). Both β-conglycinin gene family members hybridized with the 2.5-kb and 1.7-kb β-conglycinin mRNAs but were preferentially homologous with the 1.7-kb β-subunit mRNA (J.J.H. and R.B.G., unpublished results). By contrast, the nonseed protein gene was transcriptionally active during embryogenesis and was represented in leaf and root mRNAs (ref. 11; J. K. Okamuro and R.B.G., unpublished results).

We characterized the *CG-4* β-conglycinin gene by R-loop analysis to visualize its structure in the electron microscope. Fig. 1*B* shows that the *CG-4* β-conglycinin gene contains at least six exons and five introns. To verify this structure directly, we sequenced the *CG-4* β-conglycinin gene and its contiguous regions. A schematic representation of the *CG-4* gene structure is presented in Fig. 1*C*. We determined that the DNA sequence produces an open reading frame with N-terminal amino acids similar to those present in the β-conglycinin β-subunit (4), that the *CG-4* gene contains five introns as predicted by the R-loop analysis, and that it differs from the *CG-1* α′-subunit gene (7) by the absence of a 0.56-kb insertion within the first exon.

We hybridized soybean midmaturation-stage embryo mRNA with a *CG-4* β-conglycinin gene 5′ probe (Fig. 1*C*, probe I) to determine whether this gene was expressed during embryogenesis. Several fragments differing by one nucleotide in length were resistant to nuclease S1 digestion (Fig. 2, lane Soy +), indicating that the *CG-4* gene was represented in embryo mRNA. The fragment producing the strongest signal (0.24 kb) suggests that the transcription start site is 0.03 kb 3′ to a TATA consensus sequence (Fig. 1*C*). Together, these data show that *CG-4* is a functional β-conglycinin gene and that it is clustered within a genomic domain containing a related β-conglycinin gene and a differentially regulated nonseed protein gene.

Representation of the β-Conglycinin Gene Region in Transformed Tobacco Plants. We introduced a 12.3-kb DNA fragment containing the *CG-4* β-conglycinin gene (Fig. 1*A*) into tobacco plants by transforming leaf disk cells with a Ti-plasmid vector (17, 25, 27–29). Two independent transformants, designated TOB-T1 and TOB-T2, were selected for analysis. We hybridized a *CG-4* β-conglycinin probe (Fig. 1*C*, probe II) with TOB-T1 and TOB-T2 DNA gel blots to determine the β-conglycinin gene copy number within the transformed plants. As shown in Fig. 3*A* and schematically presented in Fig. 3*B*, no detectable signals were obtained

