

Purification, primary structure, and homology relationships of a chloroplast ribosomal protein

(amino acid sequence/protein evolution/organelle ribosome/acidic L7/L12 protein/*Spinacia oleracea*)

MARIUS BARTSCH, MAKOTO KIMURA, AND ALAP-RAMAN SUBRAMANIAN*

Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Ihnestr. 63–73, D-1000 Berlin 33, Germany

Communicated by A. M. Pappenheimer, Jr., August 16, 1982

ABSTRACT A chloroplast ribosomal protein that showed immunological homology to *Escherichia coli* ribosomal protein L12 was purified from spinach (*Spinacia oleracea*) leaves and its primary structure was determined by manual micro Edman degradation. The protein is composed of 130 amino acid residues and has M_r 13,576. It shows structural features characteristic of the L12 proteins of eubacterial 70S ribosomes (e.g., identical amino acid residues in about 50% of the sequence) but no apparent homology to the L12-type proteins of eukaryotic cytoplasmic 80S ribosomes. The homology to eubacterial proteins is highest in the COOH-terminal region (70%) and low in the NH₂-terminal region (<20%).

Plant cells contain three types of ribosomes—namely, an 80S in the free cytoplasm, and two distinct 70S types in the plastids (e.g., chloroplast) and in mitochondria (1–3). The organelle ribosomes synthesize a subset of organelle-specific proteins which are encoded in the organelle DNA. The products of synthesis of the chloroplast ribosomes (which includes the large subunit of the abundant leaf protein ribulosebisphosphate carboxylase) contributes up to half the total protein in green leaves (4).

Previous studies have shown that chloroplast ribosomes are similar to bacterial ribosomes in functional properties (3, 5, 6) and in the structure and organization of their RNA components (7, 8). For example, the 16S rRNA of maize chloroplast (8) shows about 70% homology to *Escherichia coli* 16S rRNA; it also carries at the 3' end the conserved oligonucleotide stretch important in prokaryotic initiation. The chloroplast rRNA genes are located (two copies each) in the chloroplast DNA (7).

Like bacterial ribosomes, chloroplast ribosomes contain between 50 and 60 ribosomal proteins (rproteins) (3). The rprotein genes are divided (unlike the rRNA genes) between the nuclear and chloroplast genomes (9), and therefore the coordinate synthesis of chloroplast rproteins depends on mechanisms (not yet elucidated) of intergenomic coregulation. The system of chloroplast rproteins and their genes is important and interesting but little work has been done in characterizing the individual components.

In the present paper we report the isolation, properties, and primary structure of a chloroplast rprotein. In terms of sequence homology, it corresponds to the functionally important and phylogenetically conserved rprotein L12 (10), which is present in *E. coli* ribosomes in four copies (11) and which, as a tetramer, forms a morphologically distinct “stalk” on the 50S subunit (12).

MATERIALS AND METHODS

Preparation of Chloroplast Ribosomes. Because of the need to process large quantities of leaves, we developed a simplified

procedure (based on refs. 13 and 14). Spinach (*Spinacia oleracea*) leaves (purchased at the central market) were cleaned and deveined, homogenized (2 liters of buffer per kg of leaves) in a Waring blender (two 10-sec runs), and filtered through cheesecloth (all operations at 0–4°C). The filtrate was centrifuged (1,200 × g, 15 min) and the pellet was washed once by resuspension and centrifugation. The homogenization and resuspension were done with 0.7 M and 0.4 M sorbitol as osmoticum in buffer A (10 mM Tris-HCl, pH 7.6/50 mM KCl/10 mM Mg acetate/7 mM 2-mercaptoethanol). The washed chloroplast pellet was suspended in lysis buffer (2% Triton X-100 in buffer A) for 30 min and, after clarification (26,000 × g, 30 min), was layered over 1 M sucrose in buffer A (Beckman Ti 45 tubes) and centrifuged (86,000 × g, 17 hr). The greenish ribosomal pellet was dissolved in a small volume of 10% (vol/vol) glycerol in buffer A and the insoluble material was removed (26,000 × g, 15 min). In some experiments the ribosomes were further purified by a second sucrose cushion pelleting. Bacterial contamination of chloroplast preparations was determined by colony assay on L-broth agar plates.

