Nuclear-encoded Tobacco Chloroplast Ribosomal Protein L24

PROTEIN IDENTIFICATION, SEQUENCE ANALYSIS OF cDNAs ENCODING ITS CYTOPLASMIC PRECURSOR, AND mRNA AND GENOMIC DNA ANALYSIS*

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Using a Nicotiana tabacum leaf cDNA library in the expression vector λgt11, two cDNAs encoding the full-length precursor polypeptide (M, 20,696) of tobacco chloroplast ribosomal protein L24 were identified and sequenced. These cDNAs encode a mature protein of 146 amino acids (M, 16,418) with a transit peptide of 41 amino acids (M, 4,278). The mature tobacco L24 protein has 78, 65, 45, and 35% sequence identity with ribosomal proteins L24 of pea, spinach, Bacillus subtilis, and Escherichia coli, respectively. The transit peptide of tobacco L24 is 54 and 57% identical with that of L24 chloroplast ribosomal proteins of pea and spinach, respectively. An expressed β-galactosidase:L24 fusion protein, bound to nitrocellulose filters, was used as affinity matrix to purify monospecific antibody to L24 protein. Using this monospecific antibody, protein L24 was identified among high performance liquid chromatography (HPLC)-purified tobacco chloroplast ribosome 50 S subunit proteins. The predicted amino terminus of the mature L24 protein was confirmed by partial sequencing of the HPLC-purified L24 protein. Northern blot analysis revealed a single mRNA band (0.85–0.90 kilobase) corresponding in size to full-length L24 cDNA. The presence of multiple genes for L24 is suggested by Southern blot hybridization and characterization of two cDNAs for L24 which only differ in their 3′-noncoding sequences.

Publication of the complete nucleotide sequences of three chloroplast genomes (Shinozaki et al., 1986; Hiratsuka et al., 1989; Ohyama et al., 1986) has allowed the prediction of most chloroplast DNA protein-coding functions. These findings have emphasized the need to identify those genes located in nuclear DNA which code for the majority of chloroplast polypeptides. Chloroplast ribosome biogenesis requires that synthesis of components which are coded by the chloroplast genome (i.e. ribosomal RNA and 21 ribosomal proteins (Tohdoh and Sugiyura, 1982; Takaiwa and Sugiyura, 1982; Shinozaki et al., 1986; Yokoi et al., 1990)) be coordinated with synthesis of those coded by the nuclear genome (about 40–50 ribosomal proteins; Schmidt et al., 1983, 1984). The primary sequences of several nuclear-encoded chloroplast ribosomal proteins from higher plants have been deduced either from direct protein sequencing (Bartsch et al., 1982), cDNA sequences (including examples from tobacco (Elhag et al., 1992; Elhag and Bourque, 1992), spinach (Giese and Subramanian, 1988; Zhou and Mache, 1989; Phua et al., 1989; Smooker et al., 1990, 1991; Carol et al., 1991), pea (Gantt, 1988), soybean, and Arabidopsis thaliana (Gantt and Thompson, 1990), and from gene sequences (including examples from pea (Gantt et al., 1991), spinach (Martin et al., 1990; Bisanz-Seyer and Mache, 1992), and A. thaliana (Thompson et al., 1992)).