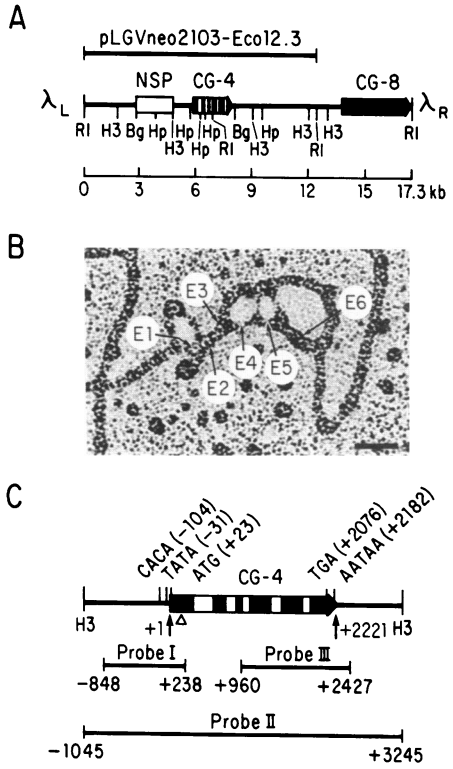

FIG. 1. The soybean *CG-4* β-conglycinin gene. (*A*) Organization of the *CG-4* β-conglycinin gene region. RI, H3, Hp, and Bg refer to *Eco*RI, *Hin*dIII, *Hpa* I, and *Bgl* II restriction endonucleases, respectively. Boxes/arrows represent gene locations/transcriptional orientations, respectively. *CG-4* exons and introns are displayed in black and white, respectively. NSP box refers to a nonseed protein gene and represents the maximum gene length. NSP and *CG-8* gene structures have not yet been characterized. pLGVneo2103-Eco12.3 is the recombinant plasmid used for the gene transfer studies. This plasmid contains the bracketed 12.3-kb *Eco*RI fragment that was obtained from a partial *Eco*RI digestion of λA16-3 phage DNA. (*B*) R-loop analysis of the *CG-4* β-conglycinin gene. λA16-3 DNA was hybridized with excess midmaturation-stage soybean embryo mRNA under conditions that form R loops (14). E1 through E6 designate exons oriented relative to the 5′ and 3′ gene ends. The bar represents approximately 0.6 kb. (*C*) A schematic representation of the *CG-4* β-conglycinin gene. Nucleotides +1 and +2221 designate the 5′ and 3′ ends, respectively. These were obtained from nuclease S1 protection studies (Fig. 2). CACA represents a consensus sequence (5′ CAACACACG 3′) that is present at least once in the 5′ flanking region of all soybean seed protein genes sequenced to date (26). Black and white areas represent exons and introns, respectively. The bracketed lines below the β-conglycinin gene represent DNA probes used in the experiments reported here. Δ refers to the 0.56-kb insertion within the *CG-1* α′-subunit β-conglycinin gene (7) that is absent from *CG-4* at nucleotide +91.

with untransformed tobacco DNA (Fig. 3*A*, lane TOB-C). By contrast, low-copy soybean DNA fragments of the expected size were present in each transformant (Fig. 3*B*, lanes TOB-T1 and TOB-T2). We determined that each tobacco plant contained a single-copy β-conglycinin gene region by using a restriction endonuclease that does not digest within vector DNA, enabling two distinct-sized border fragments to be visualized (data not shown). Together, these findings demonstrate that each tobacco transformant contains one unrearranged 12.3-kb β-conglycinin gene region.

β-Conglycinin Gene Is Expressed Correctly in Transformed Tobacco Plants. We hybridized 14- to 16-DAP tobacco seed mRNA with a *CG-4* 5′ probe (probe I, Fig. 1*C*) to determine whether the β-conglycinin gene was expressed in tobacco. The nuclease S1 assay (Fig. 2, lanes TOB-T1 and -T2) indicates that both TOB-T1 and TOB-T2 seed mRNAs

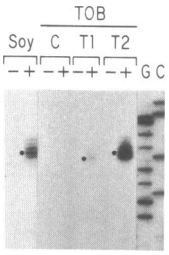

FIG. 2. Nuclease S1 analysis of β-conglycinin gene expression. Probe I (Fig. 1*C*) was hybridized with either soybean midmaturation-stage embryo mRNA or tobacco 14- to 16-DAP seed mRNA under conditions that favor DNA·RNA hybrid formation (24). Lanes: Soy, TOB C, TOB T1, and TOB T2, soybean, untransformed tobacco, transformant-1, and transformant-2 mRNAs, respectively (film exposures of lanes TOB-C, TOB-T1, and TOB-T2 differ from that of the Soy lanes); G and C, sequencing ladders from the same *CG-4* region; − and +, hybridization reactions that were not treated (−) or treated (+) with nuclease S1, respectively. The dots designate the major protected fragment in each experiment. This fragment was taken to be the 5′ terminal nucleotide of the mRNA (Fig. 1*C*).

protected DNA fragments similar in size to those produced with soybean mRNA (Fig. 2, lane Soy$^+$). This result shows that the β-conglycinin gene is expressed in each transformed plant and that the transcription start site is similar to that observed in soybean plants.

We hybridized a *CG-4* probe (Fig. 1*C*, probe II) with gel blots containing tobacco seed and leaf mRNAs to determine whether the β-conglycinin gene was regulated correctly

