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A number of different nuclear genes for the small subunit of RuBPCase are transcribed in petunia

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ABSTRACT

The sequences in the petunia genome which encode the small subunit polypeptides of the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase have been characterized. Sequence analysis of four cDNA clones indicates that there are several distinct genes transcribed in leaf tissue. There is 8-9% nucleotide divergence between the transcripts however these changes do not alter the encoded amino acid sequence. Examination of nuclear DNA by Southern hybridization and analysis of cloned small subunit genes confirm that there are a number of different genes which encode this single protein.

INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase (RuBPCase) is the primary enzyme of carbon fixation in the chloroplast (1). In higher plants the holoenzyme is composed of eight large subunits, of 52,000 to 55,000 molecular weight (M_r), encoded by a single gene in the chloroplast DNA, and eight small subunits of 12,000 to 15,000 M_r which are coded by genes in the nuclear DNA. The catalytic site of this enzyme is on the large subunit (1) there has been no function ascribed to the small subunit polypeptide. The chloroplast gene for the large subunit has been isolated and characterized from a variety of higher plant species and the complete amino acid sequence of the protein has been deduced from nucleotide sequence analysis for maize (2), and spinach (3). In pea, cDNA clones encoding the precursor to the small subunit polypeptide have been sequenced (4,5) and the amino acid sequence of the mature protein deduced from these analyses.

We report the isolation and characterization of cDNA clones and genomic sequences which encode the small subunit of RuBPCase in petunia. Our data indicate there are several distinct genes for the small subunit protein and at least four of these are transcribed in petunia leaf tissue.

MATERIALS AND METHODS

Methods used for the isolation of DNA and RNA from petunia (Mitchell

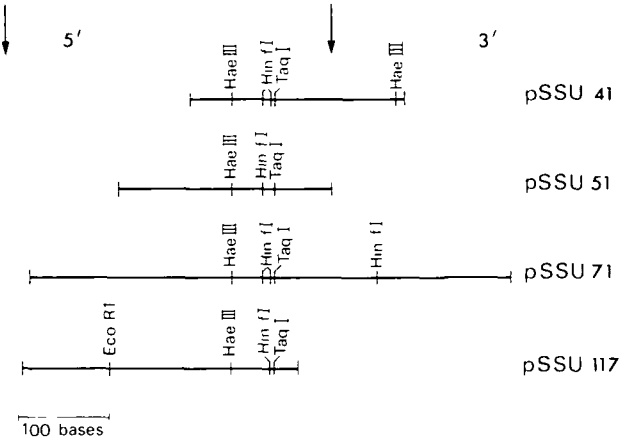

Figure 1. Restriction endonuclease cleavage maps of petunia small subunit cDNA clones. Restriction maps were determined with single and multiple enzyme digests. The maps are drawn 5'-3' and are aligned relative to each other on the basis of common restriction sites. The vertical arrows indicate the positions of the mature peptide starting point and the translation termination signal.

strain) leaf tissue, the construction of the cDNA clones, and the cloning of nuclear DNA in Charon 28 have been described in detail (6). Restriction endonucleases were purchased from Boehringer-Mannheim and Biolabs and used according to the manufacturer's specification. Nucleotide sequence analysis was by the method of Maxam and Gilbert (7). Transfer of DNA to nitrocellulose filters was according to the method of Southern (8) and subsequent hybridizations were in 50% formamide, 4xSSC (1xSSC = 0.15M NaCl 0.015M Na-citrate), 5xDenhardt's (1xDenhardt's = 0.02% Ficoll, 0.02% Bovine serum albumin, 0.02% Polyvinylpyrrolidone), 0.1% SDS, 50 mM $NaPO_4$ pH 7.0, 10% dextran sulphate and 100 µg/ml salmon sperm DNA at 42°C for [illegible] h.