Ribosomal protein L24 is not encoded in published chloroplast genome sequences and, therefore, it is assumed to be a product of a nuclear gene. cDNAs coding for ribosomal proteins with significant amino acid identities to Escherichia coli ribosomal protein L24 were described from pea (Gantt, 1988) and spinach (Carol et al., 1991). Although the function(s) of chloroplast ribosomal protein L24 has not been examined, E. coli 50 S ribosomal subunit protein L24 has several important functions resulting from its participation in specific protein-RNA interactions. L24 is one of only two primary RNA binding proteins responsible for the initiation of assembly of the 50 S ribosomal particles (Nowotny and Nierhaus, 1982; Wittmann-Liebold, 1986). The gene (rpmX), encoding ribosomal protein L24, is located in the spc operon (Cerretti et al., 1983) which also contains (from 5′ to 3′ polarity) genes for ribosomal proteins L14, L24, L5, S14, S8, L6, L18, S5, L30, and L15. Synthesis of proteins in the spc operon is regulated by ribosomal protein S8, the product of the fifth gene of the spc operon (Yates et al., 1986; Dean et al., 1981). Protein S8 binds at the beginning of the third (L5) coding sequence on the polycistronic spc mRNA and regulates translation (Cerretti et al., 1988) of the downstream coding sequences (those for S14 to L15) through indirect coupling with the L5 sequence (Mattheakis and Nomura, 1988). However, regulation of synthesis of ribosomal proteins L14 and L24 by S8 is thought to occur by nucleolytic cleavage of the spc mRNA upstream to its binding site, followed by degradation of the mRNA by 3′-to-5′ exonucleases (Matheakis et al., 1989). Earlier, L24 mutant analysis (Dabbs, 1986) provided evidence that E. coli ribosomal protein L24 is involved in regulation of several unlinked groups of ribosomal protein genes. These results imply that a hierarchy of control in addition to autoregulation might exist, whereby certain ribosomal proteins, such as L24, control expression of other sets of genes by as yet unidentified mechanisms.

This paper reports the isolation and sequence of two cDNAs coding for the complete precursor polypeptide of tobacco chloroplast ribosomal protein L24. We present results of
immunological reactions with monospecific anti-L24 antibody which permitted identification of mature protein L24 among two-dimensional PAGE1-resolved and HPLC-purified tobacco 50 S chloroplast ribosomal subunit proteins. Amino-terminal amino acid sequence analysis allowed deduction of the transit peptide cleavage site. The correspondence of the size of L24 mRNA with that of the characterized cDNA was confirmed by Northern blot analysis of poly(A)⁺ and total RNA. Evidence of multiple genes coding for tobacco ribosomal protein L24 was obtained by Southern blot analysis and by characterization of two different L24 cDNAs.

MATERIALS AND METHODS

RESULTS

cDNA Isolation and Sequence Analysis—Rabbit anti-50 S chloroplast ribosomal protein antiserum was used to screen a cDNA library in the expression vector λgt11. A cDNA (referred to as L24-4, Fig. 1A) coding for the carboxy-terminal region (amino acids 79–146, Fig. 2) of a polypeptide with sequence similarity to E. coli ribosomal protein L24 was identified, recloned, and sequenced. This partial coding cDNA fragment was used as a probe for further screening of the λgt11 library in order to obtain cDNAs containing the complete coding sequence. Three more cDNAs were identified and sequenced. The longest cDNA, 862 bp long (referred to as L24-1, Fig. 1A), contains a 5’-leader sequence of 14 nucleotides followed by a 187 amino acid (561 nucleotides) coding sequence and a 3’-noncoding sequence of 287 nucleotides (Fig. 2). This cDNA encodes a precursor polypeptide of M, 20,696. Additional experimental evidence (see below) indicated that this precursor consists of a transit peptide of 41 amino acids (Ala1 to Met27, Fig. 2) and a mature protein of 146 amino acids (Lys1 to Ser160).

