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RESEARCH ARTICLE

Light-Regulated Expression of a Pea Ribulose-1,5-Bisphosphate Carboxylase Small Subunit Gene in Transformed Plant Cells

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The enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) (E. C. 4.1.1.39) catalyzes the fixation of carbon dioxide in photosynthetic organisms. In higher plant chloroplasts the holoenzyme is composed of eight copies each of two nonidentical subunits; a large subunit (rbcL) encoded by chloroplast DNA (*1, 2*) and a small subunit (rbcS) encoded by nuclear DNA (*3*). Recent chloroplast transport studies in vitro have demonstrated that the rbcS polypeptide is synthesized as a larger precursor that is imported into chloroplasts by an energy-dependent (*4*), posttranslational process (*3*). The genes coding for rbcS exhibit a diverse array of regulatory properties. In peas, the rbcS polypeptide is a major product of cytoplasmic protein synthesis in leaves, but is either absent or present in reduced amounts in other plant parts (*5*). In plants that utilize the C_4 photosynthetic pathway, both rbcS and rbcL subunits are present in bundle sheath cells but are absent from mesophyll cells (*6*). In tissues containing Rubisco, the expression of the nuclear and chloroplast genes encoding the polypeptide subunits are controlled by light (*5, 7*), and this effect is mediated by phytochrome (*8*).

Complementary DNA (cDNA) clones encoding rbcS have been isolated from peas (*9, 10*) and wheat (*11*); they have been used to isolate the corresponding nuclear genes (*5, 11*) and study their organization within the genome. Southern blot analysis (*12*) of nuclear DNA from these and several other higher plants have revealed that the rbcS is encoded by a multigene family (*5, 11, 13–15*).

One approach to understanding the tissue-specific, light-dependent expression of rbcS is to construct mutations in putative regulatory regions and study their effects on gene expression (*16*). Such experiments require a system for plant cell transformation. We therefore used tumor-inducing (Ti) plasmid vectors to introduce a pea rbcS gene into petunia cells (*17*). During transformation of susceptible plant cells by virulent strains of *Agrobacterium tumefaciens*, a segment of the Ti plasmid, called T (transferred) DNA, is inserted and stably incorporated into the nuclear DNA (*18*). We now show that a pea rbcS gene (pPS4.0) is expressed after its transfer into petunia cells. The expression of the pea rbcS gene is under the transcriptional control of its own promoter and is regulated by light in a manner similar to that observed in pea leaves. Messenger RNA (mRNA) transcripts from the transferred pea gene are translated to yield mature rbcS polypeptides that assemble with endogenous petunia rbcL polypeptides to form heterologous holoenzymes.

Transformation of petunia protoplasts. The plasmid pMON145 intermediate vector is a variant of the previously described pMON120 (*17*). It contains the 1.6-kilobase (kb) Pvu II to Pvu I segment of pBR322 that carries the origin of replication and *bom* site (*19*) joined to a 2.7-kb segment Cla I to Eco RI of Tn7 (Fig. 1), which contains the spectinomycin-streptomycin resistance determinant

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(*20*). Additional components include a 400-bp segment (Pvu I to Sst II) of the pTiT37 Hind III–23 fragment (*21*), which contains the pTiT37 T-DNA right border (*21*) and a 2.6-kb fragment (Sst II to Cla I) of pMON128 (*17*) carrying the nopaline synthase promoter joined to the Tn5 neomycin phosphate transferase II coding sequence which is followed by the nopaline synthase polyadenylation site. This chimeric gene from pMON128 confers kanamycin resistance on transformed plant cells (*17*). The final component is a 1.6-kb fragment (Bgl II to Hind III) of the pTiB6S3 T-DNA to provide homology for cointegrate formation by recombination with a resident, octopine-type Ti plasmid in *A. tumefaciens* cells (*22*).

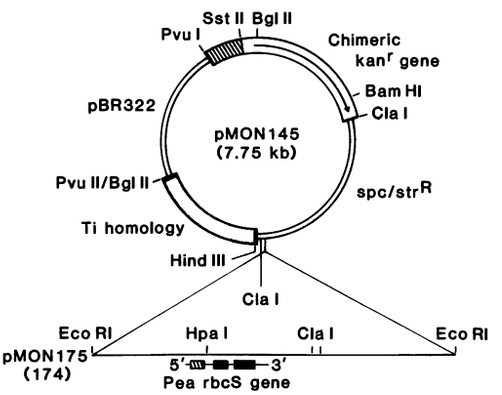
To construct pMON174 and -175 (Fig. 1) the vector, pMON145, was cleaved at the single Eco RI site, and a 4-kb Eco RI fragment encoding a pea rbcS gene (pPS4.0) (*5*) was inserted in both orientations. The chimeric plasmids were then transferred to *A. tumefaciens* cells by means of the triparental procedure (*17*) to yield cointegrates with a resident pTiB6S3 plasmid.