Cytoplasmic 80S Ribosomes. Postchloroplast supernatant from the previous experiment was centrifuged (26,000 × g, 30 min) to remove mitochondria, proplastids, and chloroplast fragments, and the ribosomes were pelleted (120,000 × g, 3 hr). The resulting mixture of 80S and 70S ribosomes was separated by centrifugation in a 10–30% sucrose gradient (in buffer A) in zonal rotor (Beckman type 15, 17,000 rpm, 19 hr).

Two-Dimensional Gel Electrophoresis. rprotein was extracted with 67% acetic acid, dialyzed against 8 M urea/0.05% 2-mercaptoethanol, and electrophoresed by the procedure of Mets and Bogorad (15). For the selective separation of acidic rproteins the procedure of Li and Subramanian (16) was used.

Ouchterlony double-immunodiffusion tests were performed as described for the semimicro method of Stöffler and Wittmann (17).

Protein Purification. Chloroplast ribosomes were treated with 1 M NH₄Cl/ethanol at 0°C and centrifuged as described by Hamel *et al.* (18). The supernatant was concentrated by rotary evaporation (20°C) and ultrafiltration (YM 5 membrane, Amicon) and dialyzed against column buffer (10 mM Tris-HCl, pH 7.6/6 M urea/0.05% 2-mercaptoethanol). It was applied on a 1 × 10 cm column of DEAE-cellulose (Whatman DE52) and eluted with a linear 200-ml gradient of 0–0.2 M NaCl in column buffer. Fractions were analyzed by NaDodSO₄ gel electrophoresis (19), and those that contained pure protein were pooled, dialyzed against 0.05% NH₄OH, and lyophilized.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: rprotein, ribosomal protein; *E. coli* L12, protein L12 of *Escherichia coli* ribosome; chloroplast L12, protein L12 of chloroplast ribosome; MeMor, *N*-methylmorpholine acetate.

* To whom reprint requests should be addressed.

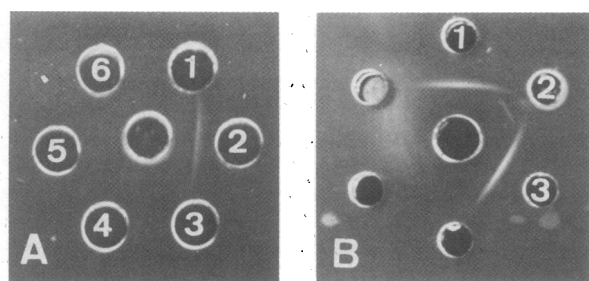


FIG. 1. Immunological homology between a chloroplast rprotein and *E. coli* L7/12. (A) Center well contained 150 μ g of spinach chloroplast rproteins; well 2 contained antiserum to *E. coli* L7/12. Other peripheral wells (which did not give precipitin lines) contained antisera to *E. coli* proteins S1, S6, NS1, and NS2 and *groE* protein. (B) Center well contained L7/12 antiserum and peripheral wells 1 and 3 contained 2 μ g of *E. coli* L7/12; well 2 contained 300 μ g of chloroplast rprotein.

Protein content was determined by the Lowry procedure with bovine serum albumin standard and by amino acid analysis of the acid-hydrolyzed samples. For protein L12 of chloroplast ribosomes (chloroplast L12), the latter gave 92% of the Lowry value.

Protein Sequence Determination Methods. Enzymatic cleavages were performed as follows.

1. *Intact protein.* TPCCK-treated trypsin (Merck) at a ratio of 1:50 in 0.02 M *N*-methylmorpholine acetate (MeMor), pH 8.1; 5.9 mg of protein. Chymotrypsin (Merck), same conditions; 1.0 mg of protein. Pepsin (Serva) at a ratio of 1:50 in 5% formic acid; 1.0 mg of protein. *Staphylococcus aureus* protease (Miles) at a ratio of 1:50 in 0.1 M ammonium acetate (pH 4.0); 0.54 mg of protein. Carboxypeptidase A (Sigma), 20 units in 0.02 M MeMor (pH 8.1), 0, 10, 20, and 40 min; 135 μ g of protein.