Identification of Mature Tobacco L24 Protein—L24 protein was identified, by immunological reactions with monospecific anti-L24 antibody, among proteins extracted from tobacco 50 S chloroplast ribosomal subunits. E. coli cells infected with recombinant phage NRT L24-4, containing L24-4 cDNA, were induced by isopropyl-1-thio-β-D-galactopyranoside to express the lacZ-L24 fusion protein coding sequence. The expressed β-galactosidase-L24 fusion protein from a plate of confluent plaques was transferred to a nitrocellulose filter which was used as an affinity matrix to isolate monospecific antibody to L24 protein from serum of rabbits immunized with unfractionated 50 S chloroplast ribosomal subunit proteins. After resolving the chloroplast 50 S ribosomal proteins by two-dimensional SDs-gel electrophoresis, a broad immunoreactive band corresponding to the location of chloroplast ribosomal protein L24 was seen (Fig. 3A). When 50 S chloroplast ribosomal proteins were further resolved on two-dimensional PAGE gels, a single 50 S ribosomal protein (approximate M, 16,000) reacted with anti-L24 monospecific antibody (Fig. 3B). The monospecific anti-L24 antibody was also used to identify an immunoreactive HPLC peak corresponding to L24 among HPLC-purified 50 S chloroplast ribosomal proteins (Fig. 3C). The amino-terminal 12 amino acids of this protein, determined by protein sequencing, matches those deduced from cDNA sequence in the 10 out of 12 positions for which the analysis was definitive (boxed sequence, Fig. 2). Thus, the amino terminus of mature L24 protein isolated from chloroplast ribosomes is located at Lys1. Accordingly, the transit peptide cleavage site is predicted to be the peptide bond between Ala1 and Lys1 (Fig. 2), assuming a one-step processing mechanism.

Predicted Properties of L24 Polypeptide—The proposed transit peptide (M, 4,278) has a high content of Ser + Thr, Leu, Pro, Ala, and Phe (Table I), it lacks Trp and Tyr, has only 1 acidic residue (Glu), and has a net positive charge. These characteristics are typical of transit peptides of nuclear-encoded chloroplast proteins (Schmidt and Mishkind, 1986). The amino acid composition of HPLC-purified L24 protein is in close agreement with that predicted for the mature protein from the cDNA sequence (Table I). The mature tobacco L24 ribosomal protein (M, 16,418) has twice the number of basic amino acids (Lys + Arg + His = 40, predominantly Lys = 27) relative to acidic amino acids (Glu + Asp = 19), giving L24 the high net positive charge characteristic of most ribosomal proteins. The amber termination codon (UAG) found in L24 occurs in about 25% of plant genes (Aota et al., 1988).

Most codons, including almost all wobble codons, occur in the ribosomal protein L24 precursor (Table II). Some codon preferences could be detected, including A at the third position for Val, Thr; T for His, Asp, Cys, Gly; C for Phe, Ser, Tyr, Asn, and G for Glu.

Relationships with Other L24 Ribosomal Proteins—Com-
proteins were resolved on a 12.5% polyacrylamide-SDS gel. Immunoblot transfers and probing with 125I-protein A. Also shown is the L24-1 fusion protein. The immunoreactive L24 protein was detected after reaction with 125I-protein A. Chloroplast ribosomal protein antiserum are shown after Western dimensional PAGE identification of 50 S chloroplast ribosomal protein L24. After electrophoresis, proteins were transferred to nitrocellulose filters by the Southern method, and then hybridized with 32P-labeled L24-1 cDNA. Three hybridizing bands (3.5, 4.0, and 5.5 kbp) were observed when DNA was digested with EcoRI (Fig. 5B). Four genomic DNA fragments (3.0, 4.0, 4.4, and 5.5 kbp) were seen when the DNA was digested with BamHI. Since recognition sites for both of these enzymes are lacking in the L24 cDNA sequence, our results indicate either the presence of more than one gene or the existence of introns which harbor recognition sites for these restriction enzymes.

**Northern Blot Analysis of Ribosomal Protein L24 mRNA**—To examine the complexity of ribosomal protein L24 mRNA, the longest cDNA (L24-1, Fig. 1A) was labeled with 32P and used as a probe to hybridize with L24 mRNA. Only one hybridizing mRNA band (0.65–0.9 kb) was observed for both poly(A)+ RNA and total leaf RNA (Fig. 5A). The size of the hybridizing RNA is as expected for the longest cDNA (L24-1, 862 bp), obtained for ribosomal protein L24. However, the broad nature of the banding pattern seen with both poly(A)+ and total RNA indicates the presence of multiple different length L24 mRNAs. These results support the observed range (770–862 bp; Fig. 1) of cDNAs which possess the complete L24 coding sequence.