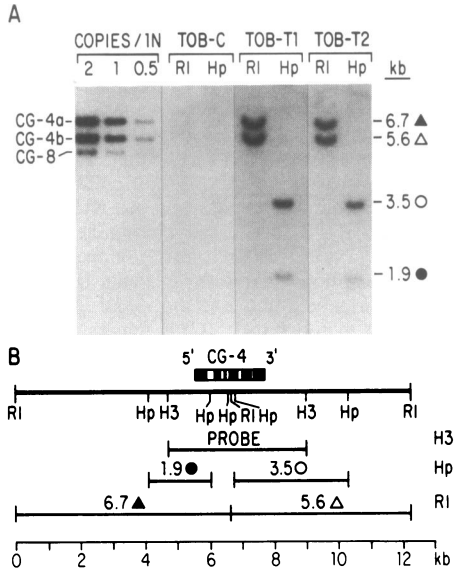

FIG. 3. Representation of the β-conglycinin gene region in transformed tobacco plants. (*A*) DNA gel blot of untransformed and transformed tobacco DNAs. Leaf nuclear DNA from individual plants was digested with restriction endonuclease, fractionated by electrophoresis, transferred to nitrocellulose, and hybridized with *CG-4* gene probe II (Fig. 1*C*). Lanes: Copies/1N, reconstruction lanes containing 0.5-, 1-, and 2-copy equivalents of *Eco*RI-digested λA16-3 DNA [DNA copy equivalents were calculated using a tobacco genome size of 2.4×10^6 kb (30)]; TOB-C, untransformed tobacco; TOB-T1, transformant 1 mRNA; TOB-T2, transformant 2 mRNA. CG-4a and CG-4b refer to the 6.7-kb and 5.6-kb *CG-4 Eco*RI fragments, while *CG-8* refers to the 5-kb *CG-8 Eco*RI fragment (Fig. 1*A*). The reduced *CG-8* hybridization signal indicates that this gene has diverged from the *CG-4* β-conglycinin gene. ▲ △, *Eco*RI DNA fragments; ● ○, *Hpa* I DNA fragments. (*B*) Map of relevant restriction endonuclease sites within the *CG-4* β-conglycinin gene region. RI, H3, and Hp refer to *Eco*RI, *Hin*dIII, and *Hpa* I restriction endonucleases, respectively. CG-4 exons and introns are represented by black and white boxes, respectively.

during the tobacco life cycle. Fig. 4 shows that the *CG-4* probe produced a 1.7-kb signal with both TOB-T1 and TOB-T2 seed mRNAs. Densitometric analysis indicated that these signals were 1/20th (TOB-T2) to 1/100th (TOB-T1) of that obtained with soybean mid-maturation stage embryo mRNA. We presume that this prevalence difference is due to position effect because TOB-T1 and TOB-T2 plants contain a single-copy β-conglycinin gene insert at distinct genomic locations (Fig. 3). By contrast, no detectable signals were obtained with untransformed tobacco seed and leaf mRNAs (Fig. 4, lanes TOB-C), or with transformed tobacco leaf mRNA (Fig. 4, lanes L of TOB-T1 and TOB-T2). Together, these results show that the 1.7-kb soybean β-conglycinin mRNA is produced in the correct developmental state within each transformed tobacco plant.

β-Conglycinin Gene Expression Is Regulated Temporally During Seed Development. We isolated transformed tobacco seed mRNA at different times after pollination and hybridized each mRNA with a β-conglycinin probe (Fig. 1*C*, probe II) to determine whether the β-conglycinin gene was regulated temporally during seed development. We constructed a timetable for tobacco seed development to specify stages from which mRNAs were isolated (data not shown). Fig. 5*A* shows that β-conglycinin mRNA accumulates and decays during tobacco seed development. Transformed tobacco leaf and untransformed tobacco seed mRNAs did not produce hybridization signals (Fig. 5*B*). At its peak prevalence (23 DAP), β-conglycinin mRNA represented approximately 0.5% of the TOB-T2 tobacco seed mRNA mass or approximately 1/20th of that observed during soybean embryogenesis (Fig. 5*C*). We conclude that the soybean β-conglycinin gene displays a precise temporal expression pattern during tobacco seed development and that this pattern is analogous to that which occurs in soybean (5, 11).

Soybean β-Conglycinin mRNA Is Concentrated Within Specific Tobacco Seed Regions. We utilized *in situ* hybridization (21) to localize β-conglycinin mRNA within transformed tobacco seed sections (22). Fig. 6*A* shows a bright-field micrograph of a 19-DAP tobacco seed longitudinal section that was stained with toluidine blue. A prominent embryo (E) with well-developed cotyledons (C) and a hypocotyl-radicle axis (A) is embedded within several layers of nonembryonic