RESULTS AND DISCUSSION

There are several distinct small subunit mRNAs in petunia leaf tissue

Six hundred cDNA clones constructed from petunia leaf $polyA^+$ RNA were screened with the pea cDNA clones pSSU 60 and pSSU 160 which encode the small subunit polypeptide of RuBPCase (4). Restriction maps of the four petunia cDNA clones which were selected in this screen show a number of common restriction sites which allow them to be aligned (Figure 1). However each cDNA has a unique restriction map suggesting that there may be at least

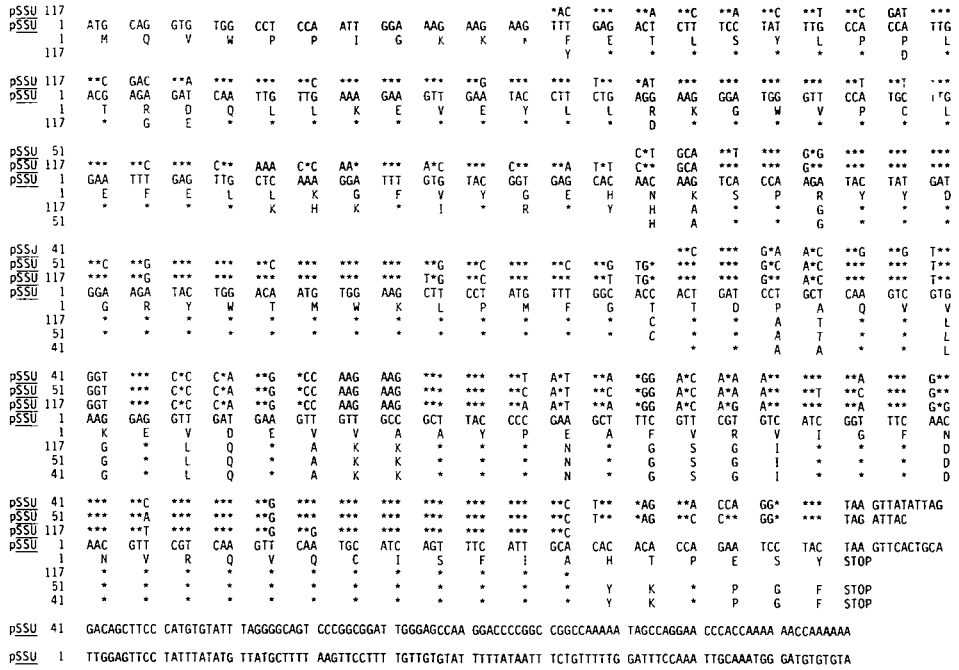

Figure 2. The nucleotide sequence and predicted amino acid sequence of small subunit cDNA clones. The nucleotide sequences of pSSU 41, pSSU 51 and pSSU 117 have been determined using the chemical sequencing procedure (9). The cDNAs were sequenced from 5' and 3' labels at the Bam H1 linkers using secondary Taq I cleavage to separate the ends. The nucleotide sequences of the coding strand for pea (pSSU 1) (4) and the petunia small subunit cDNAs (pSSU 117, pSSU 51 and pSSU 41) and part of the 3' untranslated regions are shown. The deduced amino acid sequences are also given. Only differences between the sequences are indicated; asterisks denote identical nucleotides or amino acids.

four distinct mRNAs for the small subunit polypeptide in petunia leaf cells.

The complete nucleotide sequences for three of the petunia cDNA clones are presented in Figure 2 together with that part of the published sequence for the small subunit cDNA from pea pSSU 1 which corresponds to the mature polypeptide plus part of the 3' untranslated region. The derived amino acid sequences for each of the cDNAs are also given. Alignment of the petunia cDNAs with the pea cDNA to give maximum sequence matching indicates that pSSU 117 corresponds to the nucleotide sequence encoding the amino acids 12 to 116 of the mature small subunit protein which is 123 amino acids long. The cDNA in pSSU 51 corresponds to amino acids 56 to 123, the translation termination signal TAG and 5 nucleotides of 3' untranslated sequence. The

cDNA pSSU 41 extends from amino acid 78 to 123, followed by the translation terminator TAA, and 110 nucleotides of the 3' untranslated region. There are 16 nucleotide differences between pSSU 117 and pSSU 51 within the 183 nucleotide region of overlap (8.7% divergence) however 15 of these changes are in the third codon position and do not alter the amino acid sequence; the additional alteration while in the first codon position (TTG→CTG) also does not change the coded amino acid which is leucine. The sequence of pSSU 41 differs from pSSU 117 in 9 of the 114 overlapping nucleotides (7.9% divergence) and all alterations are in the third codon position and do not affect the amino acid sequence. There are 11 nucleotide differences between pSSU 41 and pSSU 51 in the 148 nucleotides of coding region where they overlap (8% divergence).