**Analysis of L24 Coding Sequences in Tobacco Genomic DNA**—Tobacco genomic DNA isolated from young seedlings was digested to completion with either EcoRI or BamHI, transferred to nitrocellulose filters by the Southern method, and then hybridized with 32P-labeled L24-1 cDNA. Three hybridizing bands (3.5, 4.0, and 5.5 kbp) were observed when DNA was digested with EcoRI (Fig. 5B). Four genomic DNA fragments (3.0, 4.0, 4.4, and 5.5 kbp) were seen when the DNA was digested with BamHI. Since recognition sites for both of these enzymes are lacking in the L24 cDNA sequence, our results indicate either the presence of more than one gene or the existence of introns which harbor recognition sites for these restriction enzymes.

**Complexity of cDNA Species Coding for Tobacco Ribosomal Protein L24**—In the course of searching for cDNA clones coding for the complete chloroplast ribosomal protein L24 precursor, another cDNA (L24-2; Fig. 1) with an identical coding sequence to that of L24-1 was identified. Compared with L24-1, cDNA L24-2 is identical except for having nine different nucleotides (Fig. 6A) in its 3′-noncoding sequence, being 112 nucleotides shorter, and having a longer poly(A) tail (A33 compared with A41 in the L24-1 cDNA). The 3′-noncoding sequence of cDNA L24-1 contains a consensus plant polyadenylation signal, ATAAA (Heidecker and Melling, 1986), located at nucleotides 830–834 (248 bases downstream from the stop codon (Fig. 2) and 24 nucleotides upstream from the poly(A) sequence). Although lacking a perfect ATAAA signal, the sequence ATTTA (nucleotides 693–697 and 713–717; Fig. 6A) occurs 45 and 25 nucleotides 5′ to the poly(A) tail of cDNA L24-2.

The 3′-nontranslated region of these two cDNAs could be folded into predictably stable stem loop structures as shown in Fig. 6B. The two structures are somewhat different with the shorter L24-2 cDNA having a more complex branched secondary structure (ΔG = -22.4 kcal/mol) than L24-1 (ΔG = -22.4 kcal/mol). These stem loop structures could affect the rate of L24 gene expression by regulating the rate of accumulation of L24 mRNA in vivo. Similar effects have been observed in other genes (Stern et al., 1991).

**DISCUSSION**

The tobacco L24 precursor polypeptide sequence predicted from cDNA sequences should contain an amino-terminal targeting (transit) peptide which is removed upon import into chloroplasts, as would be expected for nuclear-encoded chlo-
Fig. 4. Alignment of chloroplast and procaryotic L24 ribosomal proteins. The chloroplast L24 sequences are those of tobacco, pea (Gantt, 1988), and spinach (Carol et al., 1991). Procaryotic L24 ribosomal proteins are those of B. subtilis (Henkin et al., 1989) and E. coli (Wittmann-Liebold, 1979). Symbols indicate identical amino acids (+), conservative replacements (dot), and gaps (−), relative to the tobacco L24 sequence. Nonrelated amino acids are indicated by blank spaces. Numbers above the amino acids refer to the positions of the residues of tobacco L24 protein. Shaded residues are those for which E. coli mutants have been characterized. a, percentage were calculated by dividing the number of identical amino acids by the number of amino acids of mature tobacco L24 ribosomal protein (146 residues). b, percentage were calculated by dividing the number of identical amino acids by the number of amino acids of mature tobacco L24 ribosomal protein which are optimally aligned with the procaryotic protein (residues 61–166, positions 71–177).

