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Tobacco chloroplast ribosomes contain a homologue of *E. coli* ribosomal protein L28

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The genes for ribosomal proteins L28 and L33 constitute an operon (*rpmBG*) in *E. coli*, but in plant chloroplasts L33 is encoded by the chloroplast DNA and L28 seems to be encoded by the nuclear genome. A 15 kDa protein was isolated from the 50 S subunit of tobacco chloroplast ribosomes and its N-terminal amino acid sequence was determined. A cDNA for this protein was cloned and analyzed. The cDNA encodes a 151 amino acid protein consisting of a predicted transit-peptide of 74 amino acids and a mature protein of 77 amino acids. The mature protein is homologous to *E. coli* L28, hence we named it chloroplast L28 (CL28). This is the first report on the presence of an *E. coli* L28-like protein in another organism.

Chloroplast; Ribosomal protein; CL28; Tobacco

1. INTRODUCTION

Chloroplast ribosomes are 70 S in size similar to those of *E. coli* and contain 3–5 different rRNAs and about 60 ribosomal protein species (reviewed in [1,2]). Determination of the entire chloroplast DNA sequence and analysis of chloroplast ribosomal proteins revealed the presence of 21 chloroplast loci for ribosomal proteins in tobacco [3–5], including *rpl33* encoding the ribosomal protein L33 (CL33). This gene has been localized in the large single copy region of tobacco chloroplast DNA, based on its homology to *E. coli* L33 [3]. However no ORF corresponding to *E. coli* ribosomal protein L28 has been found in tobacco chloroplast DNA, although in *E. coli* the genes encoding L28 and L33 occur together in a single operon (*rpmBG*) [6]. Since *E. coli* mutants missing L28 are viable [7], it is possible that chloroplast ribosomes lack an *E. coli* L28-type protein. The primary structure of L28 has only been reported in *E. coli* [8].

To check whether the gene encoding chloroplast L28, if there is one, is encoded by the nuclear genome, we have attempted to isolate the corresponding chloroplast ribosomal protein from tobacco leaves and to clone its cDNA. Sequence analysis revealed in fact the presence of an *E. coli* L28-type protein in tobacco chloroplast ribosomes, suggesting that L28 has a function in ribosomes.

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2. MATERIALS AND METHODS

2.1. *Isolation of ribosomal protein CL28*

Chloroplast ribosomal subunits were prepared from mature tobacco leaves (*Nicotiana tabacum* var. Bright Yellow 4) by a previously reported method [9]. Ribosomal proteins were fractionated by reverse-phase chromatography (Pharmacia FPLC PRO-RPC HR5/10) followed by SDS-polyacrylamide gel electrophoresis (details will be published elsewhere). After being electroblotted on a polyvinylidene difluoride membrane, the proteins were stained with Coomassie R350 stain (Pharmacia). A 15 kDa protein band was cut out and its N-terminal amino acid sequence was determined with a gas-phase protein sequencer (Applied Biosystems 470A-120A).

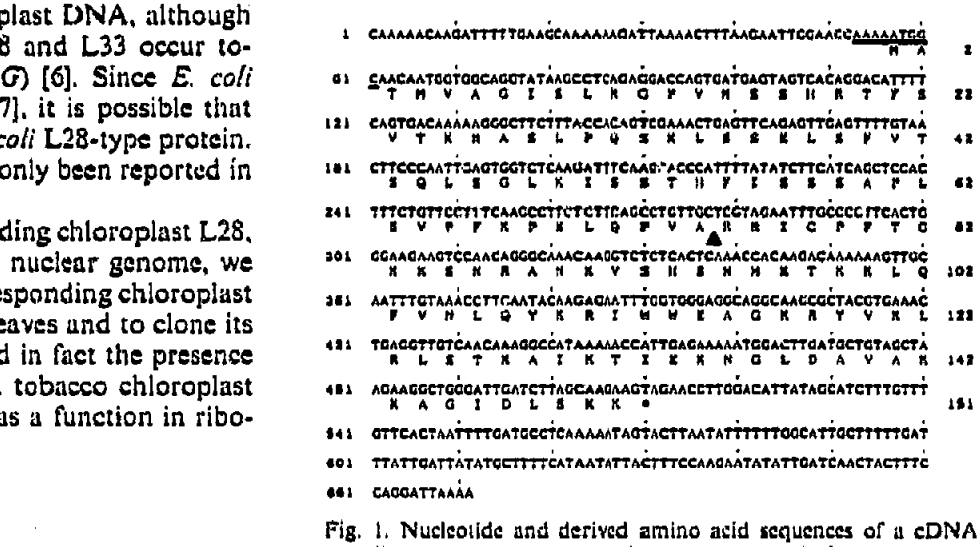

Fig. 1. Nucleotide and derived amino acid sequences of a cDNA encoding tobacco chloroplast ribosomal protein CL28. The triangle indicates the processing site. The asterisk represents the stop codon. The conserved sequence flanking the start codon in dicot plants is underlined.

