Nuclear-Encoded Chloroplast Ribosomal Protein L27 of *Nicotiana tabacum*: cDNA Sequence and Analysis of mRNA and Genes†,‡

Gasmalla A. Elhag§ and Don P. Bourque*,§,‖

Department of Biochemistry and Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721

Received February 12, 1992; Revised Manuscript Received April 27, 1992

ABSTRACT: A tobacco (*Nicotiana tabacum cv. Petite Havana*) leaf cDNA library was constructed in the expression vector λgt11. Immunological and nucleic acid hybridization screening yielded several cDNAs encoding an M_r 19 641 precursor to an M_r 14 420 mature protein which is homologous to *Escherichia coli* ribosomal protein L27. One cDNA (L27-1; 882 nucleotides long) contains 104 bp of 5′-noncoding sequence, 51 codons for a transit peptide, 128 codons for the predicted mature L27 polypeptide, and 241 bp of 3′-noncoding sequence, including the poly(A)$_{29}$ tail. A β-galactosidase–L27 fusion protein was bound to nitrocellulose filters, expressed, and used as an affinity matrix to purify monospecific antibody to L27 protein from an antiserum of rabbits immunized with 50S chloroplast ribosomal proteins. Using this monospecific antibody, protein L27 was identified among HPLC-purified tobacco chloroplast ribosome 50S subunit proteins. The predicted amino terminus of the mature L27 protein was confirmed by partial sequencing of the HPLC-purified L27 protein. The mature L27 protein has 66%, 61%, 56%, and 48% amino acid sequence identity with the L27-type ribosomal proteins of *Bacillus subtilis*, *E. coli*, *Bacillus stearothermophilus*, and yeast mitochondria (MRP7), respectively, in the homologous overlapping regions. The transit peptide of tobacco chloroplast ribosomal protein L27 has 41% amino acid sequence similarity with the MRP7 mitochondrial targeting sequence. Tobacco chloroplast L27 protein also has a 40 amino acid long carboxyl-terminal extension (compared to its bacterial counterparts) which is similar to the corresponding portion of yeast MRP7. The carboxyl end of tobacco L27 has an unusual cysteine-rich sequence (CFCCC) of unknown function. Northern blot analysis revealed a single band of mRNA corresponding in size (0.85–0.90 kb) to full-length L27 cDNAs. Two cDNAs for tobacco ribosomal protein L27 were identical in coding sequence, but differed in 3′-noncoding sequence. Hybridization of L27 cDNA probes to restriction enzyme digests of tobacco genomic DNA and to cloned genomic fragments provided additional evidence suggesting that more than one gene exists for tobacco L27.

Ribosomes are protein-synthesizing organelles found in all cells. The structure and function of bacterial ribosomes have been intensively studied (Lindahl & Zengel, 1986; Gourse et al., 1986). Recent studies have begun to reveal details about the structure and function of eucaryotic ribosomes (Planta et al., 1986; Warner et al., 1986; Wool et al., 1990; Amaldi et al., 1989; Subramanian et al., 1990). In contrast to bacteria, plant cells maintain multiple, distinctly different types of ribosomes in the cytoplasm, chloroplast, and mitochondrion (Boynton et al., 1980). These three protein-synthesizing systems differ in the mass and density of their ribosomal subunits, the number of their component proteins, and the size and degree of sequence homology of the rRNA of large and small ribosomal subunits (Boynton et al., 1980; Capel & Bourque, 1982; Bonham-Smith & Bourque, 1990).

The genes encoding chloroplast ribosomal proteins are distributed between the chloroplast and nuclear genomes. Twenty-one ribosomal protein genes occur in chloroplast genomes of tobacco (Shinozaki et al., 1986; Yokoi et al., 1990), rice (Hiratsuka et al., 1989), and the liverwort *Marchantia polymorpha* (Ohyama et al., 1986). Each of these chloroplast genomes codes for an identical complement of ribosomal proteins, with the exceptions being that the liverwort genome lacks the *rps*16 gene, while those of tobacco and rice lack the *rpl*21 gene [reviewed in Shimada and Sugiura (1991)]. The remaining 32–37 (Capel & Bourque, 1982) chloroplast ribosomal proteins are nuclear-encoded, being synthesized by cytoplasmic ribosomes as precursors and then imported into chloroplasts (Schmidt et al., 1984; Gantt & Key, 1986; Subramanian & Giese, 1989). The primary sequences of several nuclear-encoded chloroplast ribosomal proteins from higher plants have been deduced from cDNA sequences, including examples from tobacco (Bourque et al., 1991), spinach (Subramanian & Giese, 1989; Zhou & Mache, 1989; Phua et al., 1989; Smooker et al., 1990), pea (Gantt, 1988), soybean, and *Arabidopsis thaliana* (Gantt & Thompson, 1990).

Escherichia coli mutants lacking ribosomal protein L27 have severely impaired growth (Dabbs et al., 1983), probably due to loss of important function(s) of ribosomal protein L27 as part of the peptidyltransferase center. *E. coli* ribosomal protein L27 was identified as a constituent of the peptidyltransferase center, on the basis of cross-linking studies with either peptidyl- or aminoacyl-tRNA labels as well as with antibiotic derivatives (Ofengand et al., 1986). Immunoelectron microscopy located ribosomal protein L27 on the central protuberance of the large subunit of *E. coli* ribosome (Lake & Strycharz, 1981) near the subunit interface (Morrison et al., 1973), since antibodies which bind to L27 prevent the reassociation of subunits to 70S ribosomes and since L27 could be cross-linked to protein S9 in the *E. coli* 30S ribosomal subunit (Traut et al., 1980). Other studies have shown that L27, along with two other 50S subunit proteins (L2 and L15),

† This research was supported by Research Grant GM26937 from the National Institute of General Medical Sciences of the U.S. Public Health Service.

‡ The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number M75731.

* Author to whom correspondence should be addressed.

§ Department of Biochemistry.

‖ Department of Molecular and Cellular Biology.

0006-2960/92/0431-6856$03.00/0

is found on the 50S–30S subunit interface in the valley between the L1 ridge and the central protuberance (Stoffler & Stoffler, 1986; Hackle et al., 1988). This protein cluster was located at the site of contact of the aminoacyl end of tRNA with the ribosomal P site (Wower et al., 1989). Antibiotic derivatives have been cross-linked with ultraviolet irradiation to determine the contact sites of 23S ribosomal RNA and ribosomal protein L27 in *E. coli* ribosomes (Gulle et al., 1988). *E. coli* ribosomal protein L27 cross-links with analogs of chloramphenicol (Sonenberg et al., 1973), a bacteriostatic antibiotic which inhibits peptide bond formation in bacteria (Lamborg & Zamecnik, 1960), mitochondria (Kroon, 1965), and chloroplasts (Ellis, 1969). Chloramphenicol analogs specifically alkylate cysteines of proteins L2 and L27. Recently, the chloramphenicol interaction site with the 23S rRNA has been identified within the peptidyltransferase center of the 50S subunit of the *E. coli* ribosomes (Marconi et al., 1990).

Here we characterize tobacco cDNAs coding for nuclear-encoded chloroplast ribosomal protein L27. Results of immunological reactions with monospecific anti-L27 antibody which permitted identification of mature protein L27 among HPLC-resolved tobacco 50S chloroplast ribosomal subunit proteins are presented. The site of cleavage of transit peptide from mature protein was determined by amino-terminal amino acid sequence analysis. RNA blot analysis of mRNA size, genomic Southern blot analysis, and isolation of different genomic clones for tobacco L27 ribosomal protein are described. Structural and functional conservation of procaryotic and eucaryotic L27-type proteins is discussed. Possible functions of the carboxyl-terminal portion of chloroplast ribosomal protein L27 are discussed.

