Ridsdale, J. A., & Davies, J. R. (1987) *Nucleic Acids Res. 15*, 1081-1096.
Sakuma, K., Matsumura, Y., & Senshu, T. (1984) *Nucleic Acids Res. 12*, 1415-1426.
Sandeen, G., Wood, W. I., & Felsenfeld, G. (1980) *Nucleic Acids Res. 8*, 3757-3778.
Sasi, R., Huvos, P. E., & Fasman, G. D. (1982) *J. Biol. Chem. 257*, 11448-11454.
Shaw, P. A., Sahasrabuddhe, C. G., Hodo, H. G., & Saunders, G. F. (1978) *Nucleic Acids Res. 5*, 1999-3012.
Solomon, M. J., Larsen, P. L., & Varshavsky, A. (1988) *Cell 53*, 937-947.
Warnick, C. T., & Lazarus, H. M. (1975) *Nucleic Acids Res. 2*, 735-744.
Weisbrod, S., & Weintraub, H. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 631-635.

Chloroplast Ribosomal Protein L12 Is Encoded in the Nucleus: Construction and Identification of Its cDNA Clones and Nucleotide Sequence Including the Transit Peptide[†,‡]

Klaus Giese and Alap R. Subramanian*
Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Ihnestrasse 73, D-1000 Berlin 33, West Germany
Received September 16, 1988; Revised Manuscript Received December 21, 1988

ABSTRACT: An architectural feature found in all classes of ribosomes is a thin, 10-nm-long protuberance in the large subunit, generated by multiple copies of r-protein L12. The primary structure of spinach chloroplast r-protein L12 is known [Bartsch, M., Kimura, M., & Subramanian, A. R. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6871-6875], but the location of its gene, whether in the organelle or in the nucleus, has not been determined. Therefore, we synthesized four oligodeoxynucleotides based on the amino acid sequence data and used them to probe a spinach cDNA library we constructed in λgt11 vector. cDNA inserts from four of the hybridizing recombinant clones were characterized and sequenced. The data showed that they are reverse transcripts of varying length, all derived from a single poly(A+) RNA species. The longest cDNA molecule is 900 base pairs (bp) long and includes a 5′ noncoding sequence followed by two neighboring AUG codons both in the consensus, eukaryotic initiator context, a 56-codon-long transit peptide sequence (starting from the first AUG codon), the amino acid sequence of mature L12 protein, and a 238 bp long 3′ downstream noncoding sequence including a polyadenylation signal and the start of the poly(A) tail. The transit peptide sequence has an unusual amino acid composition similar to that of other known chloroplast transit peptides. Northern blot analysis of the poly(A+) RNA isolated from spinach seedlings and probed with the cDNA insert revealed the occurrence of a strong, broad, 950-nucleotide-long band of the corresponding poly(A+)-containing mRNA species. These results thus show that chloroplast L12 is nuclear-encoded. The possible significance of the two AUG codons in the presequence, and of the codon usage pattern of L12 (different from that of chloroplast-encoded r-proteins), is discussed.

Genes encoding the ribosomal proteins (r-proteins)[1] and rRNAs of the chloroplast ribosome are located in two cellular compartments. The rRNAs and several r-proteins are encoded in the organelle DNA, but the majority of the r-protein genes are believed to be located in the nuclear DNA (Bogorad, 1975). Because of this bicameral gene distribution, biosynthesis of chloroplast ribosomes must include regulatory features which would be unnecessary for the biosynthesis of bacterial or cytoplasmic ribosomes, whose structural components are encoded in single genomes. The gene dosage ratio of nuclear to chloroplast genes is also dependent on the cell type and development. It is greater than $1:10^4$ in mature leaf cells but lower in other plant cells which contain developmentally arrested proplastids or etioplasts in place of chloroplasts (Hoober, 1984).