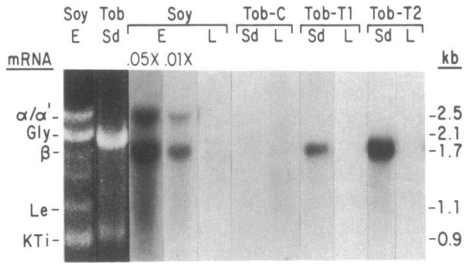

FIG. 4. β-conglycinin gene expression in transformed tobacco plants. Soybean and tobacco mRNAs were fractionated by electrophoresis on a methylmercury(II) hydroxide-agarose gel, stained with ethidium bromide to visualize mRNA bands, and transferred to nitrocellulose paper. The RNA gel blots were hybridized with *CG-4* gene probe II (Fig. 1*C*). Lanes: Soy, TOB-C, TOB-T1, and TOB-T2, soybean, untransformed tobacco, and transformed tobacco mRNAs 1 and 2, respectively; E, Sd, and L, embryo, seed, and leaf mRNAs, respectively, at 5 μg of mRNA; soybean embryo 0.05× and 0.01×, 0.25 μg and 0.05 μg of midmaturation-stage mRNA representing ≈1000 and 250 molecules per cell equivalents of β-conglycinin mRNA (5). α/α′, Gly, β, Le, and KTi refer to mRNAs encoding the β-conglycinin α/α′-subunit, glycinin, β-conglycinin β subunit, lectin, and Kunitz trypsin inhibitor seed proteins, respectively. Seed protein mRNA sizes have been described previously (5, 14). The prevalent 1.9-kb tobacco seed mRNA (lane Tob Sd) hybridized weakly with a soybean β-conglycinin gene probe (data not shown), suggesting that it encodes an endogenous tobacco storage protein.

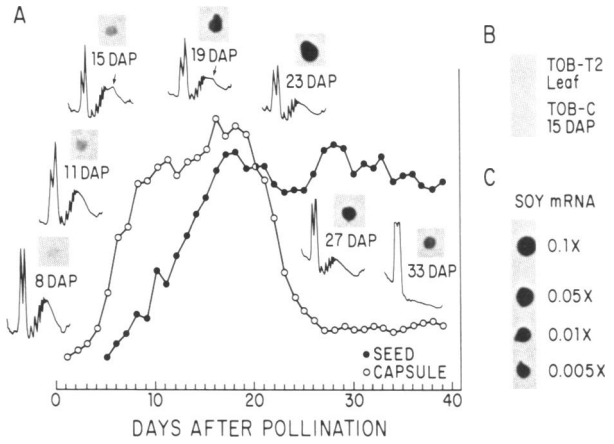

FIG. 5. β-conglycinin gene expression during tobacco seed development. (*A*) Representation of β-conglycinin transcripts in tobacco seed mRNA. One microgram of TOB-T2 mRNA from different developmental stages was spotted onto nitrocellulose and hybridized with a *CG-4* gene probe (Fig. 1C, probe II). ●, Seed dry weight in milligrams per 100 seeds; ○, fruit capsule fresh weight in grams. Fruit capsule fresh weight and seed dry weight at 19 DAP were 1.4 g and 7.7 mg, respectively. Polysome profiles for seeds at different DAP are shown below the dot-blot signals. The arrow indicates a 15-mer polysome that probably contains the 1.9-kb tobacco storage protein mRNA visualized in Fig. 4 (lane Tob Sd). (*B*) Hybridization of the β-conglycinin gene probe with transformed tobacco leaf mRNA (TOB-T2) and untransformed tobacco seed mRNA (TOB-C). Each spot contained one microgram of mRNA. (*C*) Soybean mRNA standards used to calibrate β-conglycinin mRNA prevalences. The 0.1×, 0.05×, 0.01×, and 0.005× spots contained 0.1 μg, 0.05 μg, 0.01 μg, and 0.005 μg of soybean midmaturation-stage mRNA and correspond to β-conglycinin mRNA prevalences of 1%, 0.5%, 0.1%, and 0.05%, respectively (5).

endosperm cells (En). In addition, a provascular cylinder (V) is apparent within both the cotyledons and the axis.

The dark-field micrograph (Fig. 6*B*) indicates that β-conglycinin ^{35}S-labeled anti-mRNA (Fig. 1*C*, probe III) hybridized intensely with RNA present in embryo cotyledon cells and upper axis cells. By comparison with the ^{35}S-labeled mRNA control (Fig. 6*C*), no grains above background were observed in the endosperm or in the lower axis embryonic region. Shorter exposures of a seed section analogous to that presented in Fig. 6*B* (data not shown) and hybridization with sections sliced in different planes (L. Perez-Grau and R.B.G., unpublished results) indicated that β-conglycinin mRNA was present in embryo storage parenchyma cells but undetectable in cells of the provascular cylinder. These findings indicate that the β-conglycinin mRNA concentration differs significantly within the seed and within different embryonic regions and cell types. Assuming that endosperm and embryo cells have similar mRNA contents, we estimate that the actual β-conglycinin mRNA prevalence in the embryo is at least 2% of the mRNA mass or only one-fifth that measured in soybean for the family as a whole (5). We conclude that soybean β-conglycinin mRNA is spatially regulated within the tobacco seed and that the β-conglycinin gene has an embryo-specific gene expression program similar to that observed in soybean.