MATERIALS AND METHODS

Materials. Chemicals were purchased from Sigma, BRL, or Mallinckrodt. Agarose (Molecular Biology grade) was from IBI. Nitrocellulose filters (HATF, 0.45 μm) were from Millipore. Restriction endonucleases, T4 DNA ligase, and other DNA modifying enzymes were from BRL, Boehringer Mannheim, New England Biolabs, Promega Biotech, or Stratagene. AMV reverse transcriptase was from Life Sciences. *Eco*RI linkers and isopropyl β-D-thiogalactopyranoside (IPTG) were from BRL. Predigested λgt11 arms, packaging extracts, pBS(+) and pBluescript II KS +/− vectors, and bacterial strains Y1088, Y1090, Y1090R⁻, and XL1 Blue were from Stratagene. Sequenase kit was from United States Biochemicals. [α-^{35}S]dATP was from New England Nuclear. ^{125}I-Labeled protein A was from ICN. Cyanogen bromide-activated Sepharose 4B was from Sigma.

mRNA Isolation. Young expanding leaves 2–3 cm long were collected from 3-month-old tobacco plants (*N. tabacum* var. *Petite Havana*) grown under greenhouse conditions. The leaves were washed with tap water and used immediately for RNA isolation by the guanidine isothiocyanate/CsCl method (Chirgwin et al., 1979). Poly(A)$^+$ mRNA was separated from the total leaf RNA on oligo(dT)–cellulose columns (Theologis et al., 1985). The size distribution of this poly(A)$^+$ mRNA was from <0.5 kb to >3.0 kb, as determined by agarose gel electrophoresis. When wheat germ extract in vitro translation products of this poly(A)$^+$ mRNA were analyzed by SDS and acid/urea gel electrophoresis, a broad range of translation products including numerous basic polypeptides were observed (data not shown), indicating that ribosomal protein-coding mRNAs were well represented.

Construction of the cDNA Library. cDNA was synthesized as outlined by Gubler and Hoffman (1983) using 5 μg of template poly(A)$^+$ mRNA. The size of the synthesized first and second cDNA strands was monitored on alkaline gels (Maniatis et al., 1982). The double-stranded cDNA was blunt-ended with T4 DNA polymerase, methylated with *Eco*RI methylase, ligated to *Eco*RI linkers, digested with *Eco*RI to remove excess linkers, and size-fractionated on a Bio-Gel A-50m column (Huynh et al., 1985). The cDNA was ligated to *Eco*RI-predigested and dephosphorylated λgt11 arms and packaged in vitro to produce a primary library (1.6×10^6) of infective phage stocks. The packaged library was titered and amplified in *E. coli* strain Y1088 and screened in *E. coli* strain Y1090 or Y1090R⁻.

Preparation of Antibodies to 50S Chloroplast Ribosomal Subunit Proteins. Tobacco chloroplast ribosomal subunits were isolated as previously described (Bourque & Capel, 1982). Purified proteins from 50S ribosome subunits (Capel & Bourque, 1982) were used to immunize rabbits by surgical injection in the popliteal lymph nodes (Sigel et al., 1983). Proteins were prepared for injection by mixing 1.0 mg of ribosomal proteins in 100 μL of phosphate-buffered saline emulsified with 100 μL of Freund's adjuvant. Intraperitoneal booster injections of 1 mg of antigen were given after 1 month. Immunoreactive antiserum was collected after 1 or 2 additional months.

Immunological Screening of the cDNA Library. Antiserum to tobacco 50S ribosomal subunit proteins was freed from coliform-reactive background antibodies essentially as described by Kemp and Cowman, (1981). Five milliliters of serum was mixed with 5 mL of cyanogen bromide-activated Sepharose 4B to which approximately 3 mg of a lysate of *E. coli* strains BNN97 (C_{600}-λgt11 lysogen) and Y1090 had been bound. The mixture was stirred gently overnight by end-over-end shaking at 4 °C and poured into a 5-mL syringe plugged with glasswool, and the antiserum was eluted with an equal volume of Tris-buffered saline (25 mM Tris, pH 7.4, 0.14 M NaCl, and 5 mM KCl). Antibody-containing fractions which absorbed light at 280 nm were pooled. Screening for λgt11 recombinants which expressed chloroplast ribosomal proteins was done by infecting *E. coli* Y1090R⁻ cells with 10^5–10^6 plaque-forming units (pfu) and plating on 150-mm plates. The plates were incubated at 42 °C for 3–4 h until the plaques were just pinpoints, and dry nitrocellulose filters (which had been impregnated in 10 mM IPTG) were carefully placed on the plates and incubated at 37 °C for 2 h. The filters were removed and blocked by incubation for 4 h with MNTA (3% powdered dry skim milk, 0.9% NaCl, 10 mM Tris, pH 7.5, and 0.02% sodium azide). The filters were then incubated for 21 h at 4 °C with antibody in MNTA. After three washes (30 min each) at room temperature in MNTA containing 0.1% Triton X-100, the filters were reacted with ^{125}I-protein A in MNTA at room temperature. The filters were then washed with MNTA (containing 0.1% Triton X-100 and 0.05% SDS), dried, and exposed to X-ray film to identify positive plaques.

Filter Affinity Purification of Monospecific Antibodies. Monospecific antibody was prepared essentially as described by Johnson et al. (1985). The recombinant λphage L27-4 (Figure 1A) was plated (10^4 pfu per 85-mm plate) on *E. coli* strain Y1090R⁻. The plate was incubated at 42 °C until plaques were just pinpoints. A dry nitrocellulose filter (previously soaked in 10 mM IPTG solution) was placed on the plate and the plate incubated at 37 °C overnight. The filter was removed and treated with antibody as described for cDNA library screening. After being washed to remove unbound antibody, each filter was placed (antibody side facing

up) into a sterile 50-mL plastic tube such that the filter was in contact with the inner walls of the tube. Bound antibody was eluted with 3.5 mL of 0.2 M glycine hydrochloride (pH 2.5) by hand-rolling the tube for 2.5 min, taking care that the glycine hydrochloride solution washed the entire filter surface. After the filter was removed, the antibody solution was neutralized with 1.75 mL of 1.0 M potassium phosphate buffer, pH 9.0. To identify chloroplast ribosomal protein L27, total proteins from the chloroplast 50S ribosome subunit were purified by HPLC [see Figure 3A of Elhag et al. (1992)]. The proteins were spotted (1.5 pmol each) on nitrocellulose filters, blocked with MNTA, incubated with anti-β-galactosidase–L27 antibody, and detected with ^{125}I-protein A.

Screening of the cDNA Library with Nucleic Acid Probes. A 189 bp L27-coding cDNA fragment (L27-4; cf. Figure 1A) was isolated by electroelution from an agarose gel and used as a ^{32}P-labeled probe to screen the cDNA library by in situ plaque hybridization (Maniatis et al., 1982). Fifty nanograms of the insert was labeled to about 2×10^8 cpm/μg using the random primer method (Feinberg & Vogelstein, 1983) with [α-^{32}P]dCTP and Klenow fragment and then purified on a Sephadex G-50 (fine) spun column (Maniatis et al., 1982). Prehybridization and hybridization were at 42 °C in 50% formamide, 5× Denhardt's solution, 6X SSC, 50 mM sodium pyrophosphate (pH 6.5), 100 μg/mL denatured salmon sperm DNA, 0.1% SDS, and 10 μg/mL poly(A) RNA. Dextran sulfate at a concentration of 10% was sometimes used in the hybridization mix. After hybridization (1×10^7 cpm/5 mL) for 12–18 h, the filters were washed twice in 6X SSC/0.1% SDS for 30 min each at room temperature and exposed with an intensifying screen to X-ray film at –80 °C. Plaques which gave positive signals were further purified by two additional rounds of screening (Huynh et al., 1985).