2. *Large tryptic peptides.* Thermolysin (Serva) at a ratio of 1:50 in 0.2 M MeMor (pH 8.1); 4 hr for peptides T₁, T₃, and T₁₀. Trypsin at a ratio of 1:10 in 0.2 M MeMor (pH 8.1); 8 hr for T₁. Chymotrypsin and *S. aureus* protease at a ratio of 1:50 in 0.2 M MeMor (pH 8.1); 4 hr for T₃. All digestions were done at 37°C for 4 hr if not stated differently.

Peptides were separated on cellulose acetate thin-layer plates (20). After staining with 0.3% ninhydrin or 0.004% fluram, peptides were eluted with 50% acetic acid. Tryptic digestion produced three large peptides which were separated by gel filtration on Sephadex G-75. Amino acid analysis was performed after hydrolysis for 20 hr in 5.7 M HCl/0.02% 2-mercaptoethanol on a Durrum D500 amino acid analyzer. Peptides containing tryptophan were identified by using Ehrlich's reagent. Cysteine was

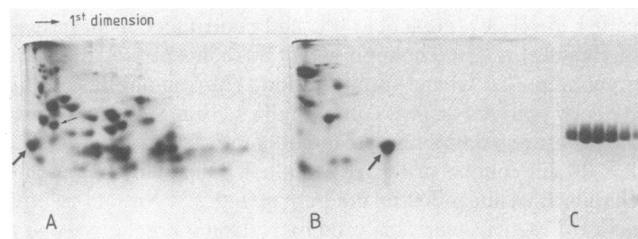


FIG. 2. (A and B) Two-dimensional gel electrophoretic patterns of spinach chloroplast rproteins and homogeneity of purified chloroplast L12. (A) Total rprotein (\approx 100 μ g). (B) Acidic rproteins. Thick arrow shows the spot of chloroplast L12. Thin arrow shows a second protein removed by ethanol treatment. (C) NaDodSO₄ gel electrophoresis of the peak fractions containing chloroplast L12.

Table 1. Amino acid compositions (mol %) of spinach chloroplast L12 and of chloroplast and *E. coli* ribosomes

Amino acid	Chloroplast L12		Ribosome	
	Analysis	Sequence	Chloroplast	<i>E. coli</i> *
Asx	7.6	—	7.1	8.3
Asp	—	6.9	—	—
Asn	—	0.8	—	—
Thr	2.7	3.1	4.7	5.2
Ser	6.6	6.9	4.8	4.4
Glx	16.8	—	11.5	10.1
Glu	—	11.5	—	—
Gln	—	3.1	—	—
Pro	4.5	4.6	5.3	3.7
Gly	6.7	6.1	15.2	8.2
Ala	16.7	16.9	7.7	11.0
Val	8.4	10.0	6.0	9.6
Met	0	0	1.6	2.4
Ile	4.3	4.6	6.1	5.5
Leu	11.1	10.8	7.1	7.4
Tyr	0	0	2.4	1.8
Phe	1.6	1.5	2.8	3.0
His	0	0	1.7	1.9
Lys	10.7	10.0	9.0	9.0
Arg	2.4	2.3	7.1	7.3
Trp	ND	0.8	ND	0.7
Cys	ND	0	ND	0.5

ND, not determined.

* Taken from Spahr (26).

determined after oxidation with performic acid.

Amino acid sequences of peptides and of the NH₂-terminus of the intact protein were determined by the sensitive double-coupling method described by Chang *et al.* (21). The hydrophobic COOH-terminal peptide T₁₂ was subjected to manual sequence analysis after attachment to aminopolystyrene by using carbodiimide (22).