These sequence data support the hypothesis that there are distinct mRNAs for the small subunit polypeptide in the petunia leaf mRNA population. The nucleotide sequences determined for these three small subunit cDNAs indicate that they do not contain premature termination codons and thus may be derived from functional mRNAs. However it is possible that they are transcripts from non-functional genes with premature translation stop codons in the 5' regions which are not contained within these clones.

The deduced amino acid sequence of the petunia small subunit protein differs from the pea small subunit sequence in 31% of the residues but the replacements which occur in the petunia sequence are consistent with the amino acid variants observed in small subunit protein sequence data available for a range of higher plant species (9). In spite of the considerable divergence between the amino acid sequences of petunia and pea small subunit polypeptides there are two long invariant sequences of amino acids, between positions 61 and 76, and 106 and 117; these regions may have some structural function in the holoenzyme.

There are a number of different small subunit genes in the petunia genome

In view of there being distinct mRNAs for the small subunit protein in the petunia leaf we have examined genomic DNA to verify whether there are a number of different genes. The hybridization profile for the pSSU 117 probe with petunia DNA which has been digested with EcoRI and transferred to nitrocellulose (8) is shown in Figure 3A. There are at least twelve discrete EcoRI fragments in the petunia genome ranging from 2 to 12 kb with homology to the pSSU 117 sequence. In the pea genome there are at least six discrete EcoRI fragments which hybridize to the pea cDNA clones (Figure 3A) pSSU 60 (corresponding to the 3' end of the gene), or pSSU 160 (corresponding to the

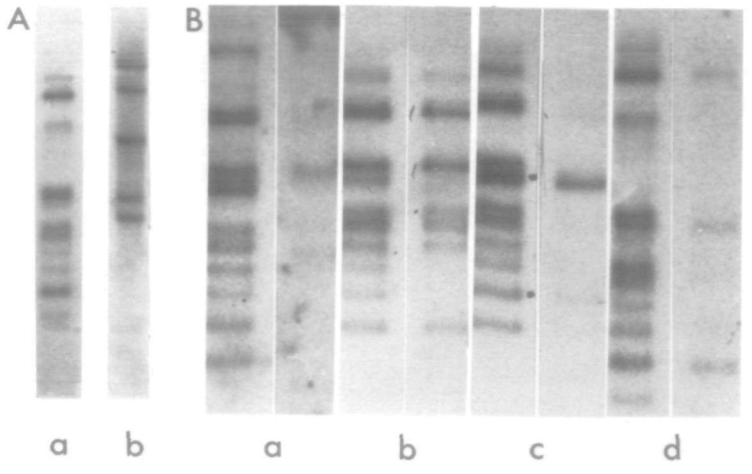

Figure 3. Hybridization of small subunit cDNA probes to plant nuclear DNA digested with EcoR1 and thermal elution of the hybrids. A. 5 µg samples of nuclear DNA from (a) petunia and (b) pea were digested to completion with the restriction endonuclease EcoR1, fractionated on 0.75% agarose gels, transferred to nitrocellulose and hybridized with (a) $5x10^6$ cpm of ^{32}P labelled pSSU 117 and (b) $5x10^6$ cpm of pSSU 60 DNA or pSSU 160 DNA ($1x10^8$ dpm/µg).

B. 5 µg. samples of petunia DNA digested with EcoR1 and fractionated as above were hybridized with the SSU cDNA clones (a) pSSU 71, (b) pSSU 51, (c) pSSU 41 and (d) pSSU 117. Each pair of autoradiographs shows the hybridization pattern after filters were washed in hybridization buffer at 45°C (left) and 55°C (right). (The samples in lanes (a), (b) and (c) were fractioned on a 1% gel and lane (d) on a 0.75% gel).

5' end of the gene), suggesting that in this species also there are several small subunit coding sequences.