RNA and Genomic DNA Blot Analysis. Northern blot analysis was done after electrophoresis of RNA in a 1.2% agarose gel in the presence of 2.2 M formaldehyde (Ogden & Adams, 1987). The resolved RNA species were transferred to nitrocellulose membranes and hybridized to a ^{32}P-labeled L27-1 cDNA insert (cf. Figure 1A). The transfer was in 10X SSC, and prehybridization and washing were as for cDNA library screening. Tobacco genomic DNA was prepared from young expanding tobacco leaves following the method of Bedbrook (1981). The DNA was digested to completion with restriction endonucleases (*Eco*RI, *Bam*HI, or *Hin*dIII) and subjected to electrophoresis in 0.8% agarose gels. The DNA was transferred to nitrocellulose filters (Southern, 1975) and hybridized to L27-1 cDNA (cf. Figure 1A) as described for cDNA library screening.

Recloning of cDNA Inserts and Sequencing Strategy. Phage DNA was purified (Silhavy et al., 1984) and digested with *Eco*RI. The cDNA inserts were isolated after gel electrophoresis and cloned into the unique *Eco*RI sites of pBS(+) and Bluescript II KS(+) and KS(–) vectors. The recombinant plasmid was amplified in transformed cells of *E. coli* strain XL1 Blue. Single-strand template DNA was prepared (Vierra & Messing, 1987) and sequenced by the dideoxy chain termination method (Sanger et al., 1977) using Sequenase (Tabor & Richardson, 1987) and [α-^{35}S]dATP as a radioactive substrate. Primers used for sequencing were T3 and M13 universal primers as well as two L27 primers (Figure 1B). The sequence data were analyzed on an IBM PS/2 Model 80 computer using programs of Mount and Conrad (1986). Related L27 ribosomal proteins were identified and aligned by searching the NBRF (National Biomedical Research Foundation) protein sequence library using the computer programs Word Search and Gap of the Wisconsin GCG package.

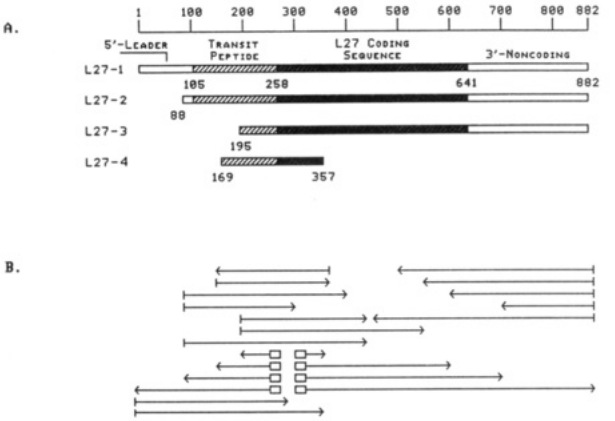

FIGURE 1: Size (A) and sequencing strategy (B) for cDNAs encoding tobacco ribosomal protein L27. Shown are the size and features of 4 of 12 characterized cDNAs encoding L27. The scale at the top designates the length in nucleotides starting at the 5′-end of the cDNA L27-1. Arrows indicate polarity of DNA sequencing. Open rectangles indicate the location of synthetic oligonucleotide primers P-L27(1), complementary to nucleotides 211–231 (Figure 3), and P-L27(2), identical to nucleotides 310–330 (Figure 3), used in DNA sequencing.

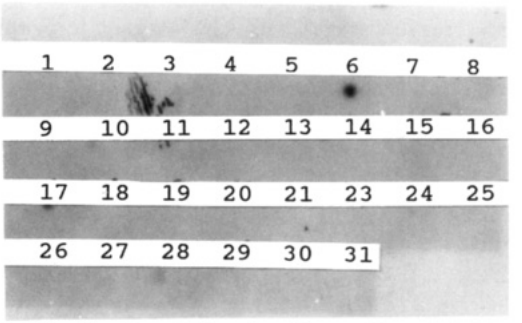

FIGURE 2: Identification of chloroplast ribosomal protein L27 among HPLC-purified 50S subunit proteins. The amino-terminal sequence (Figure 3) of protein from HPLC fraction 6 was determined as previously described (Elhag et al., 1992). Experimental details are given in the text.

RESULTS

cDNAs Coding for Chloroplast Ribosomal Protein L27. The tobacco cDNA library was screened with a mixture of antibodies to total chloroplast ribosomal proteins. An immunopositive recombinant phage contained a cDNA (cDNA L27-4, nucleotides, 169–357, Figures 1A and 3) coding for a polypeptide with 69% identity to a portion of *E. coli* ribosomal protein L27. The cDNA insert L27-4 was used as a probe to rescreen 2×10^6 recombinant phage from the library. From 12 positive plaques, 3 different size classes of cDNA larger than L27-4 were characterized (Figure 1A). The longest cDNA, L27-1, codes for a mature L27 protein of 128 amino acids (M_r 14 420), a transit peptide of 51 amino acids (M_r 5221), a 5′-leader sequence of 104 nucleotides, and 241 nucleotides of 3′-noncoding sequence, including the poly(A)$_{29}^+$ tail. The second (L27-2) and third (L27-3) size classes of L27 cDNA are identical to L27-1, except for being shorter at the 5′-end by 87 and 194 nucleotides, respectively. The length (104 nucleotides) of the 5′-leader sequence of the L27-1 cDNA is similar to that of other eucyarotic mRNAs (Kozak, 1987). The sequence context (AAAAATGGCA, Figure 3) of the proposed initiation codon is typical of plant nuclear genes (Lutcke et al., 1987), having an A at position –3 and

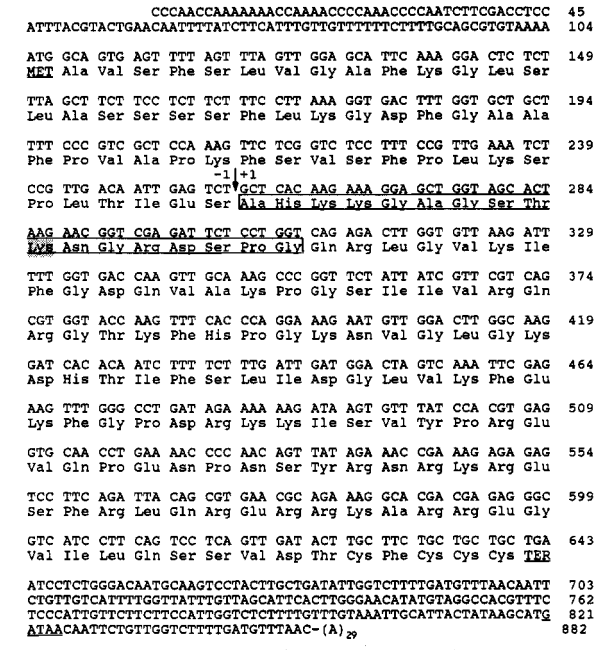

FIGURE 3: Nucleotide sequence of cDNA L27-1 and deduced amino acid sequence of the nuclear-encoded tobacco chloroplast ribosomal protein L27. The initiation codon (Met^{-51}), the termination codon, and a putative polyadenylation signal are underlined. The first 17 amino acids of the mature protein determined by protein sequencing are boxed. The shaded residue (Lys^{+10}) could not be definitively determined. The vertical arrow indicates the site of cleavage of transit peptide and mature protein.

a G at +4. A putative polyadenylation signal, GATAA (nucleotides 821–825, Figure 3), located 34 nucleotides upstream from the poly(A)$^+$ tail, matches the pentanucleotide consensus sequence for plants (Messing et al., 1983), though it differs by 1 nucleotide from the consensus polyadenylation signal (AATAA) for animal genes.

Identification of Mature Tobacco L27 Protein. *E. coli* cells were infected with recombinant phage containing L27-4 cDNA (Figure 1A) and were induced by IPTG to express the lacZ–L27 fusion protein coding sequence. The expressed L27 fusion protein was transferred to a nitrocellulose filter which was used as an affinity matrix to isolate monospecific antibody to L27 protein from serum of rabbits immunized with unfractionated 50S chloroplast ribosomal subunit proteins. The monospecific anti-L27 antibody was used to identify an immunoreactive HPLC-purified protein (fraction 6, Figure 2) corresponding to L27 among HPLC-purified tobacco 50S chloroplast ribosomal proteins. The amino-terminal 17 amino acids of this protein (boxed sequence, Figure 3), determined by protein microsequencing, correspond with those deduced from cDNA sequence.