Cells of *A. tumefaciens* that contained pMON145, -174, or -175 cointegrate plasmids were cocultivated with petunia protoplasts (*23, 24*). The cell suspension was incubated for 2 days in fresh culture medium containing carbenicillin (500 μg/ml) to prevent further bacterial growth; the cell mixture was then transferred to and spread in a thin layer on the surface of double-filter feeder plates (*25*). After 7 to 10 days, microcolonies (0.5 mm) were observable on the feeder plates, and the transfer disk was removed and placed on selection medium lacking phytohormones. Within 2 weeks, hormone-independent transformants could be distinguished as green colonies at a transformation frequency of 10^{-1} (*26*). The hormone-independent transformants were pooled and propagated on the above medium containing kanamycin (50 μg/ml) before DNA and RNA analysis.

Expression of the pea rbcS gene in petunia cells. Southern blot hybridization analysis (*12*) of DNA isolated from pMON174 and pMON175 transformants (Fig. 2) confirm the presence of the expected 4-kb Eco RI fragment of pPS4.0 encoding the rbcS gene. For this experiment a cDNA clone, pSS15 (*10*), encoding the pea rbcS polypeptide was used as a hybridization probe. The presence of a single band of hybridization corresponding to the 4-kb pea DNA fragment, indicates that, at this level of sensitivity, the probe is able to discriminate between the pea rbcS gene and endogenous petunia rbcS genes. Reconstruction experiments with the purified 4-kb Eco RI fragment reveal that the transformed petunia calli contain one to five copies of the pea rbcS gene, pPS4.0.

Abstract. *A pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase was inserted into the tumor-inducing (Ti) plasmid of* Agrobacterium tumefaciens *and transferred into petunia cells by in vitro transformation. The transferred pea rbcS gene is expressed in petunia cells under the transcriptional control of its own promoter in a light-dependent fashion similar to that observed in pea leaves. In contrast, a nonphotosynthetic chimeric gene containing a nopaline synthase promoter is expressed constitutively in both light- and dark-grown tissues. In the transformed cells, transcripts from the pea rbcS gene are processed correctly and translated to yield an authentic pea small subunit polypeptide which is localized in chloroplasts.*

Transcription of the pea rbcS gene in transformed petunia calli was studied by Northern blot analysis (*27*). Polyadenylated RNA, isolated from pea and petunia leaves and from transformed calli, was denatured, resolved by electrophoresis in a 1 percent agarose gel, and transferred to nitrocellulose filters (*27*). Initially, RNA blots were hybridized with nick-translated (*28*) pSS15. Calli transformed by pMON174 (Fig. 3A, lane 4) or pMON175 (lane 5) exhibit a 50-fold increase in hybridization as compared to calli transformed by pMON145. The weak signal in the latter can be accounted for by cross-hybridization to endogenous petunia rbcS messenger RNA's (mRNA). The extent of cross-hybridization to the pea rbcS probe can be estimated by comparing the hybridization efficiency of polyadenylated RNA from pea and petunia leaves (Fig. 3A, lanes 1 and 2). We estimate that under stringent washing conditions, the level of heterologous hybridization is approximately 2 to 5 percent of that with the homologous pea leaf mRNA. To confirm that the increase in hybridization intensity observed with pMON174 and pMON175 transformants is due to transcription of the inserted pea rbcS gene and not to activation of endogenous petunia rbcS genes, we hybridized the same RNA