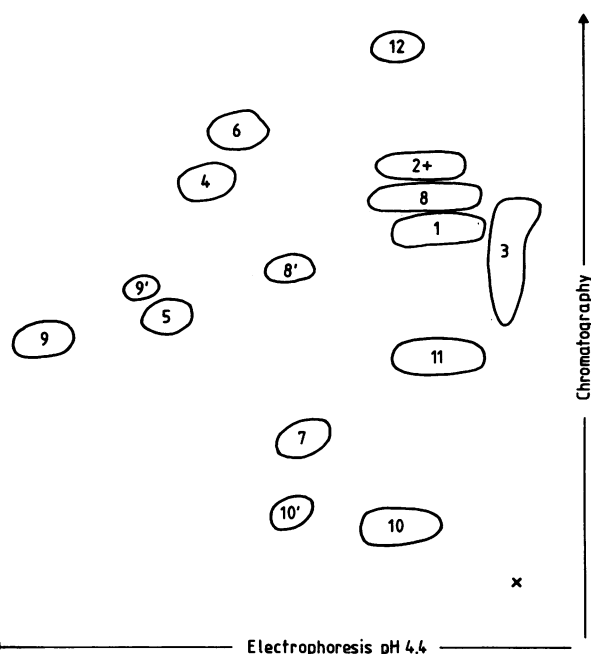


FIG. 3. Thin-layer pattern of the tryptic peptides of spinach chloroplast L12. Peptides are numbered according to their order in the sequence. +, Positive tryptophan color reaction. This pattern may be of use to detect amino acid replacements in chloroplast L12 from other plants.

RESULTS AND DISCUSSION

Chloroplast Ribosomes Contain an Immunological Homolog of *E. coli* L12. At the beginning of our work with chloroplast ribosomes we detected an immunological crossreaction between spinach chloroplast rproteins and an antiserum raised against *E. coli* rproteins L7/L12 (Fig. 1A). The spur formation (Fig. 1B) by the precipitin lines of purified L7/L12 and chloroplast rproteins indicated partial identity of the antigens. *E. coli* proteins L7 and L12 are NH₂-terminal acetylated and non-acetylated forms of the translation product of a single rprotein gene (10). The protein (the two forms are functionally alike) plays an important role in the ribosomal GTP hydrolysis steps required for protein biosynthesis (10).

We obtained 200–1,000 A₂₆₀ units of chloroplast ribosomes (A₂₈₀/A₂₆₀ = 0.53–0.56) per kg of spinach leaves, equal to 12–60 mg based on the conversion factor for *E. coli* ribosomes. The bacterial count in our chloroplast preparations was 5–8 × 10⁵ colony-forming units per 100 g of leaves, which is smaller than observed by others (23) and will cause no detectable bacterial ribosome contamination.

A two-dimensional gel electrophoretic pattern of spinach

chloroplast ribosomes is shown in Fig. 2A. There are about 45 spots in this pattern, about the same as seen in *E. coli* ribosomes separated by the same procedure (24). The chloroplast rproteins covered a wide range in pI and M_r, as did *E. coli* rproteins. Each protein pattern, however, was distinct and unique. The protein pattern of cytoplasmic 80S ribosomes from spinach was very different, as expected (data not shown).

Proteins L7 and L12 have the lowest pI of all *E. coli* rproteins and therefore they migrate the least in the first-dimension gel. The chloroplast rprotein of lowest mobility in Fig. 2A is the protein we purified (indicated by thick arrows) and it corresponds to *E. coli* rprotein L12 (*E. coli* L12). Fig. 2B shows a selective separation of the acidic chloroplast rproteins (16). The most acidic protein spot on this gel—i.e., the one with the greatest migration—is chloroplast L12.

Under the electrophoretic conditions of Fig. 2B, *E. coli* L7 and L12 separate into two distinct spots. Only one spot was observed in the case of chloroplast ribosomes, and therefore it is likely that the chloroplast protein exists in only one form.