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          1       10        20        30        40        50        60        70
          |       |         |         |         |         |         |         |
17B       XRIXPFTGKKSNRANKVSHSN
CL28      RRICPFTGKKSNRANKVSHSNHKTKKLQFVNLQYKRIWHEAGKRYVKLRLSTKAIKTIEKNGLDAVAKKAGIDLSKK
          * * ***     * **    **      **    * * *  ** * ** * *    * * * * *        *
EL28      SRVCQVTGKRPVTGNNRSHALNATKRRFLPNLHSHRFWVESEKRFVTLRVSAKGMRVIDKKGIDTVLAELRARGEKY
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Fig. 2. Comparison of the predicted amino acid sequence of tobacco chloroplast ribosomal protein CL28 (CL28) with that of *E. coli* ribosomal protein L28 (EL28). Asterisks indicate identical amino acids. The N-terminal amino acid sequence of the 17B protein is shown above CL28.

2.2. *Cloning and sequencing of the cDNA encoding CL28*

A *N. tabacum* leaf cDNA library was constructed by Dr. R.F. Whittier using a cDNA synthesis kit in λgt10 (Amersham). An oligonucleotide probe (5'-TT(TC)TTICCIGT(AG)AAIGGI(GC)(AT)-IATIC-3') was synthesized using an Applied Biosystems 318A synthesizer based on the amino acid sequence (RIXPFTGKK). Screening of the library was carried out according to the instruction manual from Amersham (cDNA synthesis kit λgt10) and [10]. The hybridization temperature was 48°C. Recombinant λDNA was prepared by a mini-lysate method [11]. The insert was digested with *Eco*RI and subcloned