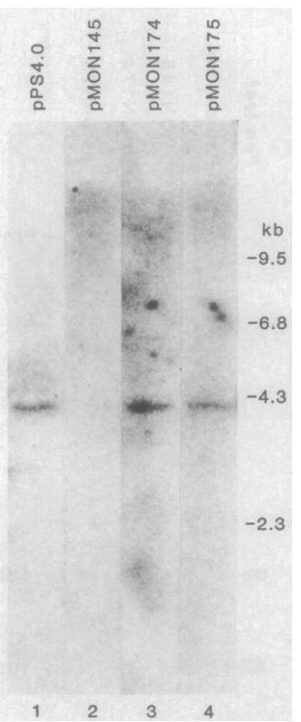


Fig. 1 (left). Structure of pMON145 intermediate vector and pMON174 and pMON175 chimeric plasmids. The plasmids pMON174 and pMON175 contain a 4.0-kb Eco RI fragment of pea DNA encoding the rbcS gene inserted into the single Eco RI site of pMON145 in the same (pMON174) or the opposite (pMON175) orientation with respect to the chimeric kan^r gene. Fig. 2 (right). Southern blot analysis (*12*) of DNA from transformed petunia calli. The hybridization probe used was a gel-purified cDNA clone (pSS15) encoding the pea rbcS gene (*10*). Lanes contained the following DNA samples: (lane 1) Five-copy reconstruction with gel-purified 4.0-kb Eco RI pea DNA fragment; (lane 2) pMON145 transformants (10 μg); (lane 3) pMON174 transformants (10 μg); and (lane 4) pMON175 transformants (10 μg). DNA was isolated from transformed calli (*5*) and samples were digested to completion with Eco RI and electrophoresed through a 0.8 percent agarose gel. Labeled DNA probes were prepared by nick translation (*28*) with the use of all four radioactive ^{32}P-labeled nucleotide triphosphates. Southern blotting, hybridization, and washing were preformed as described (*5*). Hind III fragments of lambda DNA were used as size markers.

samples to a 550-bp Dde I–Cla I fragment derived from the 3′ noncoding region of the pea rbcS gene, pPS4.0 (Fig. 4B). Individual members of rbcS multigene families show nucleotide sequence heterogeneity in their 3′ noncoding regions, and these sequences can be used as specific hybridization probes to discriminate among members of the same gene family (*5, 11*). Thus, in a Southern blot analysis of pea nuclear DNA, the 3′ noncoding probe of pPS4.0 hybridizes specifically to a single Eco RI fragment; in contrast, probes containing DNA sequences that code for the rbcS structural polypeptide hybridize to at least five different Eco RI fragments (*5*). When this probe is used in a Northern analysis of RNA from transformed calli (Fig. 3B), only those calli containing the pea rbcS gene (pPS4.0) show strong hybridization signals (lanes 4 and 5). No hybridization can be detected with RNA from petunia leaves or control pMON145 transformants (lanes 2 and 3).

As a positive control for those samples that do not hybridize, the RNA blots were also probed with a cDNA clone encoding pea polypeptide 15, the major chlorophyll a and b binding protein (*10*). This probe is homologous to RNA encoding polypeptide 15 from both pea and petunia leaves, as well as from transformed calli (Fig. 3C).

The pea rbcS gene (pPS4.0) used to construct pMON174 and pMON175 is of sufficient size to contain putative transcriptional regulatory sequences in addition to those coding for the rbcS structural polypeptide. Our Northern hybridization experiments show that the pea rbcS gene is transcribed in petunia calli regardless of its orientation (with respect to the chimeric kan^r gene) in the pMON174 and pMON175 plasmids. Moreover, the size of the RNA transcript detected in both instances is identical to that in pea leaves. Together, these results suggest that transcription initiation of the pea rbcS gene is controlled from its own promoter rather than from sequences located elsewhere in the plasmid vector.

The 5′ transcription initiation site of the rbcS gene, pPS4.0, in pea leaves is

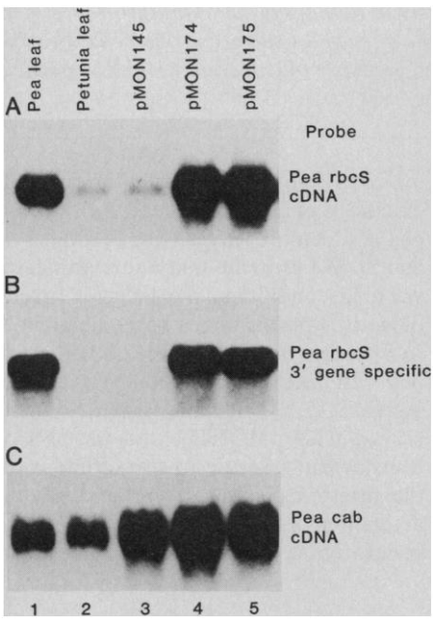


Fig. 3. Northern blot analysis of petunia callus transcripts (*27*). Lanes 1 to 5 contained polyadenylated RNA samples isolated from: (lane 1) pea leaf (60 ng); (lane 2) petunia leaf (60 ng); (lane 3) pMON145 transformants (3 μg); (lane 4) pMON174 transformants (3 μg); and (lane 5) pMON175 transformants (3 μg). The RNA blots were hybridized with (A) pea rbcS cDNA insert (pSS15) (*10*); (B) a probe specific for the 3′ nontranslated region of the pea rbcS gene (see Fig. 5B); and (C) pea cab cDNA insert (pAB96) coding for polypeptide 15, the major thylakoid polypeptide that binds chlorophyll a and b (*10*). RNA was isolated with guanidinium thiocyanate as a protein denaturant (*43*), and polyadenylated RNA was isolated by chromatography on polyuridylate-Sepharose (*10*). The filters were hybridized with nick-translated (*28*) probes (specific activity 1×10^8 to 4×10^8 cpm/μg) (*5, 11*) and were washed twice in 2× SSC, 0.5 percent sarkosyl, 0.2 percent sodium pyrophosphate for 1 hour at 25°C and then for 1 to 2 hours at 55°C in either the same solution (panel C) or a solution containing 0.1× SSC, 0.05 percent sarkosyl, and 0.2 percent sodium pyrophosphate (panels A and B).