Fig. 5. Analysis of tobacco chloroplast ribosomal protein L24 mRNA and genes. A, Northern blot analysis of poly(A)+ mRNA and total leaf RNA for chloroplast ribosomal protein L24 message. Formaldehyde-denatured poly(A)+ mRNA (2 μg) and total leaf RNA (5 μg) from tobacco seedlings were electrophoresed in a 1.5% agarose gel containing 2.2 M formaldehyde, transferred to nitrocellulose paper, and hybridized with the 32P-labeled cDNA of clone L24-1 (cf. Fig. 1). The left lane shows the location of RNA size markers electrophoresed in the same gel. The arrow points to the L24 message (0.85–0.9 kb). B, Southern blot analysis of tobacco genomic DNA cut with the restriction enzymes EcoRI and/or BamHI and probed with the L24-1 cDNA. The digested DNA was electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose paper, and probed with 32P randomly labeled L24-1 cDNA. The mobility of molecular weight markers in kilobase pairs are indicated in the left lane. Arrows indicate restriction fragments which hybridize to the L24 probe.

Fig. 6. Sequence and computer generated folding patterns of the 3′-noncoding region of L24 mRNAs. A, the coding sequence of L24-1 is identical to that of L24-2 except for variations in the 3′-noncoding sequences as indicated. The sequence shown starts at nucleotide 576. The termination codon TAG is shaded for reference. The numbering of nucleotides on the right of the sequences is the same as in Fig. 2. B, predicted folding pattern of mRNAs corresponding to these two cDNAs.
(Fig. 2) of mature tobacco L24 protein is Lys$^{+4}$ of the precursor polypeptide. Acidic amino analysis of the HPLC-purified mature L24 protein (Table 1) indicates that it includes the entire 146 amino acids predicted from the cDNA sequences. Thus, if there is only one processing step, we conclude that the tobacco L24 transit peptide cleavage site for tobacco L24 protein occurs between Ala$^{-1}$ and Lys$^{+1}$ (Fig. 2). Comparison of predicted amino acid sequences (Fig. 4) of tobacco, pea, and spinach L24 precursors reveals that remarkably high sequence identity occurs at the proposed transit peptide cleavage sites of these proteins, about 20 residues upstream from the start of homology with bacterial L24 proteins. Based on these considerations, we predict that the transit peptide cleavage sites of pea and spinach L24 proteins are Lys$^{+51}$ and Arg$^{-47}$, respectively.

When compared with their E. coli counterpart (Fig. 4), chloroplast L24 ribosomal proteins have additional elements including the transit peptide (41 residues in tobacco), 19 more residues prior to the start of amino-terminal homology with bacterial counterparts, and a carboxyl-terminal extension of 21–22 amino acids. The high degree of similarity between chloroplast L24 proteins suggests that all three of these regions are important to their structure and/or function. Amino- and carboxyl-terminal extensions, relative to bacterial ribosomal proteins, are common features of mature nuclear-encoded chloroplast ribosomal proteins (Gantt, 1986; Gantt and Thompson, 1990; Smooker et al., 1990, 1991; Elhag and Bourque, 1992). Chloroplast ribosomal protein L13 of spinach, which has both amino- and carboxyl-terminal extensions (Phua et al., 1989), was expressed and assembled into functional bacterial ribosomes (Giese and Subramanian, 1991). That the amino-terminal extension could be removed by mild protease digestion indicated its location on the surface of the “mosaic” ribosome. Whether these amino- and carboxyl-terminal extensions are essential for proper assembly and/or function of chloroplast ribosomes and whether these regions have function(s) unique to the chloroplast ribosome remains to be investigated. Experimental evidence at the protein level is required to reveal the role of these amino- and carboxyl-terminal protein extensions in functional chloroplast ribosomes.