DISCUSSION

Soybean *CG-4* β-Conglycinin Gene Region. We utilized a 12.3-kb genomic segment containing a β-subunit β-conglycinin gene for the gene transfer studies reported here (Fig. 1). Closely linked to the *CG-4* gene is a β-subunit gene relative (*CG-8*) and an unidentified gene that is expressed during embryogenesis and in the mature plant. DNA walking experiments showed that within 45 kb no other β-conglycinin genes are contiguous to *CG-4* and *CG-8* (unpublished data). By contrast, other regions exist that contain up to five interspersed α′/α-subunit and β-subunit β-conglycinin genes (unpublished data). Close linkage of differentially expressed genes also occurs in the glycinin, lectin, and leghemoglobin gene regions (14, 17, 31) and appears to be a general characteristic of the soybean genome (17). We infer that each gene within the *CG-4* β-conglycinin region has a unique regulatory domain that programs its expression during the soybean life cycle, at least at the transcriptional level.

***CG-4* β-Conglycinin Gene Is Expressed Correctly in Transformed Tobacco Plants.** Both genetic and molecular analyses indicated that a single-copy soybean gene is present in each transformed tobacco plant (Fig. 3). Nuclease S1 (Fig. 2), RNA gel-blot (Fig. 4), and RNA dot-blot (Fig. 5) studies demonstrated that the 1.7-kb β-subunit mRNA is present at prevalent levels in developing tobacco seeds, that this mRNA

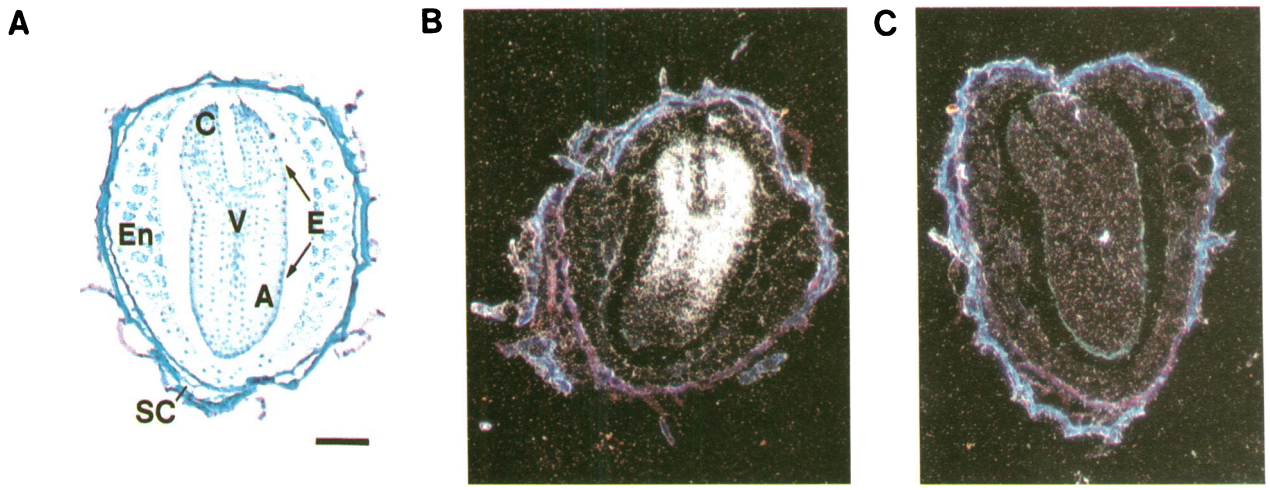

FIG. 6. Localization of β-conglycinin mRNA in transformed tobacco seeds. TOB-T2 19-DAP tobacco seeds were fixed, embedded in paraffin, sectioned, and hybridized *in situ* with single-stranded ^{35}S-labeled RNA probes (Fig. 1*C*, probe III) as outlined. (*A*) Longitudinal section of a tobacco seed. SC, E, En, A, C, and V refer to seed coat, embryo, endosperm, hypocotyl-radicle axis, cotyledons, and provascular cylinder, respectively (22). The photograph was taken using bright-field microscopy. (Bar = 100 μm.) (*B*) Hybridization with a ^{35}S-labeled anti-mRNA probe. White grains represent regions containing RNA·RNA hybrids. The photograph was taken with dark-field microscopy. (*C*) Hybridization with a ^{35}S-labeled mRNA probe. White grains represent background hybridization levels. The photograph was taken with dark-field microscopy.