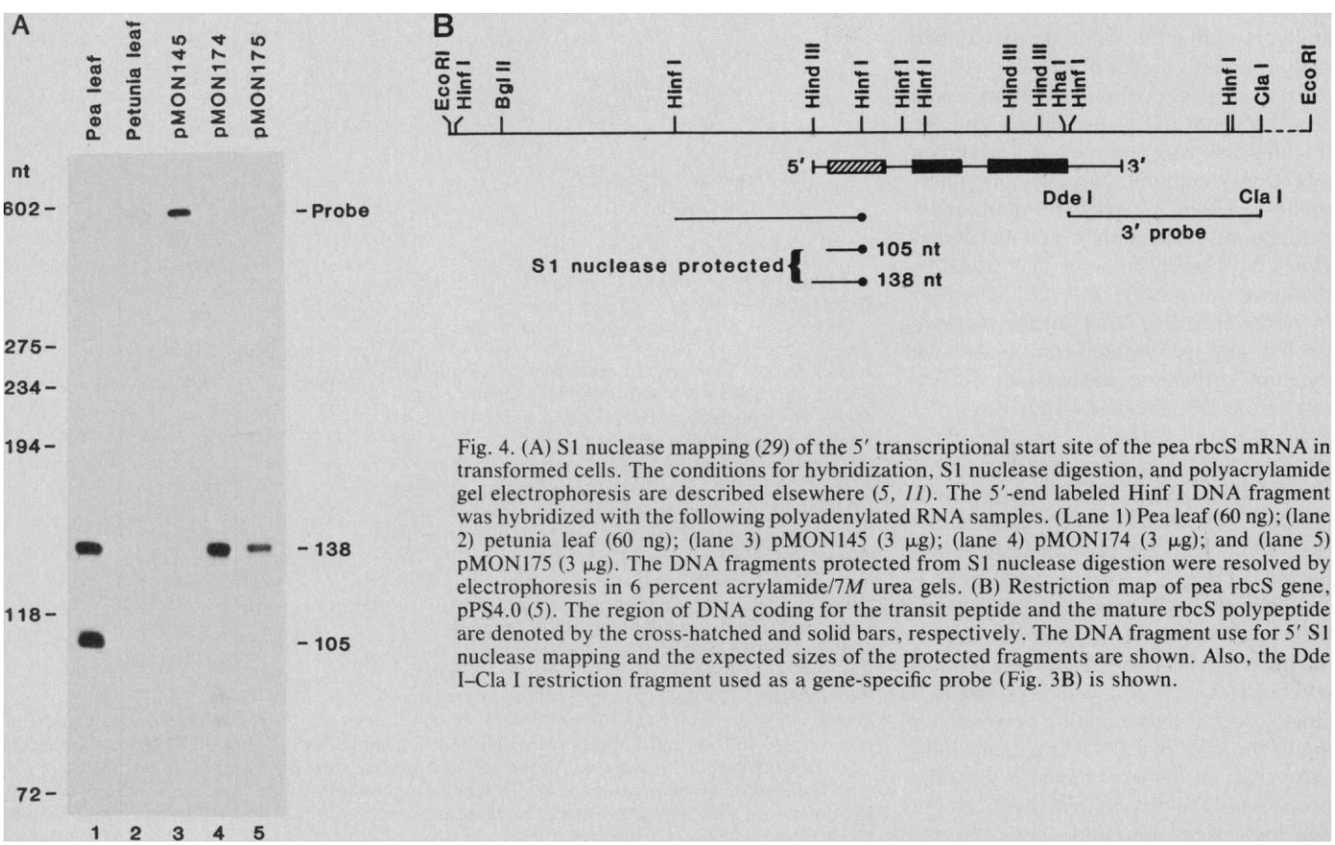


Fig. 4. (A) S1 nuclease mapping (*29*) of the 5′ transcriptional start site of the pea rbcS mRNA in transformed cells. The conditions for hybridization, S1 nuclease digestion, and polyacrylamide gel electrophoresis are described elsewhere (*5, 11*). The 5′-end labeled Hinf I DNA fragment was hybridized with the following polyadenylated RNA samples. (Lane 1) Pea leaf (60 ng); (lane 2) petunia leaf (60 ng); (lane 3) pMON145 (3 μg); (lane 4) pMON174 (3 μg); and (lane 5) pMON175 (3 μg). The DNA fragments protected from S1 nuclease digestion were resolved by electrophoresis in 6 percent acrylamide/7*M* urea gels. (B) Restriction map of pea rbcS gene, pPS4.0 (*5*). The region of DNA coding for the transit peptide and the mature rbcS polypeptide are denoted by the cross-hatched and solid bars, respectively. The DNA fragment use for 5′ S1 nuclease mapping and the expected sizes of the protected fragments are shown. Also, the Dde I–Cla I restriction fragment used as a gene-specific probe (Fig. 3B) is shown.

situated 33 nucleotides upstream of the initiator ATG (A, adenine; T, thymine; G, guanine) (*5*). To determine the 5′ terminus of the pea rbcS mRNA in transformed calli we performed an S1 nuclease mapping experiment (*29*). The probe was a 5′ end-labeled Hinf I fragment that encodes the first 35 amino acids of the precursor polypeptide, and 430 nucleotides of DNA sequence situated 5′ to the initiator ATG (Fig. 4B). After hybridization to pea leaf mRNA, two DNA fragments of approximately 105 and 138 nucleotides are protected from digestion with S1 nuclease (Fig. 4A, lane 1). The larger fragment results from hybridization to mRNA transcripts derived from the cognate rbcS gene, and the smaller fragment can be explained by hybridization to mRNA's transcribed from other pea rbcS genes which have divergent 5′ nontranslated regions (*5*). When this probe is hybridized to RNA isolated from pMON174 and pMON175 transformants (Fig. 4A, lanes 4 and 5), a single DNA fragment, identical in size to the larger fragment in lane 1, is protected from S1 nuclease digestion owing to the presence of a single pea rbcS gene in the transformants. No DNA fragments are protected from S1 digestion after hybridization to mRNA from petunia leaves or pMON145 transformants (Fig. 4A, lanes 2 and 3). These results show that the site of transcription initiation of the pea rbcS gene in the transformed cells is identical to that in pea leaves.