Evolutionary Relationship with Other L27 Proteins. Comparisons of the predicted amino acid sequence of tobacco L27 with those in the NBRF (National Biomedical Research Foundation) protein library revealed homologies with procaryotic ribosomal proteins from *B. subtilis*, *E. coli*, and *B. stearothermophilus* (Figure 4). The identities of the predicted mature tobacco ribosomal protein L27 (residues 52–139) with these procaryotic L27 protein sequences are 66%, 61%, and 56%, respectively, for *B. subtilis*, *E. coli*, and *B. stearothermophilus*. Allowing conservative amino acid replacements, the similarities between tobacco L27 and the procaryotic proteins are 75% (*B. subtilis*), 77% (*E. coli*), and 72% (*B. stearothermophilus*) occurring throughout the aligned sequences of the four proteins. The tobacco L27 protein is 86% and 50% identical with partial amino-terminal sequences of mature L27-type chloroplast ribosomal proteins of spinach (A. R. Subramanian, personal communication) and *Chlamydomonas* [protein L18; Liu et al., 1988; nomenclature of Schmidt et al. (1983); annotated L27(L18) hereafter]. Tobacco chloroplast ribosomal protein L27 has significant amino acid sequence similarity to the nuclear-encoded yeast mitochondrial ribosome large subunit protein MRP7 (Fearon & Mason, 1988). When the portions of MRP7 (residues 28–117) and mature tobacco L27 (residues 52–139) which are optimally aligned with the *E. coli* L27 protein are compared, tobacco L27 is 48% identical (66% similarity, including conservative amino acid substitutions; Figure 5) with MRP7. There is also 41% similarity between the aligned portions of the proposed amino-terminal 27-residue targeting peptide of MRP7 (Fearon & Mason, 1988) and the predicted tobacco chloroplast L27 transit peptide (Figure 5). The carboxyl-terminal extension of tobacco L27 (residues 140–179) may also be related (36% similar, identities plus conservative substitutions) to the corresponding portion of yeast mitochondrial ribosomal protein MRP7 (Figure 5).

Predicted Properties of the L27 Precursor Protein. The transit peptide (amino acids Met^{-51} to Ser^{-1}) of tobacco L27 lacks Arg, His, Tyr, Asn, Cys, Trp, and Gln (Figure 3), but there is a relatively high content (mole percent) of other amino acids [Ser and Thr (23.6%), Phe (13.7%), and Ala and Leu (each 11.8%)]. The basic amino acid content (7.8% Lys) is about twice that of the acidic residues (4% Asp/Glu). These characteristics are typical of chloroplast protein transit peptides, i.e., a high content of Ser and Thr, absence of Trp and Tyr, none or few acidic amino acids, relatively high content of centrally-located Pro (3 for L27), a net positive charge, and a conserved amino acid composition with no significant amino acid sequence homology (Schmidt & Mishkind, 1986). Like other chloroplast targeting sequences (von Heijne et al., 1989), the L27 transit peptide has at least three distinct regions. The amino-terminal 10 amino acids are mostly uncharged and lacking in Pro; no corresponding region occurs in mitochondrial targeting peptides. The central region is rich in Ser and contains few acidic amino acids. As in other nuclear-encoded chloroplast ribosomal proteins, the amino-terminal dipeptide of the L27 precursor protein is Met-Ala (von Heijne et al., 1989). These observations and the amino acid sequence similarities mentioned above with mature spinach L27 and *Chlamydomonas* L27(L18) chloroplast ribosomal proteins support our conclusion that the tobacco L27 sequences reported here are of chloroplast, not mitochondrial, origin.

The mature tobacco L27 protein lacks Met and Trp. The content of basic amino acids (25% Arg/Lys/His) is more than double that of the acidic residues (9.4% Glu/Asp), giving L27 a net positive charge characteristic of ribosomal proteins. The stop codon is TGA, present in about one-third of higher plant nuclear-encoded genes (Aota et al., 1989). The coding sequence for the L27 precursor protein shows a preference for codons ending in T (for Leu, Val, Ser, Ala, Asp, and Gly), C (for His, Asn, and Cys), A (for Arg), and G (for Gln, Glu, and Lys). Thus, the gene for ribosomal protein L27 differs from chloroplast DNA-encoded ribosomal protein genes (Subramanian et al., 1983; Shinozaki et al., 1986), in which codons ending in A or T are more abundant. This difference probably reflects, in part, the higher GC content of tobacco nuclear DNA, relative to chloroplast DNA (Kirk & Tilney-Basset, 1978).

Amino-Terminal Processing of L27. Since the amino terminus of mature L27 protein isolated from tobacco chloroplast ribosomes is located at Ala^{+1}, the transit peptide

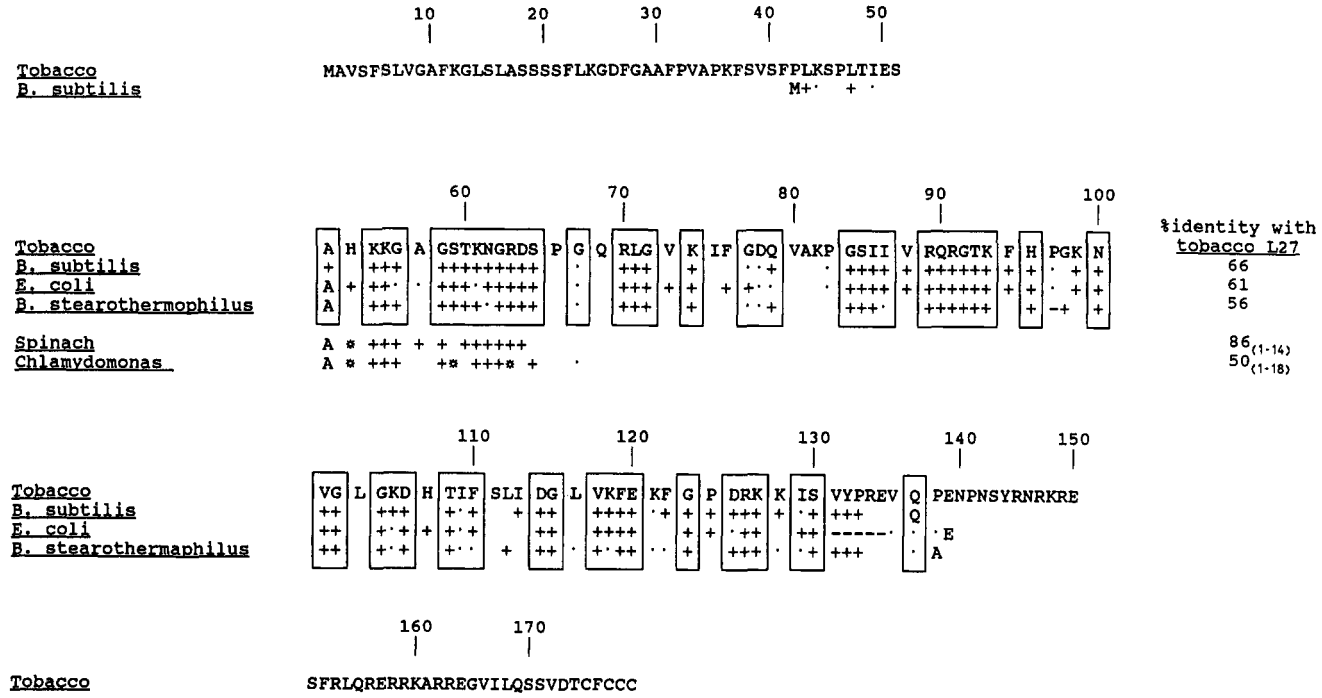

FIGURE 4: Alignment of chloroplast and procaryotic L27-type ribosomal proteins. The chloroplast L27 sequences are those of tobacco, spinach (amino-terminal 14 amino acids; A. R. Subramanian, personal communication), and *Chlamydomonas* [first 18 amino acids of L27(L18) protein; Liu et al., 1988]. The procaryotic L27 proteins are those of *B. subtilis*, *E. coli*, and *B. stearothermophilus* (NBRF). Symbols indicate (+) identical amino acids, (dots) conserved replacements, (–) gaps, and (asterisks) unidentified amino acids relative to the tobacco L27 sequence. Nc ıconservative amino acids are indicated by blank spaces. The terminal amino acids are explicitly indicated for each protein. Invariant and conserved residues in the tobacco and procaryotic proteins are boxed. The numerical scale refers to the tobacco L27 amino acid sequence. Sequences for spinach and *Chlamydomonas* proteins were determined by protein sequencing.