The subcloning and characterization of organelle-located chloroplast r-protein genes have been reported by us [e.g., see Subramanian et al. (1983) and Giese et al. (1987)] and by several other groups [tabulated in Prombona et al., 1989)]. The determination of the complete nucleotide sequence of two chloroplast genomes (Shinozaki et al., 1986; Ohyama et al., 1986) has, however, shifted the interest from organelle-located genes to those which are located in the nucleus. These nuclear genes are particularly interesting from three points of view. (1) Their mRNA is likely to be polyadenylated and monocistronic. The transcriptional regulation of such species would differ considerably from that of the prokaryotic-type, polycistronic mRNA produced in the chloroplast from the organelle DNA. (2) The chloroplast proteins encoded by nuclear genes and synthesized on the cytoplasmic 80S ribosomes always carry a transit sequence necessary to enter the organelle [reviewed in Schmidt and Mishkind (1986)]. The isolation of nuclear-located chloroplast r-protein genes will provide more transit sequences (and clones) useful to understand this process.

[†] This paper is part of the doctoral dissertation (Biochemistry) of K.G. to be submitted to the Free University of Berlin.
[‡] The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J02849.
* Address correspondence to this author.

[1] Abbreviations: r-protein, ribosomal protein; SSC, 0.15 M NaCl/0.015 M sodium citrate (pH 7.0); Denhardt's, 0.02% bovine serum albumin, 0.02% Ficoll, and 0.02% poly(vinylpyrrolidone); HPLC, high-pressure liquid chromatography; SDS, sodium dodecyl sulfate.

0006-2960/89/0428-3525$01.50/0

(3) The endosymbiont theory of the origin of organelles proposes that chloroplasts are derived from photosynthetic prokaryotes [discussed in Bogorad (1975)]. If this theory were correct, the nuclear-located r-protein genes would form a distinct category of prokaryotic-type genes incorporated into the nucleus at a relatively late stage of nuclear evolution. As such, their chromosomal location, transcriptional signals, intron–exon arrangement, and gene copy number would be of general interest.

We have previously purified and completely sequenced a chloroplast r-protein from spinach, shown to be the homologue of the structurally and functionally important r-protein L12 (Bartsch et al., 1982). It is the only r-protein present in multiple copies in the ribosome, and dimeric units of it generate a thin protuberance in the 50S subunit necessary for the GTP hydrolysis reactions of protein biosynthesis [reviewed in Matheson et al. (1980) and Wittmann (1986)]. The chloroplast L12 also occurs in multiple copies but does not show the N-terminal acetylation which characterizes the *Escherichia coli* L12 (Bartsch et al., 1982).

Ribosomal protein L12 is not encoded in the two published chloroplast genome sequences (Shinozaki et al., 1986; Ohyama et al., 1986). Therefore, it was likely to be a nuclear gene. Here we present experimental evidence for the nuclear location of chloroplast r-protein L12 and report the nucleotide sequence of a full-length cDNA clone encoding the mature L12 protein and its 56-residue-long transit peptide. Features of the nucleotide sequence that can enhance its translational efficiency are discussed, and a correction is noted in the previously determined (Bartsch et al., 1982) primary structure.

Materials and Methods

Construction of cDNA Libraries. Total RNA was extracted by the guanidinium thiocyanate/CsCl method (Chirgwin et al., 1979) from the upper 5-mm part of spinach seedlings (*Spinacia oleracea*, cv Matador) grown for 9 days in the dark at 15 °C and illuminated for 14 h at 20 °C before cutting and freezing in liquid N_2. Poly(A+) RNA was prepared by affinity chromatography on oligo(dT)–cellulose (Theologis et al., 1985). Double-stranded cDNA was synthesized by the procedure of Gubler and Hoffman (1983) using 5 μg of poly(A+) RNA. The subsequent steps (*Eco*RI methylation to block internal *Eco*RI sites, DNA polymerase I fill-in, attachment of *Eco*RI linker, removal of excess linker, and size fractionation on a Bio-Gel A-50 column) were done according to Huynh et al. (1985). Approximately 0.6-μg portions of cDNA were ligated to dephosphorylated arms of λgt10 and λgt11 vectors and were packaged in vitro (arms and packaging mix purchased from Genofit, Heidelberg). The packaged λgt10 library was plated and amplified in *Escherichia coli* K802 and screened on *E. coli* C600 *hfl*A150; the λgt11 library was titrated and amplified in *E. coli* Y1088 and screened on *E. coli* Y1090 (Huynh et al., 1985).