Light-dependent expression of the pea rbcS gene in transformed calli. The steady-state level of pPS4.0 mRNA transcripts increases 50 to 100 times on illumination of pea leaves (*5*). We therefore undertook to determine whether transcription of this gene in petunia calli is also regulated by light. Calli transformed by pMON174 or pMON175, were transferred to complete darkness. After 2 weeks, the new growth, which appeared white, was excised, transferred to fresh medium, and incubated for a further 2 weeks in the dark before harvesting. Northern blot analysis of RNA isolated from light- and dark-grown pea leaves and calli transformed by pMON174 (Fig. 5A) show that the amounts of small subunit mRNA's in leaves and transformed calli (lanes 1 and 3, respectively) grown in the light, are at least 50-fold higher than in their corresponding dark-grown samples (lanes 2 and 4, respectively). Similar results have been obtained for light- and dark-grown pMON175 transformants (data not shown).

To ensure that RNA from dark-grown calli was not degraded during the isolation procedure, the RNA samples were also hybridized with the plasmid pBRneo (*30*). This probe was used to detect transcripts derived from the chimeric kan^r gene, which contains the 5′ and 3′ regulatory regions of nopaline synthase (*17*). Since nopaline synthase appears to be expressed in both photosynthetic and nonphotosynthetic tissues of regenerated plants containing this gene (*31*), we expect the level of the chimeric kan^r mRNA to be constant in both light- and dark-grown calli. This is verified by the Northern blot shown in Fig. 5B. These results provide evidence for a selective light-dependent regulation of the pea rbcS gene expression in the transformed cells.

Although a trivial explanation for the extremely low levels of pea rbcS mRNA's in dark-grown calli is that the inserted pea gene had been deleted, rearranged, or translocated during growth in darkness, Southern blot analysis of DNA isolated from dark-grown calli confirmed the presence of the expected 4-kb Eco RI fragment (data not shown). In addition, when the dark-grown calli were reexposed to light they turned green within 2 to 3 weeks, and the amount of pea rbcS transcripts had been restored to the level found in light-grown calli (Fig. 5C). From our Northern blot experiments we estimated that the level of pea rbcS mRNA in green petunia calli is approximately 50 times less than in green pea leaves (Fig. 3A, lanes 1, 4, and 5). In spite of these different levels of expression, the response of the pea rbcS gene to light is similar in both instances (Fig. 4A), an indication that light regulation is independent of the tissue type. This finding is consistant with recent results obtained with various tissues of light- and dark-grown pea plants (*5*).