Two-dimensional gel patterns of cytoplasmic 80S ribosomes of spinach did not contain a spot in the position of chloroplast



FIG. 4. Alignment of peptides and the complete primary structure of spinach chloroplast L12: T, C, P, and SP, peptides derived from digestions with trypsin, chymotrypsin, pepsin, and *S. aureus* protease, respectively; TT, TC, TTh, and TSP, peptides from large tryptic peptides cleaved with indicated enzymes; arrows, sequences determined by micro Edman degradation.

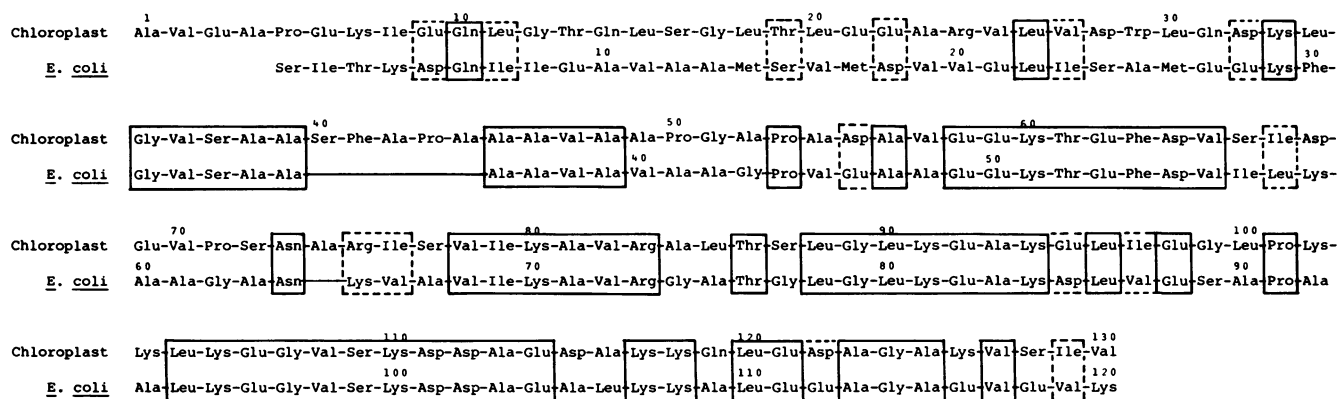


FIG. 5. Homology between chloroplast L12 and *E. coli* L12. Identical residues are enclosed by solid lines. Conservative replacements (Glu/Asp; Lys/Arg; Leu/Ile/Val; and Ser/Thr) are enclosed by broken lines.

L12. Thus, this important protein is apparently not shared between the two classes of ribosomes within the same leaf cells.

Chloroplast L12: Purification and Properties. *E. coli* L7/L12 (18) and, to a lesser extent, a pentameric complex between L7/L12 and L10 (25) are released from ribosomes by ethanol/ NH_4Cl . Two proteins, chloroplast L12 and a protein of higher M_r , which could correspond to *E. coli* L10, were released from chloroplast ribosomes by this procedure. Upon DEAE-cellulose chromatography, chloroplast L12 eluted from the column at 0.07 M NaCl, in homogeneous form as checked by Na-DodSO₄ gel electrophoresis (Fig. 2C) and by two-dimensional gel electrophoresis. The second protein did not bind to the ion exchanger. The yield was 10 mg of purified chloroplast L12 from $2 \times 10^4 A_{260}$ units (1.2 g) of chloroplast ribosomes.

Amino acid compositions of purified chloroplast L12 and of total chloroplast rprotein determined after acid hydrolysis are shown in Table 1. As noted with *E. coli* and other eubacterial L12 proteins (10), alanine was the most abundant amino acid; cysteine, histidine, and tyrosine were absent. Methionine was absent from chloroplast L12 but it is present in L12 proteins of some bacteria.

Comparison of total protein of spinach chloroplast ribosome with that of *E. coli* (Table 1) shows a strikingly greater content

of glycine; the contents of most other amino acids are similar. Glycine contents of chloroplast L12 and *E. coli* L12 are similar.