Synthetic Oligonucleotides. Deoxyoligonucleotides were synthesized on an Applied Biosystems DNA synthesizer (Model 380A) and purified by reversed-phase HPLC on an ODS-Hypersil (5 μm) column. The wobble base deoxyinosine (C-I, A-I, and T-I base pairs) was used to reduce isomeric complexity (Martin et al., 1985). In the case of the longest oligonucleotide (38-mer), we also used the codon usage data on the eukaryotic genes (Lathe, 1985). The oligonucleotides were labeled with [γ-^{32}P]ATP (5000 Ci/mmol, Amersham) in the presence of T4 polynucleotide kinase and purified on a column of Sephadex G-50 (Maniatis et al., 1982).

Screening, Subcloning, and Nucleotide Sequencing. Aliquots of amplified library corresponding to ca. 10^5 pfu were plated on 145-mm plates, lifted onto nitrocellulose filters, and screened with ^{32}P-labeled oligonucleotide by the in situ plaque hybridization method (Benton & Davis, 1977). Prehybridization and hybridization were in 6 × SSC, 5× Denhardt's, 0.1 mg/mL *E. coli* tRNA, 50 mM sodium pyrophosphate (pH 6.5), and 0.1% SDS (Maniatis et al., 1982). Prehybridization was at 65 °C for 4 h, and hybridization was at 56 °C for 4–12 h. The filters were washed twice in 6 × SSC/0.1% SDS for 15 min at room temperature followed by a 5-min wash at 56 °C and were dried and exposed with an intensifying screen to X-ray film at −80 °C for 12–48 h. Northern blot analysis was carried out (Thomas, 1983) on glyoxal-treated poly(A+) RNA using, as probe, L12 cDNA labeled with [α-^{32}P]dCTP (3000 Ci/mmol) by nick-translation. Hybridization was at 65 °C, 16 h (buffer composition as given for plaque hybridization), and washings were in 2 × SSC/0.1% SDS for 30 min at room temperature followed by twice in 0.1 × SSC/0.1% SDS for 30 min at 65 °C.

Plaques which gave positive hybridization signals were purified by secondary and tertiary screening (Davis et al., 1986), and phage DNA was isolated by using *E. coli* TAP90 (Patterson & Dean, 1987). The cDNA inserts were cloned into pUC18/19 and M13mp18/19 vectors according to standard procedures (Davis et al., 1986). The nucleotide sequence was determined by the dideoxy chain termination method (Sanger et al., 1977). Primers for sequencing included synthetic oligonucleotides (see Figure 1) and also Pos. 270 (5′-GAGTTCCGAGTTGTTCG) and Pos. 448 (5′-ACTTCATCAATTGAGAC). Nucleotide sequences were aligned and compared by means of version 4A of the UWGCG program (Devereux et al., 1984) on a VAX 8600 computer.