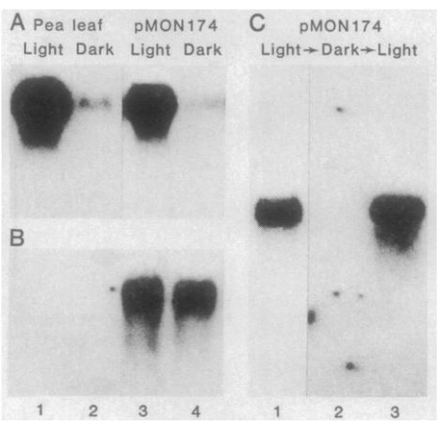


Fig. 5. Northern blot analysis of pea rbcS transcripts in light- and dark-grown tissue. Lanes contained the following RNA samples: (A and B) (lanes 1 and 2) total RNA (10 μg) from light- and dark-grown pea leaves, respectively; (lanes 3 and 4) polyadenylated RNA (3 μg) from light- and dark-grown pMON174 transformants, respectively. (C) (Lane 1) Total RNA (10 μg) from light-grown pMON174 transformants; (lane 2) total RNA (10 μg) from dark-grown pMON174 transformants; (lane 3) total RNA (10 μg) from dark-grown calli after transfer to the light for 2 to 3 weeks. The RNA blots in (A) and (C) were hybridized with gel-purified pea rbcS complementary DNA insert (*10*) and in (B) with the plasmid pBRneo containing the coding region for the bacterial neomycin-kanamycin resistance gene [obtained from P. Southern (*30*)]. The conditions for electrophoresis, hybridization, and washing are described in the legend to Fig. 3.

Identification and subcellular localization of the rbcS polypeptide in transformed petunia calli. The presence of the pea rbcS mRNA in transformed petunia calli raises the question of whether it is translated correctly to yield the pea rbcS polypeptide. Since calli contain mostly undifferentiated chloroplasts (*32*) their Rubisco content could be very low, rendering identification of the rbcS polypeptide difficult. Therefore, we compared the level of rbcS in petunia calli to that in petunia leaves by an ELISA (enzyme-linked immunosorbent assay) (*33*), using monospecific antibodies raised against pea rbcS polypeptide with purified petunia Rubisco as a standard. These antibodies have been shown to cross-react with rbcS polypeptides of several higher plants (*34*).

Our results revealed that in petunia leaves the rbcS polypeptide accounts for 2.6 percent of the total soluble protein while in petunia calli this number is reduced to less than 0.1 percent. To determine whether the pea rbcS polypeptide is present in transformed calli, we labeled the cultured tissues in vivo with carrier-free $^{35}SO_4$ and enriched for rbcL and rbcS polypeptides from the labeled extracts by immunoselection under nonstringent conditions with either a mixture of antibodies to rbcL and antibodies to rbcS (data not shown) or antibody to rbcL (Fig. 6) coupled to protein A–Sepharose beads (*35*). The immunoselected polypeptides were combined with a mixture of unlabeled holoenzymes purified from pea and petunia leaves and analyzed by two-dimensional gel electrophoresis (*36*) (Fig. 6). These gel systems provide adequate resolution of pea and petunia rbcS polypeptides reflecting differences in both charge and molecular weight. The most abundant radioactively labeled species from pMON145 transformants comigrate with purified petunia leaf rbcL and rbcS (Fig. 6A); other radioactively labeled polypeptides present are due to nonspecific adsorption of more abundant callus proteins which were not completely removed by the nonstringent

washing conditions employed. Analysis of the immunoselected polypeptides from pMON174 (Fig. 6B) and pMON175 (Fig. 6C) transformants reveal an additional radioactive polypeptide which comigrates with authentic pea rbcS.

The identification of correctly processed mature pea rbcS polypeptides in transformed calli raises questions regarding its subcellular localization in cells of callus tissues. In leaf cells, the rbcS polypeptide is synthesized as a precursor on free cytoplasmic polyribosomes and processed during or shortly after its posttranslational transport into chloroplasts. Since the processing enzyme is localized in the chloroplast stroma (*37*), the mature form of the rbcS polypeptide is expected to be found only within the chloroplasts. Therefore, the recovery of mature pea rbcS polypeptides from the transformed calli indicates that the pea rbcS precursor has been imported by petunia chloroplasts and processed correctly to yield the mature form.

The rbcS polypeptides are normally found in association with the rbcL polypeptides to form the holoenzyme, and only very low amounts of unassembled rbcS can be detected in the chloroplast stroma (*38*). To determine whether the pea rbcS is associated with the petunia rbcL in transformed calli, ^{35}S-labeled polypeptides were immunoselected with antibodies to rbcL only. Both pea rbcS and petunia rbcS are selected along with petunia rbcL demonstrating the assembly of pea rbcS with petunia rbcL to form hybrid holoenzymes in transformed tissues. Since rbcL is a chloroplast product (*1, 2*), the results provide further evidence that pea rbcS is localized in the same subcellular compartment.