is undetectable in the tobacco leaf, and that β-conglycinin mRNA accumulates and decays during tobacco seed development. This regulated β-conglycinin gene expression program is qualitatively indistinguishable from that observed in the soybean plant (5, 6, 11).

We have not yet carried out transcription experiments with the transformed tobacco plants analogous to those reported for soybean (11). However, we infer from our results that the transcriptional controls responsible for activating and repressing β-conglycinin gene expression during seed development and the posttranscriptional processes that operate to degrade β-conglycinin mRNA prior to seed dormancy are highly conserved in tobacco and soybean. This inference probably applies to legume seed protein genes in general because several different gene classes (e.g., storage protein, trypsin inhibitor, and lectin) have been shown to be expressed correctly in solanaceous plants (refs. 12, 13, 17, and 32; K. D. Jofuku and R.B.G., unpublished results). We presume that molecules regulating soybean seed protein gene expression have counterparts within the tobacco seed. Whether these molecules are identical or highly related to those in soybean remains to be determined; however, they must be sufficiently homologous in order to recognize soybean control sequences that participate in transcriptional and posttranscriptional regulatory processes.

β-Conglycinin mRNA Is Present Within Specific Tobacco Embryo Cell Types. A clue to the distribution of molecules that control β-conglycinin gene expression within the tobacco seed was obtained from the *in situ* hybridization experiments presented in Fig. 6. No detectable hybridization above background was observed within the endosperm (Fig. 6 *B* and *C*). By contrast, β-conglycinin mRNA was detected within the embryo and was preferentially localized within the cotyledons and upper axis cells (Fig. 6*B*). Greenwood and Chrispeels (33) and Sengupta-Gopalan *et al.* (32) showed that the bean storage protein phaseolin is present primarily in the embryo of transformed tobacco seeds, although a small quantity of protein is found in the endosperm. Our mRNA localization studies indicate that soybean β-conglycinin mRNA is only detectable in the tobacco embryo. Thus, β-conglycinin mRNA is either absent entirely from the tobacco endosperm or is present at a concentration too low to be detected by our *in situ* hybridization procedure.

By comparison with tobacco, soybean seeds are composed almost entirely of embryonic cells and possess a vestigial endosperm (34). During early seed development, however, a significant mass of endosperm tissue surrounds the developing embryo (34). Recently, we localized β-conglycinin mRNA sequences within developing soybean seeds, using *in situ* hybridization (L. Perez-Grau and R.B.G., unpublished data). β-Conglycinin transcripts are only detectable during the maturation phase of embryogenesis and are confined to the embryo. Thus, the distribution of β-conglycinin mRNA within the tobacco seed approximates that which is observed within soybean. We conclude that sequences required for embryonic expression, temporal control, and cell specificity are contained within the 12.3-kb β-conglycinin DNA segment and are probably less than 1 kb 5′ and/or 4 kb 3′ to the *CG-4* β-subunit gene (Fig. 1*A*). Whether the temporal and spatial β-conglycinin gene expression programs are controlled by a single regulatory element or several different elements, and the relationship, if any, between α′-subunit (12, 13) and β-subunit control sequences is not yet known.

We showed recently that a soybean embryo DNA binding protein interacts with a specific soybean lectin gene 5′ region and that its activity parallels lectin gene transcriptional levels during the soybean life cycle (35). The β-conglycinin gene competes with the lectin gene for this DNA binding protein (C. Reeves and R.B.G., unpublished results). Although we have no information regarding the physiological relevance of this DNA binding protein, it may perform some role in regulating seed protein gene transcription. A major conclusion of the present study is that factors regulating seed protein gene expression are compartmentalized within specific regions of soybean and tobacco seeds. What these factors are and how they become spatially distributed during embryogenesis remain to be determined.