AMINO ACID SEQUENCES

```
                 61                                  67
-GLU-GLU-LYS-THR-GLU-PHE-ASP-
   70                    75
-ILE-ASP-GLU-VAL-PRO-SER-
113                                 120                 126
-LYS-ASP-ASP-ALA-GLU-ASP-ALA-LYS-LYS-GLN-LEU-GLU-ASP-ALA
```

OLIGODEOXYNUCLEOTIDES SYNTHESIZED:

```
5′ TCIAACTCIGTCTTCTCCTC 3′   20-MER (61-67)
      T     T T T

5′ GICGGCACCTCITCIAT 3′   17-MER (70-75)
   C I   I T

5′ ITCCTCCGCITCITCCTT 3′   18-MER (113-118)
     T I      T

5′ GCITCCTCCAICTGCTTCTTCGCITCCTCCGCITCITC 3′   38-MER (114-126)
                          I     T I
```

FIGURE 1: Amino acid sequences and the nucleotide sequences of the mixed oligonucleotides used for isolating cDNA clones of chloroplast r-protein L12. I, deoxyinosine.

Results

In preliminary experiments, the synthetic 38-mer oligonucleotide (Figure 1) gave the clearest hybridization signal, and, therefore, it was exclusively used for all subsequent screenings. The other oligonucleotides were used, as noted, as primers for sequencing. Screening a total of 4 × 10^5 recombinant phages from the library gave 54 plaques (one out of 7400) that hybridized to the 38-mer oligonucleotide on duplicate filters. One of these clones was purified by replating, and its insert DNA was completely sequenced (L12-1, Figure 2). The UWGCG program located a reading frame in the 477 bp long sequence identical with the published amino acid sequence of spinach chloroplast L12, from residue 57 (Val) to the C-terminus. This sequence was followed by a termination codon (TAA), 238 bases of downstream sequence rich in T, and a stretch of 14 adenines.

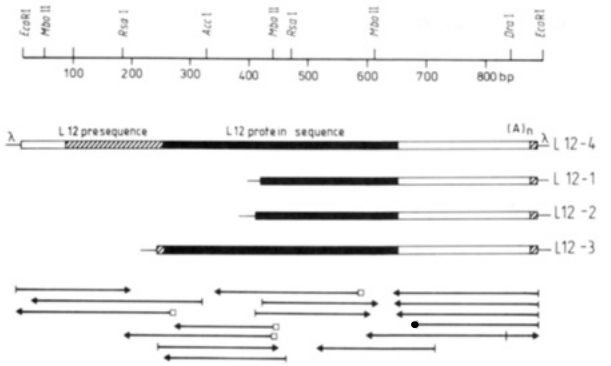

FIGURE 2: Four sequenced cDNA clones of chloroplast r-protein L12: their restriction map, coding stretches, and nucleotide sequencing strategy. Arrows show directions and lengths of sequenced fragments (those with rectangles were sequenced using synthetic oligonucleotides described in the text).

The insert of cL12-1 was used to screen for full-length cDNA clones. DNA was isolated from 10 of the positive recombinant phage plaques and digested with *Eco*RI, and a Southern blot of the digest was hybridized to ^{32}P-labeled cDNA from cL12-1. Autoradiograms showed different lengths of cDNA in all 10 clones. Three were taken for purification and sequencing. The nucleotide sequence of the cDNA in all three of these clones was identical in the 3′ part but contained additional sequences at the 5′ part: clone L12-2 encoded three more amino acids, while clones L12-3 and L12-4 encoded the complete published amino acid sequence of spinach chloroplast L12. Clone L12-4 in addition encoded a leader peptide of 56 amino acid residues and a further 5′ leader nucleotide sequence of 78 bp; clone L12-3 encoded two amino acids (Ile-Ala) of the leader peptide (Figures 2 and 3).

The cDNA clone L12-4 (Figure 3) consists of 886 bp [excluding the poly(A) tail], and its open-reading frame encodes 189 amino acids. The first ATG codon (79–81) has the context AACA*ATG*GCA that exactly fulfills the consensus context AACA*ATG*GC commonly found in functional plant initiator codons (Lütcke et al., 1987). Five codons downstream from this ATG a second ATG codon occurs, also in exactly the same context as the first one, and therefore a potential initiator codon. In line with other eukaryotic mRNAs (Kozak, 1987), the first consensus ATG would probably be the functional initiator. The length of the 5′ leader sequence in L12-4 (78 nucleotides) is within the observed range of leader sequences (20–100 nucleotides) for the majority of eukaryotic mRNAs (Kozak, 1987).