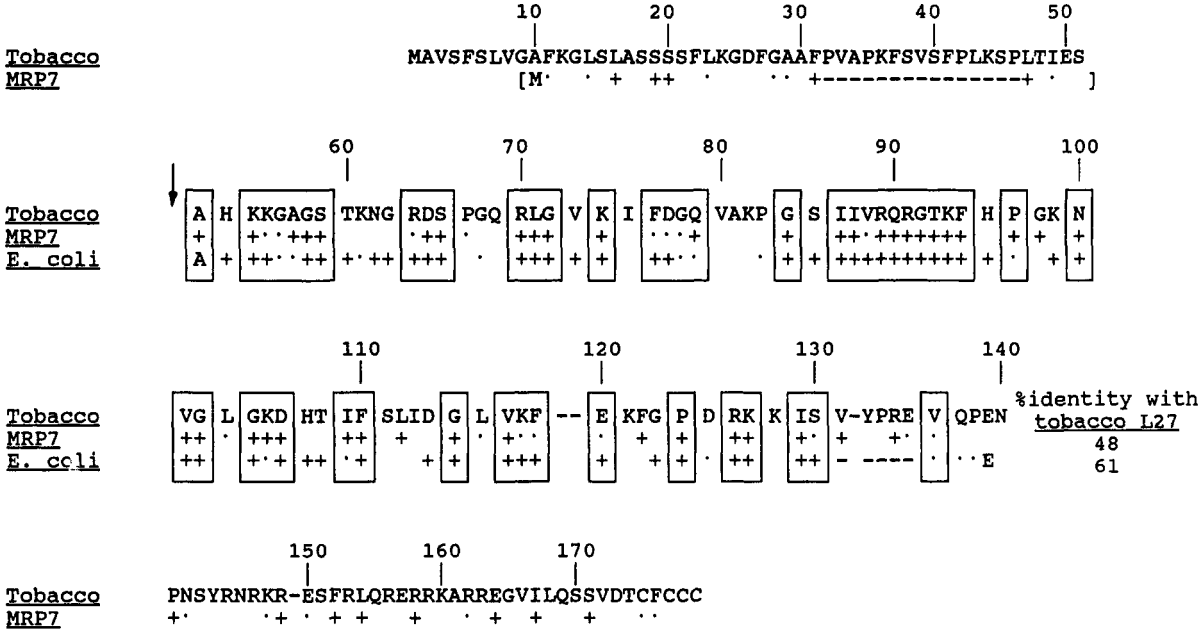

FIGURE 5: Alignment of the tobacco L27 ribosomal protein sequence with that of MRP7, a protein of the large subunit of yeast mitochondrial ribosomes and the ribosomal protein L27 of *E. coli*. The first 27 amino acids of the MRP7 protein represent the proposed cleavable amino-terminal leader sequence (in brackets) which targets the protein into the mitochondria (Fearon & Mason, 1988). The transit peptide of the tobacco L27 ribosomal protein which targets the protein into chloroplasts consists of the first 51 amino acids of the tobacco precursor protein. Symbols indicate (+) identical amino acids, (dots) conserved replacements, and (–) gaps relative to the tobacco L27 sequence. Nonconserved amino acids are indicated by blank spaces. The numerical scale refers to the tobacco L27 protein amino acid sequence. The vertical arrow indicates the tobacco L27 transit peptide cleavage site. Only the amino-terminal 156 amino acids of the 371 amino acids of MRP7 are shown.

cleavage site is predicted to be the peptide bond between Ala^{+1} and Ser^{-1} (Figure 3). This conclusion is supported by several similarities with related L27 proteins. Cleavage at this site would give a 51-residue-long transit peptide. There is a close match between the amino-terminal sequences of tobacco, spinach, and *Chlamydomonas* chloroplast L27 ribosomal proteins, with similar conserved sequences of L27 from *E. coli* and two *Bacillus* species (Figure 4), and with the proposed amino terminus of the mature mitochondrial protein MRP7 (Figure 5). Ala is the amino-terminal amino acid of all of these L27 proteins. Finally, in-frame translation of the sequence upstream from Met^{-51} gives a predicted polypeptide sequence whose amino acid composition is unlike that of a typical transit peptide (for example, the Ser/Thr content would