Although the location of poly(A) sites differ by over 150 nucleotides, a consensus plant polyadenylation signal, AATAAA (Heidecker and Messing, 1986), occurs in L24–1 cDNA but is lacking in L24–2 cDNA (Fig. 6). Both cDNA sequences contain a variant signal (ATTAA), differing from the consensus by only 1 base. That this sequence might function as a polyadenylation signal is suggested by its proximity to the poly(A) tail of cDNA L24–2. Location of polyadenylation signal sequences varies, but is within 15–35 nucleotides 5′ to the poly(A) tail in about 90% of plant mRNAs (Heidecker and Messing, 1986; Joshi, 1987; Hunt et al., 1987). The ATTAA sequence has been suggested to function as a polyadenylation signal in spinach nuclear-encoded chloroplast ribosomal protein L13 mRNA (Phua et al., 1989). Mature mRNA of plant genes can both possess more than one polyadenylation signal (Dhaese et al., 1983) and be polyadenylated at alternative sites in vivo (Dean et al., 1986). The ATTAA sequences in cDNA L24–1 could be alternate polyadenylation sites in vivo, although no cDNAs were isolated with the predicted sequences. Typical polyadenylation signals are lacking from a number of higher plant genes (Dean et al., 1986), but the capacity of these mRNAs to be polyadenylated appears to be unaffected (Joshi, 1987). More stringent requirements for sequence conservation of the AATAAA polyadenylation signal exist for animal mRNAs (Humphrey and Proudfoot, 1988; Sheets and Wickens, 1989). Variants (1 or 2 bases) of the animal consensus sequence have been identified and suggested to function as poly(A) addition signals in mRNA for other nuclear-encoded chloroplast ribosomal proteins (Phua et al., 1989; Smooker et al., 1990; Gantt and Thompson, 1990).

We have sequenced two different cDNAs coding for the complete tobacco chloroplast ribosomal protein L24 precursor polypeptide. The cDNAs characterized are identical in their coding sequence with differences existing only in their 3′ noncoding sequence. Sequences of two different cDNAs were also characterized (in each case) for tobacco ribosomal proteins L12 (Elhag et al., 1992) and L27 (Elhag and Bourque, 1992), and two different L27 genomic clones likely to represent different L27 genes have been isolated (Elhag and Bourque, 1992). Since tobacco (Nicotiana tabacum) is an allotetraploid species believed to have arisen by chromosome doubling after hybridization of the diploid parents Nicotiana sylvestris and Nicotiana tomentosiformis (Smith, 1975, 1979), multiple expressed genes for other nuclear-encoded tobacco chloroplast ribosomal proteins would be expected to occur. Analysis of hybridization of L24 probes to restriction enzyme digests of genomic DNA from the progenitor species should permit tracing the phylogenetic origin of different L24 genes, as has been described for acetolactate synthase (Lee et al., 1988). Multiple genes occur for eucaryotic cytoplasmic ribosomal proteins in yeast (Fried et al., 1981), Xenopus laevis (Bocconii et al., 1981), and the mouse (Monk et al., 1981). Only one mouse ribosomal protein (L32) gene is expressed, whereas the remainder (15 genes) are processed pseudogenes (Dudov and Perry, 1984). In yeast, two genes for yeast ribosomal proteins RP51 and L16 are functional, differing in levels of expression (Abovich and Rosbach, 1984; Leer et al., 1985; Rotenberg and Woolfard, 1986).

Single genes have been reported for chloroplast ribosomal proteins in the diploid species spinach (proteins L12 (Giese and Subramanian, 1989), L21 (Martin et al., 1990), and S22 (Bisanz-Seyer and Mache, 1992)), pea (protein L22 (Gantt et al., 1991)), and A. thaliana (proteins S17, L9, and L15 (Thompson et al., 1992)). More than one expressed nuclear gene for a chloroplast ribosomal protein might compensate, in part, for the presence of a single set of nuclear genes in mesophyll cells which can possess a few hundred chloroplasts (each with many chloroplast DNA molecules) and thousands of copies of expressed chloroplast DNA-encoded ribosomal proteins (see review in Bonham-Smith and Bourque, 1990). Stoichiometric amounts of chloroplast and nuclear-encoded chloroplast ribosomal proteins are necessary for chloroplast ribosome biogenesis. The limited number (one to several) of cellular copies of nuclear genes for these ribosomal proteins must possess features permitting high levels of expression in order to assure synthesis and transport into the chloroplast of sufficient quantities to match that produced by expression of numerous copies of chloroplast DNA-encoded ribosomal protein genes.