A plant polyadenylation signal, ATAAA (Heidecker & Messing, 1986), occurs 217 nucleotides downstream from the stop codon (Figure 3): the poly(A) sequence starts 17 nucleotides later. A large stem–loop structure (ΔG = −21.9 kJ/mol), similar to that reported in many plant mRNAs (Heidecker & Messing, 1986), can be computer-generated from the 3′-sequence positions 765–885 before the beginning of the poly(A) tract.

The size of the L12 mRNA in the cytoplasm was estimated from a Northern blot of poly(A+) RNA hybridized with the cDNA insert (Figure 4). There was a broad, apparent doublet band, 900–1000 nucleotides in length. Excluding its poly(A) tail, the cytoplasmic mRNA of r-protein L12 thus appears to be of approximately the same length as the isolated L12-4 clone.

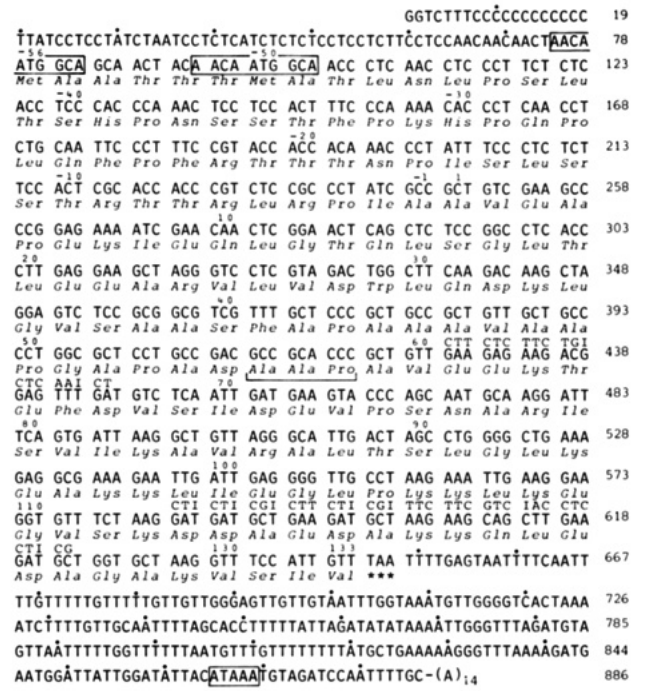

FIGURE 3: Nucleotide sequence of cDNA encoding chloroplast r-protein L12 and its transit peptide. The two ATG codons in the presequence, both in plant eukaryotic initiator context, are boxed. Assuming initiation at the first ATG, the transit peptide would be 56 residues (−1 to −56) long. The mature protein sequence, beginning at Ala and ending at Val (133 residues), is in complete agreement with the previously determined primary structure (Bartsch et al., 1982) except at the (underlined) tripeptide. Two of the synthetic oligonucleotide sequences (positions 61–67 and 114–126) are shown at their complementary sites. Arrow indicates the cleavage site of the transit peptide.

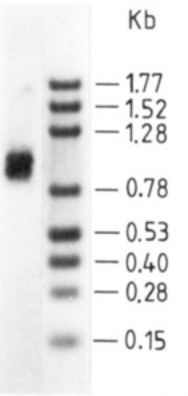

FIGURE 4: Northern blot analysis of the poly(A)-containing mRNA of chloroplast r-protein L12. Glyoxal-denatured poly(A+) RNA (10 μg) isolated from spinach seedlings was electrophoresed in a 1.5% agarose gel, transferred to nitrocellulose, and hybridized with the ^{32}P-labeled cDNA of clone L12-4. The right lane shows RNA size markers electrophoresed in the same gel.