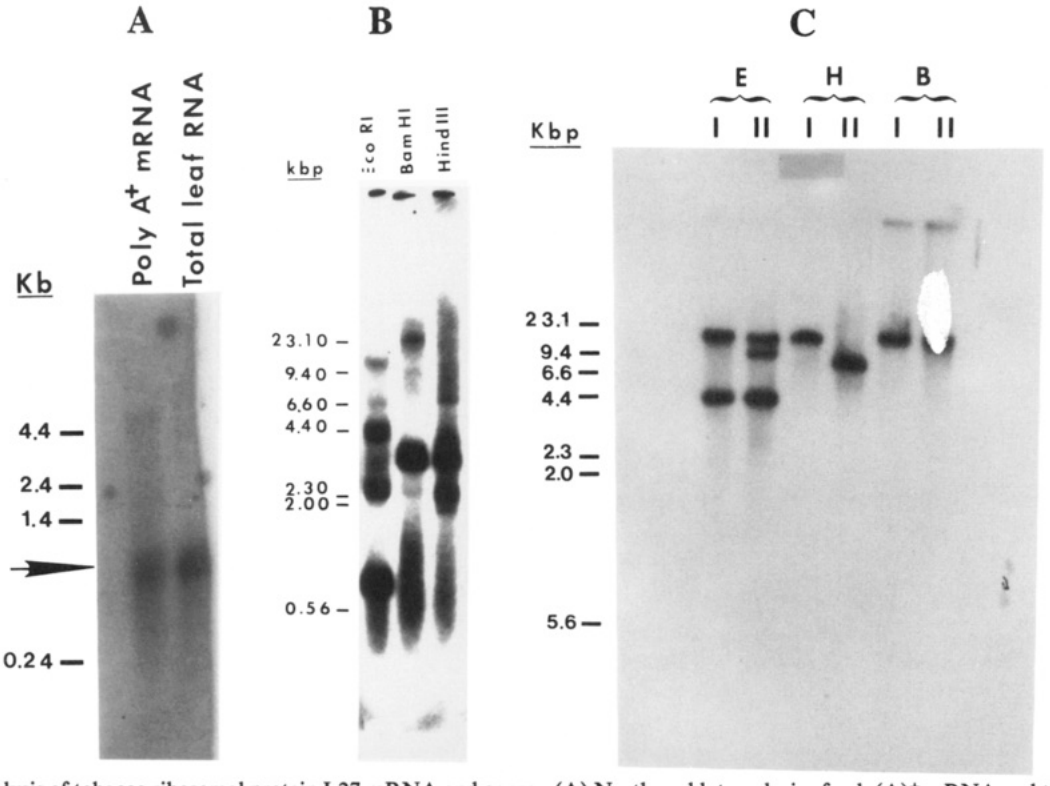

FIGURE 6: Analysis of tobacco ribosomal protein L27 mRNA and genes. (A) Northern blot analysis of poly(A)$^+$ mRNA and total leaf RNA probed with ^{32}P-labeled L27-1 cDNA. Poly(A)$^+$ mRNA (2 μg) and total leaf RNA (5 μg) from tobacco seedlings were denatured and electrophoresed in a 1.2% agarose gel containing 2.2 M formaldehyde. Indicated on the left is the mobility of RNA size markers. The arrow points to L27 mRNA (0.85–0.90 kb). (B) Southern blot analysis of tobacco genomic DNA probed with L27-1 cDNA. DNA (10 μg) was digested to completion with either *Eco*RI, *Bam*HI, or *Hin*dIII, electrophoresed on an 0.8% agarose gel, transferred to nitrocellulose filters, and probed with the ^{32}P-labeled L27-1 cDNA. The size and mobility of molecular weight markers are indicated on the left. (C) Hybridization of cloned tobacco ribosomal protein L27 genes to L27 cDNA. DNA from two EMBL-3 clones carrying genes (referred to as I and II) for the L27 ribosomal protein was cut with either *Eco*RI (E), *Hin*dIII (H), or *Bam*HI (B) and electrophoresed on an 0.8% agarose gel. The DNA was transferred to nitrocellulose filters and hybridized to the full-length L27-1 cDNA. Molecular weight markers are indicated in kbp on the left.

be only 0.06%). The second amino acid of mature tobacco L27 is His. Phosphorylation of histidine at this position may have prevented its determination in the case of *Chlamydomonas* and spinach L27-type proteins.

L27 Carboxyl-Terminal Region. The carboxyl terminus of tobacco L27 protein extends 40 amino acids beyond that of the homologous procaryotic proteins (Figure 4) and is as basic (24.4%, Arg/Lys; 13.3%, Glu/Asp) as the evolutionarily conserved portion (residues 52–139) of the protein, with 47% charged amino acids and a central cluster (residues 156–163) of basic amino acids (RERRKARR). Carboxyl-terminal extensions of 20 or more residues have been reported for other nuclear-encoded chloroplast ribosomal proteins, including the L24-type proteins from tobacco (Elhag and Bourque, unpublished results; Bourque et al., 1991) and pea (Gantt, 1988), the L21 protein from spinach (Smooker et al., 1990), and the S17-type proteins from pea and spinach (Gantt & Thompson, 1990). A very unusual, cysteine-rich sequence, CFCCC, terminates the carboxyl end of the L27 precursor polypeptide sequence. Computer analysis of protein sequence databases indicates that this pentapeptide is unique.

L27 mRNA and Genes. To examine the size of L27 mRNA, a cDNA (L27-1, cf. Figure 1A) coding for the complete tobacco ribosomal protein L27 precursor polypeptide was hybridized to poly(A)$^+$ mRNA and total leaf RNA from young tobacco leaves. As shown in Figure 6A, the cDNA hybridized to a broad band of about 0.85–0.9 kb of both poly(A)$^+$ mRNA and total leaf RNA. This result suggests that the longest cDNAs isolated which code for the L27 ribosomal protein are representative of full-length mRNA.

While screening the cDNA library to obtain full-sized cDNAs encoding the L27 ribosomal protein, several cDNAs were isolated. One of these (L27-1a) was identical to L27-1 cDNA except for (1) an A to G substitution at position 651, (2) the lack of an AAC (nucleotides 851–853), and (3) a shorter poly(A)$^+$ tail (Figure 7A). A similar stem–loop secondary structure (ΔG = –39.5 kcal/mol), which might affect mRNA stability (Stern et al., 1991), can be predicted for the 3′-noncoding sequence of both mRNAs (Figure 7B). If these secondary structures occur in vivo, their proposed contribution to mRNA stability could affect L27 mRNA translation rates, as observed in other systems (Inglebrecht et al., 1989; Jackson & Standart, 1990).

The genomic complexity of L27 coding sequences was probed by Southern blot analysis (Figure 6B). Three major (0.9, 2.4, and 4.4 kbp) and two minor (6.4 and 11.5 kpb) restriction fragments were detected upon digestion of tobacco genomic DNA with *Eco*RI. Cleavage with *Bam*HI resulted in one major (3.5 kbp) and one minor (23.1 kpb) fragment, while cleavage with *Hin*dIII resulted in two major (2.3 and 3.3 kpb) and one minor (6.9 kpb) fragment. The nucleotide sequence of the cDNA encoding protein L27 does not possess internal recognition sites for either *Eco*RI, *Bam*HI, or *Hin*dIII. These data suggest that tobacco has more than one gene coding for ribosomal protein L27.

Characterization of cDNAs L27-1 and L27-1a, in addition to the Southern hybridization data, prompted an attempt to search for the existence of multiple genes for the tobacco L27 ribosomal protein. An L27-1 cDNA probe was used to screen a λEMBL3 primary tobacco genomic DNA library for clones

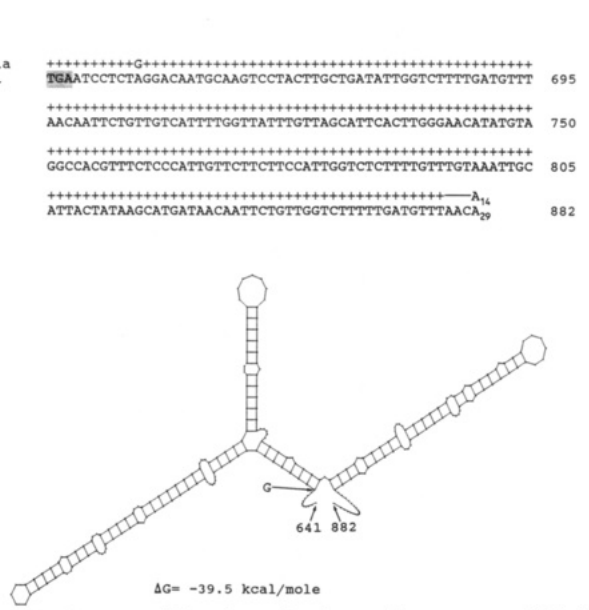

FIGURE 7: Sequence (A) and predicted secondary structure (B) of the 3′-noncoding region of two tobacco L27 ribosomal protein cDNAs. The numbering of nucleotides on the right of the sequence in (A) is as in Figure 3. The secondary structure shown in (B) is that of L27-1 with the site of the G at position 651 in L27-1a indicated by the horizontal arrow. The secondary structure was generated using the computer programs Fold and Squiggles of the Wisconsin GCG package.

harboring ribosomal protein L27 genes. DNA from two different positive plaques was digested with the restriction endonucleases *Eco*RI, *Bam*HI, or *Hin*dIII, electrophoresed in a 0.8% agarose gel, transferred to nitrocellulose paper, and probed with L27-1 cDNA. Different restriction fragment patterns (Figure 6C) resulted from cleavage of DNA from the two positive plaque isolates. The possibility that these clones are derived from different L27 genes is consistent with our data, but confirmation awaits sequence analysis of these genomic clones.

DISCUSSION

This report provides the first complete cDNA sequence coding for a higher plant nuclear-encoded chloroplast L27 ribosomal protein. The homology between tobacco and *E. coli* L27 ribosomal proteins is among the highest observed (61% identity; 77% similarity, allowing conservative replacements) between related higher plant and bacterial ribosomal proteins. Excluding the transit peptide sequence and the carboxyl-terminal extension of chloroplast L27, the identities between the two L27 proteins are distributed throughout the length of the bacterial protein. The considerable homology (48% identity, 66% similarity) also observed between a eucaryotic mitochondrial ribosomal protein (MRP7 of yeast; Fearon & Mason, 1988) and the mature chloroplast ribosomal protein L27 suggests that L27-type proteins are universally associated with the peptidyltransferase center involved in GTP hydrolysis. The amino acid similarities between coding sequences and the possibly significant similarities between targeting peptides and immediately adjacent carboxyl-terminal sequences suggest that tobacco L27 and yeast MRP7 have common phylogenetic origins.