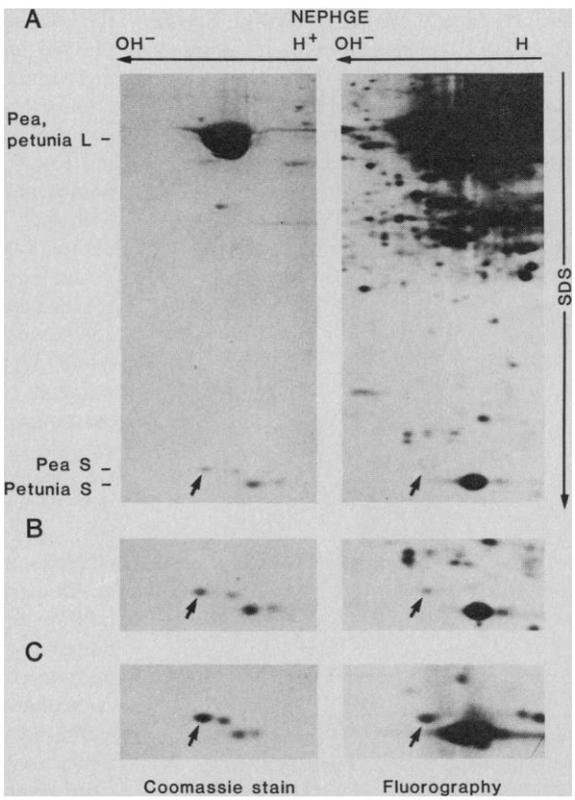


Fig. 6. Two-dimensional gel electrophoretic analysis of immunoselected Rubisco polypeptide subunits from transformed calli. Two-week-old calli (15 g, fresh weight) were grown for 20 hours (2000 lux illumination; room temperature) in 10 ml of 20 m*M* potassium phosphate buffer (*p*H 7) containing 10 mCi of carrier-free $^{35}SO_4$. The calli were homogenized in 10 ml of a solution containing 50 m*M* Hepes (*p*H 7.5), 100 m*M* NaCl, 5 m*M* β-mercaptoethanol, 1 m*M* ϵ-aminocaproic acid, 1 m*M* phenylmethylsulfonylfluoride, and 5 m*M* benzamidine and passed through four layers of cheesecloth. The filtrate was centrifuged at $50{,}000g_{max}$ for 20 minutes and the proteins were precipitated by adjusting the supernatant to 75 percent saturation with powdered ammonium sulfate. After a 30-minute incubation on ice, the precipitate was collected by centrifugation at $6{,}000g_{max}$ for 10 minutes. The protein pellet was redissolved in 2 ml of the homogenization buffer and dialyzed against the same buffer overnight. The dialyzed calli extract (1 ml) was incubated at 4°C for 3 to 4 hours with 60 μl (packed volume) of protein A–Sepharose containing covalently bound monospecific immunoglobulin G (IgG) to rbcL (8.18 mg/ml). The Sepharose beads were washed five times with 1-ml portions of phosphate-buffered saline, twice with distilled water, and finally resuspended in 1 ml of 0.2*M* glycine-HCl (*p*H 2.8). After centrifugation, the supernatant was removed and adjusted to a solution consisting of 0.1*N* HCl and 80 percent acetone to precipitate the eluted Rubisco fraction. The precipitate was collected by centrifugation at $10{,}000g_{max}$ for 10 minutes and dissolved in 50 μl of sample buffer (*44*) containing 70 μg each of purified pea and petunia Rubisco as internal markers. Samples were analyzed by two-dimensional gel electrophoresis (*36*), and the gels were processed for fluorography (*10*). The dried gels were exposed to Kodak XAR film for 5 to 20 days. (A) Two-dimensional gel analysis of in vivo labeled polypeptides immunoselected from pMON145 transformants with antibody to rbcL on Sepharose beads. (B and C) Small subunit region of two-dimensional gel of in vivo labeled polypeptides from pMON174 (B) and pMON175 (C) transformants after immunoselection with antibody to rbcL on Sepharose beads (C). L and S refer to rbcL and rbcS polypeptides, respectively.