Discussion

The amino acid sequence of spinach chloroplast r-protein L12, as deduced from the nucleotide sequence of its cDNA clones, showed one stretch of disagreement with the published primary structure (Bartsch et al., 1982), i.e., the insertion of a tripeptide sequence, Ala-Ala-Pro, after position 55 (Figure 3). Reviewing the protein–chemical sequencing data in our hands, it appears probable that such a tripeptide, at that position, could have been overlooked. It occurs within a long hydrophobic stretch with many Ala and several Pro residues including a second Ala-Ala-Pro sequence. We therefore think that the cDNA-derived sequence (133 amino acid residues; M_r 13816.7) is the correct one. Work in progress on isolation and sequencing of the genomic L12 clones from a spinach nuclear DNA library should, however, provide the final answer.

Table I: Codon Usage in the Nuclear-Located Gene for Chloroplast Ribosomal Protein L12

	T	C	A	G	
T	Phe 2	Ser 3	Tyr 0	Cys 0	T
	Phe 3	Ser 8	Tyr 0	Cys 0	C
	Leu 0	Ser 2	End 1	End 0	A
	Leu 4	Ser 1	End 0	Trp 1	G
C	Leu 3	Pro 9	His 0	Arg 2	T
	Leu 9	Pro 3	His 2	Arg 2	C
	Leu 1	Pro 2	Gln 4	Arg 0	A
	Leu 3	Pro 1	Gln 2	Arg 0	G
A	Ile 6	Thr 5	Asn 1	Ser 0	T
	Ile 2	Thr 7	Asn 3	Ser 2	C
	Ile 0	Thr 3	Lys 5	Arg 0	A
	Met 2	Thr 1	Lys 9	Arg 3	G
G	Val 6	Ala 13	Asp 6	Gly 2	T
	Val 4	Ala 6	Asp 3	Gly 2	C
	Val 2	Ala 6	Glu 9	Gly 2	A
	Val 1	Ala 3	Glu 6	Gly 2	G

The complete nucleotide sequences of the chloroplast genomes of two plant species that have been recently published do not encode r-protein L12 (Shinozaki et al., 1986; Ohyama et al., 1986). This finding and the results presented here show that chloroplast r-protein L12 is encoded exclusively in the nuclear DNA. Gantt and Key (1986) have reported the isolation of cDNA clones for six putative pea chloroplast r-proteins; most of them have also been sequenced (Gantt, personal communication). In spinach, cDNA clones for chloroplast r-protein L13 have been isolated and fully characterized (Phua et al., 1988). So far, L12 is the only chloroplast r-protein for which structural data are available at both the protein and nucleic acid levels. Therefore, in this case, the precise cleavage point of the transit sequence from the mature protein is known.

The 56-residue-long presequence, beyond the N-terminus of the mature protein, would represent the transit sequence containing routing and precursor–cleavage information. From the known primary structure of the mature protein, the cleavage in the precursor must occur at the Ala–Ala bond in the sequence Pro-Ile-Ala-Ala-Val-Glu.

The presequence contains no cysteine, glycine, valine, tyrosine, tryptophan, or acidic amino acids, and its contents of proline (14%) and threonine + serine (34%) are remarkably high. These characteristics are similar to those of chloroplast transit peptides described so far, which show a common amino acid composition motif but no significant sequence similarity, and differ in both these respects from the signal sequences of animal secretory proteins (Schmidt & Mischkind, 1986). Comparing the presequence of L12 to the presequences of five nuclear-encoded chloroplast proteins, i.e., plastocyanin (Smeekens et al., 1985a), chlorophyll *a/b* binding protein (Cashmore, 1984), ferredoxin (Smeekens et al., 1985b), the large subunit of ribulose-1,5-bisphosphate carboxylase (Corruzzi et al., 1983), and r-protein L13 of spinach (Phua et al., 1988), there is only 10–30% amino acid sequence similarity but striking resemblance in amino acid composition: little or no acidic amino acids, no tyrosine or tryptophan, high content of serine and threonine, and relatively high content of proline. Ferredoxin and the carboxylase are localized in the chloroplast stroma like the ribosome, but their transit sequences did not reveal any closer resemblance to that of L12; similarly, the transit sequences of the two known spinach chloroplast r-proteins (L12 and L13) show only 30% sequence identity (Phua et al., 1988).