Curiously, all four cysteine residues in the tobacco L27 protein are clustered at the carboxyl end (CFCCC) of the precursor polypeptide coding sequence. In procollagen, cysteines at the carboxyl-terminal propeptide provide disulfide bridges which assure proper folding of the protein (Fessler et al., 1981). The cysteines of chloroplast ribosomal protein L27 could provide for (reversible) disulfide bridges which might be involved in providing important structural features to the L27 protein. Experimental evidence is necessary to show whether the CFCCC sequence is retained in L27 protein after ribosome assembly.

On the basis of cDNA and amino acid sequence comparisons, processing of an M_r 19 641 precursor polypeptide of tobacco L27 is likely to give an M_r 5221 transit peptide and a mature tobacco L27 protein of M_r 14 420, similar in size to that estimated (M_r 15 500) for the mature *Chlamydomonas* L27(L18) protein (Schmidt et al., 1985). The *Chlamydomonas* L27(L18) precursor polypeptide (Schmidt et al., 1984) is sequentially processed by a fast reaction (probably during transport into chloroplasts) followed by a slow step (Schmidt et al., 1985) which occurs during ribosome assembly and which has been suggested to involve carboxyl-terminal processing (Liu et al., 1988). Kinetically, slow processing is characteristic of carboxyl-terminal cleavage, such as observed for glucagon (Tager & Steiner, 1973; Patzelt et al., 1979) and procollagen (Fessler et al., 1975, 1981). In chloroplasts, processing of the carboxyl end of the 32-kDa D1 protein of chloroplast photosystem II is required for its assembly into the photosystem II complex (Marder et al., 1984). Assuming that the fast step is transit peptide cleavage, if a carboxyl-terminal extension similar to that of tobacco L27 also exists in the *Chlamydomonas* L27(L18) protein, it could include the site of the second, kinetically-slow step of *Chlamydomonas* L27(L18) precursor processing. If the entire carboxyl-terminal extension of tobacco L27 were to be removed during processing, the resulting protein (M_r 9562) would be much smaller than the mature *Chlamydomonas* L27(L18) protein. Since the sizes of the two M_r 1500 (estimated) peptides reported to be cleaved from *Chlamydomonas* L27(L18) protein differ from both the putative transit peptide of tobacco L27 and its proposed carboxyl-terminal extension (M_r 4858), it is possible that these two proteins differ in the (1) lengths of transit peptides and/or carboxyl-terminal regions and the (2) nature and details of specific processing events. These observations and the 36% similarity with the corresponding portion of yeast mitochondrial ribosomal protein MRP7 suggest that a carboxyl-terminal extension may be important in the function of chloroplast L27 proteins. Additional experimental evidence is necessary to resolve these possibilities.

Sequences of two different cDNA clones and Southern blot analysis of tobacco genomic DNA and isolated genomic clones provide preliminary evidence that at least two different expressed genes exist for tobacco L27 protein, although other interpretations of these data are possible. *Nicotiana tabacum* is an allotetrapolyploid species resulting from hybridization of the diploid progenitors *Nicotiana sylvestris* and *Nicotiana tomentosiformis* (Smith, 1975, 1979). Genomic DNA fragments which hybridize to L27 cDNA probes could represent restriction fragment polymorphism among multiple L27 alleles which are expected to be present in this species. It should be possible to trace the phylogenetic origin of different L27 genes by analysis of Southern hybridization of L27 probes to restriction enzyme digests of genomic DNA from the progenitor species. Similar analysis has traced the origin of multiple genes for the enzyme acetolactate synthase (Lee et al., 1988).

Ribosome synthesis is tightly coordinated both in procaryotes and in eucaryotes. In *E. coli*, translational feedback regulation of ribosomal protein synthesis by ribosomal proteins themselves plays a major role in balancing the synthesis of

ribosomal proteins relative to cellular rRNA levels (Nomura et al., 1984). *E. coli* ribosomal protein L27 is not part of a large operon nor does it appear to be an autoregulatory protein (Takata, 1978). It is possible that carboxyl-terminal sequences are involved in regulation of organellar ribosome biogenesis. For instance, the carboxyl terminus of ribosomal protein L27 might become involved in disulfide bond linkages (mediated by the terminal pentapeptide CFCCC), resulting in facilitation of transport into the chloroplast, ribosome assembly, or protein synthesis. The addition of carboxyl-terminal extensions during evolution of chloroplast and mitochondrial ribosomal proteins might have provided these proteins with new features which could function as regulatory elements. This hypothesis provides a testable model for a role of nuclear-encoded chloroplast ribosomal proteins in regulating chloroplast ribosome structure and function.

ACKNOWLEDGMENT

We thank Steve Carls and Jane Huff for their assistance in preparation of the manuscript and Barbara Tharp for technical assistance.

REFERENCES

Amaldi, F., Bozzoni, I., Beccari, E., & Amaldi-Pierandrei, P. (1989) *Trends Biochem. Sci. 14*, 175–178.

Aota, S., Gojobori, T., Maruyama, T., & Ikemura, T. (1988) *Nucleic Acids Res. 16*, r315–r402.

Bedbrook, J. (1981) *Plant Mol. Biol. Newsl. 2*, 24.

Bonham-Smith, P. C., & Bourque, D. P. (1990) in *Chromosomes: Eucaryotic, Procaryotic, and Viral* (Adolph, K., Ed.) Vol. II, pp 179–216, CRC Press Inc., Boca Raton, FL.

Bourque, D. P., & Capel, M. S. (1982) in *Methods in Chloroplast Molecular Biology* (Edelman, M., Hallick, R. B., & Chua, N. H., Eds.) pp 617–628, Elsevier Biomedical, New York.

Bourque, D. P., Elhag, G. A., Bonham-Smith, P. C., Thomas, F., McCreery, T., & Glinsmann-Gibson, B. (1991) in *The Translational Apparatus of Photosynthetic Organelles* (Mache, R., Stutz, E., & Subramanian, A. R., Eds.) pp 85–93, Springer-Verlag, Berlin.

Boynton, J. E., Gillham, N. W., & Lambowitz, A. M. (1980) in *Ribosomes: Structure, Function and Genetics* (Chamblis, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 905–950, University Park Press, Baltimore, MD.

Capel, M. S., & Bourque, D. P. (1982) *J. Biol. Chem. 257*, 7746–7755.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.

Dabbs, E. R., Hasenbank, R., Kastner, B., Rak, K.-H., Wartusch, B., & Stoffler, G. (1983) *Mol. Gen. Genet. 192*, 301–308.

Elhag, G. A., Thomas, F. J., McCreery, T. P., & Bourque, D. P. (1992) *Nucleic Acids Res. 20*, 689–697.

Ellis, R. J. (1969) *Science 158*, 477–478.

Fearon, K., & Mason, T. L. (1988) *Mol. Cell. Biol. 8*, 3636–3646.

Feinberg, A. P., & Vogelstein, B. (1983) *Anal. Biochem. 132*, 6–13.

Fessler, L. I., Morris, N. P., & Fessler, J. H. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4905–4909.

Fessler, L. I., Timple, R., & Fessler, J. H. (1981) *J. Biol. Chem. 256*, 2531–2537.

Gantt, J. S. (1988) *Curr. Genet. 14*, 519–528.

Gantt, J. S., & Key, J. L. (1986) *Mol. Gen. Genet. 202*, 186–193.

Gantt, J. S., & Thompson, M. D. (1990) *J. Biol. Chem. 265*, 2763–2767.

Gourse, R. L., Sharrock, R. A., & Nomura, M. (1986) in *Structure, Function and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) pp 766–788, Springer-Verlag, New York.

Gubler, U., & Hoffman, B. J. (1983) *Gene 25*, 263–269.

Gulle, H., Hoppe, E., Osswald, M., Grewer, B., Brimacombe, R., & Stoffler, G. (1988) *Nucleic Acids Res. 16*, 815–832.