The petunia cDNA clones pSSU 41, pSSU 51 and pSSU 71 give similar profiles of hybridization to petunia genomic DNA as pSSU 117 under normal stringency conditions (42°C), however, if these hybridization filters are washed under more stringent conditions (55°C in hybridization solution) then each of the four different cDNA clones hybridizes to a different subset of the twelve EcoRI genomic fragments (Figure 3B). Since the cDNA clones correspond to overlapping regions of the small subunit coding sequence it is most likely that the bands of hybridization in nuclear DNA reflect many small subunit coding sequences in the genome which are distinct but closely related.

Isolation of petunia nuclear fragments containing small subunit genes

Petunia DNA, partially digested with EcoRI, was cloned into the lambda

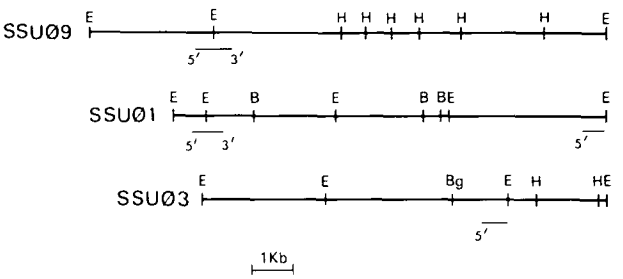

Figure 4. Restriction endonuclease maps of petunia genomic DNA clones with homology to the small subunit cDNA clones. The distribution of restriction sites in the phage was determined by comparison of fragments produced by single and double enzyme digests. Restriction sites are abbreviated E-EcoR1, B-Bam H1, H-Hind 111 and Bg-Bgl 11. The location of the small subunit coding sequences was determined by hybridization analysis (8).

vector, Charon 28 (10) and the recombinant phage screened (11) with petunia cDNA clone pSSU 117. Three different phage were isolated (Figure 4). The regions of the cloned petunia fragments which correspond to the small subunit polypeptide coding sequences have been determined by hybridizations of cDNA probes to recombinant phage DNAs after restriction endonuclease cleavage and transfer to nitrocellulose (8). A comparison of hybridizations with the probe pSSU 41 (which corresponds to the 3'-end of the small subunit coding sequence) and pSSU 117 (which extends into the 5'-end of the coding sequence) indicates the orientation of the cloned small subunit genes (which are all characterized by internal EcoRI restriction sites). The precise limits of the small subunit coding regions have not yet been determined. The occurrence of two separate regions in the phage SSU Ø1 which hybridize with pSSU 117 suggests that in the genome there may be two distinct genes arranged in the same orientation but separated by seven kilobases. This pattern is not consistent with the presence of a large (7 kb) intron in the 3' end of the coding sequence since the second region of hybridization (the right hand gene depicted in Figure 4) corresponds to a 5' but not a 3' region since it hybridizes with pSSU 117 but not pSSU 41.

It will not be possible to specify the number of small subunit protein coding sequences in the petunia nuclear genome until we have cloned all of the hybridizing sequences. We cannot estimate this number from the number of bands hybridizing in EcoRI digests of genomic DNA since we know that some of the genes have internal EcoRI sites (for example pSSU 117) while others do not (for example pSSU 71). We can place a lower limit on

the number of genes at four (which corresponds to the cDNA clones we have isolated) and an upper limit around twelve since this is the number of 3' end fragments in genomic hybridizations.

CONCLUSIONS

There are a number of distinct genes in the petunia nuclear genome which code for the small subunit polypeptide of RuBPCase. In spite of up to 9% divergence in the nucleotide sequences of the different genes, the polypeptides encoded are probably identical in amino acid sequence. This apparent conservation of the small subunit protein sequence is not so surprising when one considers that eight small subunit polypeptides interact structurally with eight identical large subunit polypeptides to form the RuBPCase holoenzyme. Yet in comparison with the amino acid sequence replacements which have occurred between petunia and pea (31% divergence), this conservation which occurs within a species is quite striking. It may reflect an amplification of small subunit genes in petunia which has occurred since the separation of pea and petunia although then one would expect the 9% sequence divergence which occurs in the petunia genes to be randomly distributed, and not confined to the third codon position.

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