The codon usage of chloroplast r-protein L12 (Table I) shows distinct preferences for codons ending in C for Leu, Ser, His, and Asn; G for Lys; T for Ile, Val, Pro, Ala, and Asp; and A for Gln and Glu. Thus, it does not share the high overall preference for codons ending in A or T displayed by the r-protein genes located in the chloroplast genome [e.g., see Subramanian et al. (1983) and Shinozaki et al. (1986)]. The chloroplast DNA of higher plants has the relatively low G + C content of 37% (Hoober, 1984). The coding as well as noncoding regions of the chloroplast DNA reflect this fact and have similar low G + C contents (Shinozaki et al., 1986; Ohyama et al., 1986). The G + C content of the nuclear DNA of higher plants is variable, i.e., 34–50% (Shapiro, 1970); the value reported for spinach is 37%. Computer analysis of the plant nuclear nucleotide sequences available in the EMBL database shows that the G + C content of the coding regions of the nuclear DNA (45–58%) is significantly higher ($\Delta \sim$ 16%) than the G + C content of its noncoding regions (30–42%). The G + C contents of the coding and noncoding regions of L12 cDNA are 53% and 30%, respectively, very similar to the G + C content of nuclear genes. Thus, although the chloroplast L12 protein might have had a prokaryotic origin from endosymbiosis (Bogorad, 1975), its gene now has the G + C content and codon usage pattern characteristic of nuclear genes.

The L12 proteins in all classes of ribosomes display the same acidic, alanine-rich composition (and form the stalk structure in the large ribosomal subunit), but two distinct types of them occur, one type in prokaryotic and chloroplast ribosomes and the other type in the eukaryotic cytoplasmic and archaebacterial ribosomes (Matheson et al., 1980; Strobel et al., 1988). The prokaryotic type shows 40–55% sequence identity within its group, but only 20–30% identity to the other type. The chloroplast L12 protein is prokaryotic type and thus has retained its type even though it is encoded in the nucleus.

The frequency of L12 cDNA clones in the λgt11 library of our construction, as determined from the primary screening, is 1 in 7500 (i.e., 0.013%). It indicates a nonabundant mRNA species for this protein. The large stem–loop structure near the 3′ end of L12 mRNA (see Results) could, however, prevent stoichiometric cDNA synthesis and lead to an underestimation of the frequency. From the length of the cDNA clone and from direct size determination, the mRNA appears to be about 900–1000 nucleotides long, with 567 bases of protein coding sequence, 319 bases of noncoding sequence, and a relatively short poly(A) tail. The apparent doublet band in the Northern blot could arise from a distinct heterogeneity in the poly(A) tail length, but it needs further analysis. Preliminary Southern blot hybridization experiments on spinach nuclear DNA have suggested that the L12 gene is a single-copy gene in the nucleus (unpublished data).

The chloroplast rpL12 mRNA would represent an efficiently translated transcript in that the first methionine codon is in initiation context and no other noncontext AUG codons occur further upstream. The second methionine codon that is also in the consensus context could act as a backup initiation codon for ribosomes that have somehow bypassed the first AUG triplet. The six-residue shorter transit peptide so synthesized would still retain the characteristic features of chloroplast transit peptides. Whether such a mechanism is demonstrable in vitro is under investigation.

Registry No. cDNA, 119593-04-3; L12 protein, 119593-06-5; mature L12 protein, 119593-05-4.

References

Bartsch, M., Kimura, M., & Subramanian, A. R. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6871–6875.

Benton, W. D., & Davis, R. W. (1977) *Science 196*, 180–182.