Hackle, W., Stoffler-Meilicke, M., & Stoffler, G. (1988) *FEBS Lett. 233*, 119–123.

Hiratsuka, J., Shimada, H., Whittier, R., Ishibashi, T., Sakamoto, M., Mori, M., Kondo, C., Honji, Y., Sun, C.-R., Meng, B.-Y., Li, Y.-Q., Kanno, A., Nishizawa, Y., Hirai, A., Shinozaki, K., & Sugiura, M. (1989) *Mol. Gen. Genet. 217*, 185–194.

Huynh, T. V., Young, R. A., & Davis, R. W. (1985) in *DNA Cloning* (Glover, D. M., Ed.) Vol. I, pp 49–78, IRL Press, Oxford.

Ingelbrecht, I. L. W., Herman, L. M. F., Dekeyser, R. A., Van Montagu, M. C., & Depicker, A. G. (1989) *Plant Cell 1*, 671–680.

Jackson, R. J., & Standart, N. (1990) *Cell 62*, 15–24.

Johnson, L. M., Snyder, M., Chang, L. M. S., Davis, R., & Campbell, J. L. (1985) *Cell. 43*, 369–377.

Kemp, D. J., & Cowman, A. F. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4520–4522.

Kirk, T. T. O., & Tilney-Basset, R. A. E. (1978) *The Plastids*, Elsevier, New York.

Kozak, M. (1987) *Nucleic Acids Res. 15*, 8125–8148.

Kroon, A. M. (1965) *Biochim. Biophys. Acta 108*, 275–284.

Lake, J. A., & Strycharz, W. A. (1981) *J. Mol. Biol. 153*, 979–992.

Lamborg, M. R., & Zamecnik, P. C. (1960) *Biochim. Biophys. Acta 42*, 206–211.

Lee, K. Y., Townsend, J., Tepperman, J., Black, M., Chui, C. F., Mazur, B., Dunsmuir, P., & Bedbrook, J. (1988) *EMBO J. 7*, 1241–1248.

Lindahl, L., & Zengel, M. J. (1986) *Annu. Rev. Genet. 20*, 297–326.

Liu, Z.-Q., Gillham, N. W., & Boynton, J. E. (1988) *Mol. Gen. Genet. 214*, 588–591.

Lutcke, 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.

Marconi, R. T., Lodmell, J. S., & Hill, W. E. (1990) *J. Biol. Chem. 265*, 7894–7899.

Marder, J. B., Goloubinoff, P., & Edelman, M. (1984) *J. Biol. Chem. 259*, 3900–3908.

Messing, J., Geraphty, D., Heidecker, G., Hu, N.-T., Kridl, J., & Rubenstein, I. (1983) in *Genetic Engineering of Plants* (Kosuge, T., Meredith, C. P., & Hollaender, A., Eds.) pp 211–227, Plenum, New York.

Morrison, C. A., Garrett, R. A., Zeichhardt, H., & Stoffler, G. (1973) *Mol. Gen. Genet. 127*, 359–368.

Mount, D. W., & Conrad, B. (1986) *Nucleic Acids Res. 14*, 443–454.

Nomura, M., Gourse, R., & Baughman, G. (1984) *Annu. Rev. Biochem. 53*, 75–118.

Ofengand, J., Ciesiolka, J., Denman, R., & Nurse, K. (1986) in *Structure, Function and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) pp 473–494, Springer-Verlag, New York.

Ogden, R. C., & Adams, D. A. (1987) *Methods Enzymol. 152*, 61–87.

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 322*, 572–576.

Patzelt, C., Tager, H. S., Carroll, R. J., & Steiner, D. F. (1979) *Nature 282*, 260–266.

Phua, S. H., Srinivasa, B.-R., & Subramanian, A. R. (1989) *J. Biol. Chem. 264*, 1968–1971.

Planta, R. J., Mager, W. H., Leer, R. J., Woudt, L. P., Roue, H. A., & El Baradi, T. T. A. L. (1986) in *Structure, Function and Gentics of Ribosomes* (Hardesty, B., & Krammer, G., Eds.) pp 699–718, Springer-Verlag, New York.

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.

Schmidt, R. J., Richardson, C. B., Gillham, N. W., & Boynton, J. E. (1983) *J. Cell Biol. 96*, 1451–1463.

Schmidt, R. J., Meyers, A. M., Gillham, N. W., & Boynton, J. E. (1984) *J. Cell Biol. 98*, 2011–2018.

Schmidt, R. J., Gillham, N. W., & Boynton, J. E. (1985) *Mol. Cell. Biol. 5*, 1093–1099.

Shimada, H., & Sugiura, M. (1991) *Nucleic Acids Res. 19*, 983–995.

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.

Sigel, M. B., Sinha, Y. N., & Vanerlaan, W. P. (1983) *Methods Enzymol. 93*, 3–12.

Silhavy, T. J., Berman, M. L., & Engwit, L. W. (1984) *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Smith, H. H. (1975) *Handb. Genet. 2*, 281–314.

Smith, H. H. (1979) in *Nicotiana: Procedures for Experimental Use* (Durbin, R. D., Ed.) pp 1–16, USDA Technical Bulletin 1586, Academic Press, New York.

Smooker, P. M., Kruft, V., & Subramanian, A. R. (1990) *J. Biol. Chem. 265*, 16699–16703.

Sonenberg, N., Wilcheck, M., & Zamir, A. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 1423–1426.

Southern, E. M. (1975) *J. Biol. Chem. 98*, 503–517.

Stern, D. B., Radwanski, E. R., & Kindle, K. L. (1991) *Plant Cell 3*, 285–297.

Stoffler, G., & Stoffler-Meilicke, M. (1986) in *Structure, Function and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) pp 28–46, Springer-Verlag, New York.

Subramanian, A. R., & Giese, K. (1989) *Biochemistry 28*, 3525–3529.

Subramanian, A. R., Steinmetz, A., & Bogorad, L. (1983) *Nucleic Acids Res. 11*, 5277–5286.

Subramanian, A. R., Smooker, P. M., & Giese, K. (1990) in *The Ribosome: Structure, Function and Evolution* (Hill, W. F., Dahlberg, A., Garrett, R. A., Moore, P. B., Schlessinger, D., & Warner, J. R., Eds.) pp 655–663, American Society for Microbiology, Washington, D.C.

Tabor, S., & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 4767–4771.

Tager, H. S., & Steiner, D. F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2321–2325.

Takata, R. (1978) *Mol. Gen. Genet. 160*, 151–155.

Theologis, A., Huynh, T. V., & Davis, R. W. (1985) *J. Mol. Biol. 183*, 53–68.

Traut, R. R., Lambert, J. M., Boileau, G., & Kenny, J. W. (1980) in *Ribosomes: Structure, Function and Genetics* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 89–110, University Park Press, Baltimore, MD.

Vieira, J., & Messing, J. (1987) *Methods Enzymol. 153*, 3–11.

von Heijne, G., Stepphun, J., & Herrmann, R. G. (1989) *Eur. J. Biochem. 180*, 535–545.

Warner, J. R., Elion, E. A., Dabeva, M. D., & Schwindinger, W. F. (1986) in *Structure, Function and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) pp 719–732, Springer-Verlag, New York.

Wool, I. G., Endo, Y., Chan, Y.-L., & Gluck, A. (1990) in *The Ribosome, Structure, Function and Evolution* (Hill, W. E., Dahlberg, A., Garret, R. A., Moore, P. B., Schlessinger, D., & Warner, S. R., Eds.) pp 203–214, American Society for Microbiology, Washington, D.C.

Wower, J., Hixon, S. S., & Zimmerman, R. A. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 5232–5236.

Yokoi, F., Vassileva, A., Hayashida, N., Torazawa, K., Wakasugi, T., & Sugiura, M. (1990) *FEBS Lett. 276*, 88–90.

Zhou, D.-X., & Mache, R. (1989) *Mol. Gen. Genet. 219*, 204–208.