Discussion

Several methods are available for the transfer of foreign genes into plants via the Ti plasmid of *A. tumefaciens* (*17, 31, 39*). The implementation of these methods depends on the faithful transcription of the introduced gene and expression of the gene product in the correct subcellular compartment. The foregoing data indicate that after transfer to petunia cells, the pea rbcS gene is transcribed with fidelity and regulated in a fashion similar to that observed in leaves. Our conclusions are supported by the following. (i) The pea rbcS transcript is polyadenylated and is of the correct size (850 bp); (ii) the same transcription start site is utilized; and (iii) the steady-state level of the pea rbcS mRNA is modulated 50-fold by light. A similar light-induced increase in the steady state amount of rbcS mRNA is also observed in pea leaves.

Experiments by Gallagher and Ellis (*40*) demonstrated that the effect of light on pea rbcS expression is at the transcriptional rather than posttranscriptional level. While we have not addressed this question directly, it is likely that the light regulation of the pea rbcS gene in petunia also occurs at the transcriptional level and not at the level of mRNA stability. If this is the case, we can conclude that the pea rbcS gene, pPS4.0, contains the necessary and sufficient DNA sequence information for its responsiveness to light.

The pea rbcS gene, pPS4.0, contains two introns; the first intron (79 bp) is situated between the second and third amino acid and the second intron (86 bp) between the 47th and 48th amino acid of the mature rbcS polypeptide (*5*). The production of functional mRNA depends on correct splicing of these two introns. Since pea rbcS polypeptides of the right size and isoelectric point are recovered from the transformed calli, we infer that splicing of the pea rbcS transcript occurs accurately in petunia.

In our experiments, the expression of the introduced pea rbcS gene is reflected not only at the RNA level but also at the protein level. Significantly, we have obtained two lines of evidence that the gene product is localized within the correct subcellular compartment of the petunia calli: (i) the processing enzyme of the precursor to the rbcS polypeptide is localized in the chloroplast (*36*) stroma. Since we recovered the mature forms of the pea rbcS polypeptides, the latter must be located in the same organelle; and (ii) the pea rbcS polypeptides are

found assembled with the petunia rbcL polypeptides, which are localized exclusively in the chloroplasts.

Although the catalytic site of Rubisco resides on rbcL, rbcS is also required for both carboxylase and oxygenase activities (*41*). The finding of hybrid holoenzymes in transformed petunia suggests the possibility of altering the enzymatic activity of this important enzyme by the introduction of novel genes coding for the rbcS polypeptide. Furthermore, the regulatory regions and transit sequence of the rbcS gene, when fused to a foreign gene, may be used to effect its light-induced expression and to target the gene product into chloroplasts. After the completion of this work, Murai *et al.* (*42*) demonstrated the expression of a bean phaseolin gene in sunflower cells after transfer via Ti plasmid vectors.

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45. We thank Dr. Carol Edwards for performing the ELISA experiments, Dr. Ben Dharm for preparing IgG–protein A–Sepharose beads, and Sara Adams, Nadera Ahmed, and Sarah Wagner for technical assistance. This work was supported in part by a Winston Foundation fellowship (to R.B.) and NIH postdoctoral fellowship GM07776 (to G.C.), and in part (for the work performed at the Rockefeller University) by grants GM-31500 and BRSG S17RR08065 from the National Institutes of Health and a grant from Monsanto Co.

26 January 1984; accepted 1 March 1984

RESEARCH ARTICLE

Expression Cloning of Human EGF Receptor Complementary DNA: Gene Amplification and Three Related Messenger RNA Products in A431 Cells

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Epidermal growth factor (EGF), a peptide residue having 53 amino acids (*1*) derived from a 128-kD precursor predicted by recombinant DNA analysis (*2, 3*), effects both growth and induction of specific differentiated functions in target tissues (*4*). The actions of EGF are exerted through binding to a specific 170-kD plasma membrane glycoprotein receptor (*5–8*), which has a protein core of about 140 kD (*9–11*). The EGF receptor is a phosphoprotein (*12*) with intrinsic kinase activity specific for tyrosine residues (*13*); the receptor is autophosphorylated at a tyrosine residue (*12, 13*) and is regulated by phosphorylation at threonine and serine residues by the Ca^{2+}, phospholipid-dependent protein kinase (*14, 15*). A critical question has been to identify the mechanism or mechanisms by which binding of EGF to the plasma membrane receptor alters the phenotypic and growth properties of the cell.

EGF and thyrotropin-releasing hormone (TRH) produce a rapid (within minutes) stimulation of prolactin gene transcription (*16–18*), and specific 5′ flanking prolactin genomic sequences transfer EGF transcriptional regulation to other genes (*19*). These data suggest that rapid, gene-specific transcriptional regulation is likely to be one crucial determinant of EGF action. A series of genetic modifications of the EGF receptor would be useful for further dissecting molecular mechanisms by which the binding of EGF to its receptor regulates specific transcriptional effects.

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