Bogorad, L. (1975) *Science 188*, 891–898.
Cashmore, A. R. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 2960–2964.
Chirgwin, J. M., Przybyla, A. E., Macdonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.
Coruzzi, G., Broglie, R., Cashmore, A. R., & Chua, N.-H. (1983) *J. Biol. Chem. 258*, 1399–1402.
Davis, L. G., Dibner, M. D., & Battey, J. F. (1986) *Basic Methods in Molecular Biology*, Elsevier, Amsterdam.
Devereux, J., Haeberli, P., & Smithies, O. (1984) *Nucleic Acids Res. 12*, 387–395.
Gantt, J. S., & Key, J. L. (1986) *Mol. Gen. Genet. 202*, 186–193.
Giese, K., Subramanian, A. R., Larrinua, I. M., & Bogorad, L. (1987) *J. Biol. Chem. 262*, 15251–15255.
Gubler, U., & Hoffman, B. J. (1983) *Gene 25*, 263–269.
Heidecker, G., & Messing, J. (1986) *Annu. Rev. Plant Physiol. 37*, 439–466.
Hoober, J. K. (1984) *Chloroplasts*, Plenum Press, New York.
Huynh, T. V., Young, R. A., & Davis, R. W. (1985) in *DNA Cloning, Volume I. A Practical Approach* (Glover, D. M., Ed.) pp 49–78, IRL Press, Oxford.
Kozak, M. (1987) *Nucleic Acids Res. 15*, 8125–8148.
Lathe, R. (1985) *J. Mol. Biol. 183*, 1–12.
Lütcke, H. A., Chow, K. C., Mickel, F. S., Moss, K. A., Kern, H. F., & Scheele, G. A. (1987) *EMBO J. 6*, 43–48.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Martin, F. H., Castro, M. M., Aboul-ela, F., & Tinoco, I., Jr. (1985) *Nucleic Acids Res. 13*, 8927–8938.
Matheson, A. T., Möller, W., Amons, R., & Yaguchi, M. (1980) in *Ribosomes, Structure, Function and Genetics* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 297–332, University Park Press, Baltimore, M.D.
Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H., & Ozeki, H. (1986) *Nature (London) 322*, 572–574.
Patterson, T. A., & Dean, M. (1987) *Nucleic Acids Res. 15*, 6298.
Phua, S. H., Srinivasa, B. R., & Subramanian, A. R. (1989) *J. Biol. Chem.* (in press).
Prombona, A., Ogihara, Y., & Subramanian, A. R. (1989) *Methods Enzymol.* (in press).
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Schmidt, G. W., & Mishkind, M. L. (1986) *Annu. Rev. Biochem. 55*, 879–912.
Shapiro, H. S. (1970) in *Handbook of Biochemistry* (Sober, H. A., Ed.) pp H90–91, The Chemical Rubber Co., Cleveland, OH.
Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B. Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H., & Sugiura, M. (1986) *EMBO J. 5*, 2043–2049.
Smeekens, S., de Groot, M., van Binsbergen, J., & Weisbeek, P. (1985a) *Nature 317*, 456–458.
Smeekens, S., van Binsbergen, J., & Weisbeek, P. (1985b) *Nucleic Acids Res. 13*, 3179–3194.
Strobel, O., Köpke, A. K., Kamp, R. M., Böck, A., & Wittmann-Liebold, B. (1988) *J. Biol. Chem. 263*, 6538–6546.
Subramanian, A. R., Steinmetz, A., & Bogorad, L. (1983) *Nucleic Acids Res. 11*, 5277–5286.
Theologis, A., Huynh, T. V., & Davis, R. W. (1985) *J. Mol. Biol. 183*, 53–68.
Thomas, P. S. (1983) *Methods Enzymol. 100*, 255–265.
Wittmann, H. G. (1986) in *Structure, Function and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) pp 1–27, Springer-Verlag, Berlin.