The significant amino acid sequence identity between the well aligned portions of chloroplast ribosomal protein L24 and its bacterial counterparts shows that these proteins are evolutionarily conserved and suggests that they have similar functions. E. coli ribosomal protein L24 binds to 23 S ribosomal RNA stoichiometrically (Nierhaus, 1980), protecting a fragment (nucleotides 13–527) near the 5′ terminus of 23 S ribosomal RNA (Branlant et al., 1973, 1977; Sloof et al., 1978). The secondary structure of this fragment is predicted to be maintained both by base-pairing and by binding of protein L24 (Krol et al., 1978). The ability of the homologous region
(nucleotides 134–537) of chloroplast 23 S rRNA to form a similar secondary structure has been proposed previously (Takaiwa and Sugiiura, 1982). This observation, as well as the conserved amino acid sequences, suggest that procarotic and chloroplast L24 proteins have similar functions in ribosome assembly and structure. Analysis of a mutation at amino acid position 84 (Gly to Asp) in E. coli resulted in a conditional lethal phenotype with a decreased binding affinity to 23 S ribosomal RNA (Nishi and Schnier, 1986). Two other L24 mutants (Gly to Asp at position 56 and Glu to Lys at position 62) lacked the conditional lethal phenotype (Nishi et al., 1987). Glycine residues 56 and 84 were implicated in formation of hairpin loops in the protein necessary to maintain structural features required for specific binding of L24 to 23 S ribosomal RNA (Nishi et al., 1987). All 3 amino acids in E. coli ribosomal protein L24 which were altered in the mutants described (i.e. Gly, Glu, and Gly) are conserved (Fig. 4) in chloroplast as well as procarotic ribosomal proteins L24 (shaded residues, Fig. 4). This observation suggests that these residues play important functional properties to chloroplast as well as procarotic L24 proteins. Effects of mutations at these positions in tobacco ribosomal protein L24 could provide evidence supporting this hypothesis and a rationale for evolution of conservation of these residues.

L24 proteins of both chloroplast and bacterial ribosomes are likely to play similar roles in assembly, structure, function, and possibly in regulation of ribosomal protein gene expression. Of particular interest is the importance of L24 binding to 23 S chloroplast rRNA during ribosome assembly and whether L24 is involved in regulation of expression of other chloroplast ribosomal protein genes. Also deserving of attention are the structural and functional roles of those portions of the chloroplast L24 polypeptide which differ from procarotic L24 proteins.

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Nuclear-encoded Tobacco Chloroplast Ribosomal Protein L24

Supplement to

Tobacco Chloroplast Ribosomal Protein L24: Isolated in the Nucleus

Isolation and Sequencing Analysis of cDNAs Encoding

In Chloroplast Precursor

by

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MATERIALS AND METHODS

Mapping: Tobacco (Nicotiana tabacum × N. sylvestris) cv. 324A, cv. Samsun, cultivated in greenhouse conditions. Young expanding leaves (5 × 5 cm) were collected, with ice over and stored immediately for RNA isolation by the guanidine thiocyanate-CsCl method (Chomczynski et al., 1993). Pilot A2 DNA was separated from the total RNA on oligo(dT) column (Srivastava et al., 1985). Double-stranded DNA was prepared as outlined by Gubler and Hoffman (1983) using T3 and T7 polymerases. The DNA was methylated with E. coli methylase, ligated to E. coli linkers, digested with E. coli restriction enzymes and cloned into a Bio-Lancear vector (Knapp et al., 1984). Plasmids, chosen to contain full-length cDNAs, were ligated into E. coli pilgramed and propagated in E. coli strain and packaged with the linear rainsery cDNA.