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1. Hill, J. E. & Breidenbach, R. W. (1974) *Plant Physiol.* **53,** 742–746.
2. Hill, J. E. & Breidenbach, R. W. (1974) *Plant Physiol.* **53,** 747–751.
3. Thanh, V. H. & Shibasaki, K. (1978) *J. Agric. Food Chem.* **26,** 692–695.
4. Coates, J. B., Medeiros, J. S., Thanh, V. H. & Nielsen, N. C. (1985) *Dev. Biol.* **83,** 218–231.
5. Goldberg, R. B., Hoschek, G., Ditta, G. S. & Breidenbach, R. W. (1981) *Dev. Biol.* **83,** 218–231.
6. Beachy, R. N., Jarvis, N. P. & Barton, K. A. (1981) *J. Mol. Appl. Genet.* **1,** 19–27.
7. Doyle, J. J., Schuler, M. A., Gudette, W. D., Zenger, V., Beachy, R. N. & Slightom, J. L. (1986) *J. Biol. Chem.* **261,** 9228–9238.
8. Ladin, B. F., Tierney, M. L., Meinke, P., Hosangadi, P., Veith, M. & Beachy, R. N. (1987) *Plant Physiol.* **84,** 35–41.
9. Davies, C. S., Coates, J. B. & Nielsen, N. C. (1985) *Theor. Appl. Genet.* **71,** 351–358.
10. Meinke, D. W., Chen, J. & Beachy, R. N. (1981) *Planta* **153,** 130–139.
11. Walling, L., Drews, G. N. & Goldberg, R. B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2123–2127.
12. Beachy, R. N., Chen, Z. L., Horsch, R. B., Rogers, S. G., Hoffman, N. J. & Fraley, R. T. (1985) *EMBO J.* **4,** 3047–3053.
13. Chen, Z. L., Schuler, M. A. & Beachy, R. N. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8560–8564.
14. Fischer, R. L. & Goldberg, R. B. (1982) *Cell* **29,** 651–660.
15. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
16. Goldberg, R. B., Hoschek, G., Tam, S. H., Ditta, G. S. & Breidenbach, R. W. (1981) *Dev. Biol.* **83,** 218–231.
17. Okamuro, J. K., Jofuku, K. D. & Goldberg, R. B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8240–8244.
18. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
19. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3693–3697.
20. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
21. Cox, K. H., DeLeon, D. V., Angerer, L. M. & Angerer, R. C. (1984) *Dev. Biol.* **101,** 485–502.
22. Avery, G. S. (1933) *Am. J. Bot.* **20,** 309–327.
23. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–722.
24. Rosbash, M., Blank, D., Fahrner, K., Hereford, L., Ricciardi, R., Roberts, B., Ruby, S. & Woolford, J. (1979) *Methods Enzymol.* **68,** 454–469.
25. Horsch, R. B., Fry, J. E., Hoffman, N. L., Eichholtz, D., Rogers, S. E. & Fraley, R. T. (1985) *Science* **227,** 1229–1231.
26. Goldberg, R. B. (1986) *Philos. Trans. R. Soc. London Ser. B* **314,** 343–353.
27. DeBlock, M., Herrera-Estrella, L., Van Montagu, M., Schell, J. & Zambryski, P. (1984) *EMBO J.* **3,** 1681–1689.
28. Zambryski, P., Joos, H., Genetello, C., Leemans, J., Van Montagu, M. & Schell, J. (1983) *EMBO J.* **2,** 2143–2150.
29. Hain, R., Czernilofsky, A. P., Steinbiss, H. N., Herrera-Estrella, L. & Schell, J. (1985) *Mol. Gen. Genet.* **199,** 161–168.
30. Okamuro, J. K. & Goldberg, R. B. (1985) *Mol. Gen. Genet.* **198,** 290–298.
31. Lee, J. S., Brown, G. G. & Verma, D. P. S. (1983) *Nucleic Acids Res.* **11,** 5541–5553.
32. Sengupta-Gopalan, C., Reichert, N. A., Barker, R. F., Hall, T. L. & Kemp, J. D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3320–3324.
33. Greenwood, J. S. & Chrispeels, M. J. (1985) *Plant Physiol.* **79,** 65–71.
34. Carlson, J. B. (1973) in *Soybeans: Improvement, Production, and Use*, ed. Caldwell, B. E. (Am. Soc. Agronomy, Madison, WI), pp. 17–95.
35. Jofuku, K. D., Okamuro, J. K. & Goldberg, R. B. (1987) *Nature (London)* **328,** 734–737.