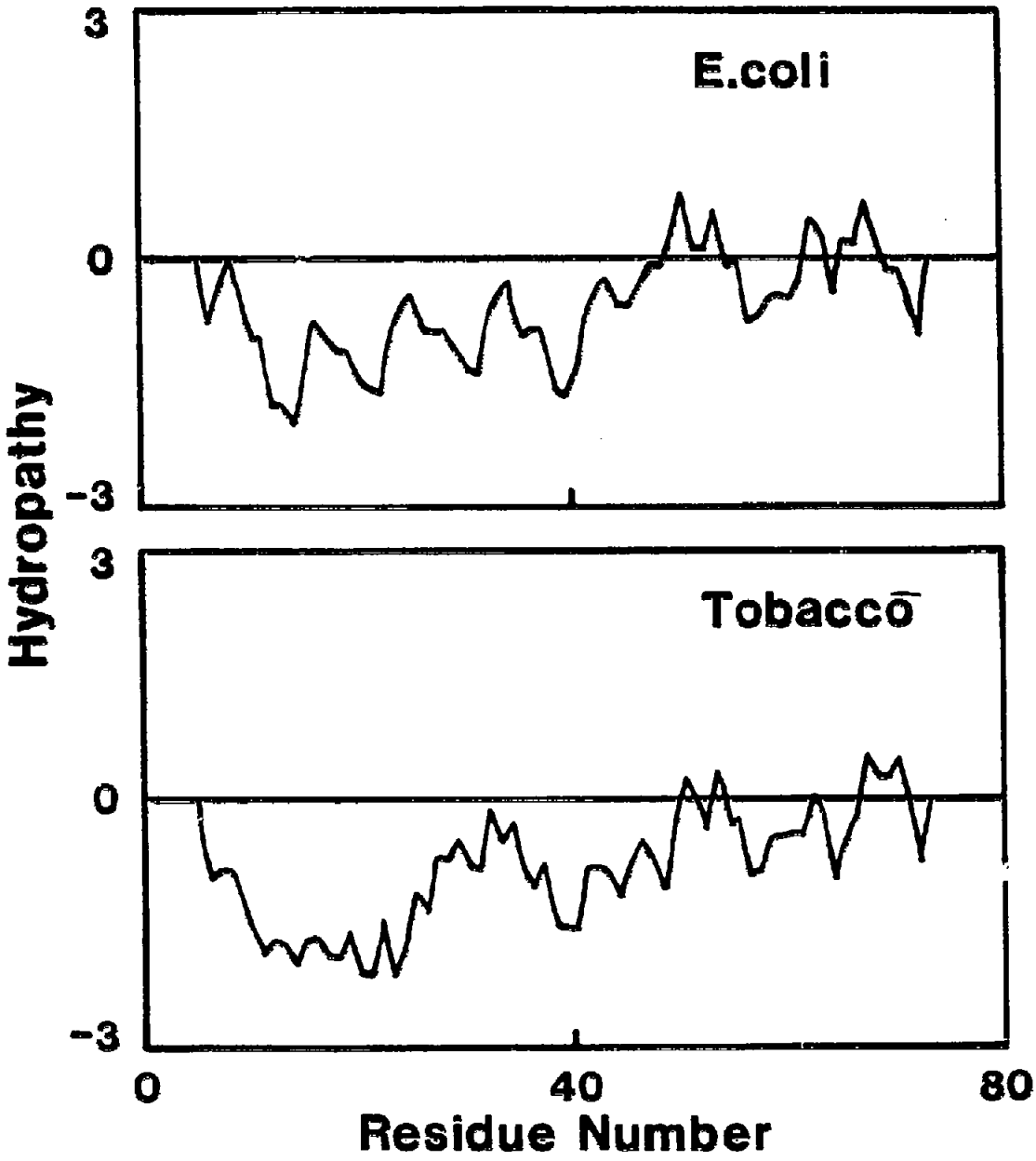

Fig. 3. Hydropathy plots of tobacco CL28 and *E. coli* L28. Hydropathy was calculated with an 11-point window according to Kyte and Doolittle [16].

into the Bluescript plasmid, SK+ (Stratugene). The nucleotide sequence was determined by the dideoxy chain termination method [12] using deaza-sequenase sequencing kits (United States Biochemical).

3. RESULTS AND DISCUSSION

3.1. *Isolation of ribosomal protein CL28*

Reverse-phase chromatography of 50 S ribosomal proteins from tobacco leaves produced at least 12 sharp peaks (details will be published elsewhere). The fourth peak was resolved further into 4 bands by SDS-PAGE. A 15 kDa protein (band 17B) on the membrane was subjected to protein microsequencing. Twenty-one amino acids of the N-terminus were analyzed and 19 of these residues were clearly identified (Fig. 2).

3.2. *Sequence of the CL28 cDNA*

A cDNA clone was isolated using an oligonucleotide, which was synthesized based on the N-terminal amino acid sequence of the 15 kDa protein. This cDNA was sequenced and contains an insert of 671 bp with a reading frame of 151 amino acid residues (Fig. 1). This reading frame contains three possible initiation codons (residues 1, 4 and 15). The first ATG codon occurs in the sequence AAAAATGGC, which matches the consensus sequence flanking functional start codons in dicot plants [13]. This suggests that the first ATG codon is the functional initiation codon. The 21 amino acids in the 15 kDa protein which were determined by amino acid sequencing match the predicted sequence of residues 75–95. Thus, the mature protein is 77 amino acids long. The preceding peptide is probably 74 amino acids in length, of which 38 are hydrophobic (51%) and 9 are basic (12%). This motif is similar to the amino acid composition of other transit peptides [14], suggesting that the preceding sequence is a transit peptide.

The size of the predicted mature protein is the same as that of *E. coli* L28 (77 amino acids). It is a basic protein (12 lysine residues out of 77) and has a calculated molecular weight of 8924. Discrepancies between observed and calculated molecular weights are often found in ribosomal proteins (e.g. [4]). The mature protein has 36% amino acid identity to *E. coli* L28 (Fig. 2) and their hydropathy profiles are similar (Fig. 3). Therefore, we conclude that the chloroplast protein is a homologue of *E.coli* L28 and designate it CL28.

The function of L28 is not clear because mutants which lack L28 are viable in *E. coli* [7]. The primary structure of L28 has only been reported in *E. coli* [8]. The tobacco CL28 sequence is the second report of this protein. Rat and yeast L28s have no homology with *E. coli* L28. Based on the conserved structure between *E. coli* L28 and tobacco CL28, L28 is thought to have a function as yet unclear in ribosomes. In *E. coli* the L28 gene (*rpmB*) is cotranscribed with the L33 gene (*rpmG*) [6]. In land plants the CL33 gene is located in the chloroplast genome [1] while the CL28 gene is in the nucleus. This suggests a chloroplast to nucleus gene relocation during evolution (e.g. [15]).

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