The yield of L12 protein from our purification procedure corresponded to 1.6 mol of protein per mol of ribosome. Because protein recovery is probably 50% or less, the data suggest that there may be as many as four copies of protein L12 in chloroplast ribosomes, as is the case in *E. coli* (11).

Amino Acid Sequence of Chloroplast L12. The protein sequence was completely determined manually. The NH₂ terminus of chloroplast L12 was degraded nine steps. All 12 tryptic peptides (which covered the complete length of the protein chain) and additional overlapping peptides were separated on thin-layer sheets (Fig. 3) and isolated. They were subjected to as many sequence analysis steps as possible. The four amino acids of the COOH-terminal peptide (T₁₂) were identified after attachment to a solid support. Carboxypeptidase A released these four amino acids in the expected order and molar ratio, confirming their sequence.

From digestions with chymotrypsin, pepsin, and *S. aureus* protease and from digestions of isolated tryptic peptides, the complete sequence with the required overlaps and confirmations was obtained. The complete data are given in Fig. 4.

Amino acid compositions of peptides (Fig. 4) and intact pro-

Chloroplast	A	V	E	A	P	E	K	I	E	Q	L	G	T	Q	L	S	G	L	T	L	E	E	A	R	V	L	V	D	W	L	Q	D	K	L	G	V	S	A	A	S	F	A	P	A	A	A	V	A					
<i>E. coli</i>																																																					
<i>B. subt.</i>																																																					
<i>M. lyso.</i>																																																					
<i>S. gris.</i>																																																					

Chloroplast	A	P	G	A	P	A	D	A	V	E	E	K	T	E	F	D	V	S	I	D	E	V	P	S	N	A	R	I	S	V	I	K	A	V	R	A	L	T	S	L	G	L	K	E	A	K	E	L	I	E					
<i>E. coli</i>																																																							
<i>B. subt.</i>																																																							
<i>M. lyso.</i>																																																							
<i>S. gris.</i>																																																							

	100	110	120	130	Total	Identical																												
Chloroplast	G	L	P	K	K	L	K	E	G	V	S	K	D	D	A	E	D	A	K	K	Q	L	E	D	A	G	A	K	V	S	I	V	130	-
<i>E. coli</i>																																	120	56
<i>B. subt.</i>																																	122	52
<i>M. lyso.</i>																																	118	56
<i>S. gris.</i>																																	125	54

FIG. 6. Amino acid sequences in eubacterial L12 proteins. +, Identical residue. Proteins compared are from spinach chloroplast, *E. coli* (27), *Bacillus subtilis* (28), *Micrococcus lysodeikticus* (29), and *Streptomyces griseus* (30). Two slightly different alignments for *E. coli* L12 are used in Figs. 5 and 6.

tein (Table 1) were in excellent agreement with those derived from the primary structure. No modified amino acids were detected in chloroplast L12.

Characteristics of the Primary Structure. Chloroplast L12 of *S. oleracea* is a single polypeptide chain of 130 amino acid residues with the composition: Asp₉Asn₁Thr₄Ser₉Glu₁₅Gln₄Pro₆Gly₈Ala₂₂Val₁₃Met₀Ile₆Leu₁₄Tyr₀Phe₂His₀Lys₁₃Arg₃Trp₁Cys₀. There are 24 acidic and 16 basic amino acid residues, thus accounting for the protein's acidic character. The $E_{280}^{1\%}$ of the protein calculated from its aromatic amino acid content is 4.1, in reasonable agreement with our observed value of 4.5. The M_r of chloroplast L12 is 13,576 from the amino acid sequence.

The charged amino acids of chloroplast L12 are unevenly distributed: the NH₂-terminal half (positions 1–65) has 12 acidic and only 4 basic residues and thus will be highly acidic; the COOH-terminal half has 12 acidic and 12 basic residues and thus will be neutral. Four of the 6 prolines and all of the aromatic residues (1 tryptophan and 2 phenylalanines) are in the acidic NH₂-terminal half, which also contains 11 alanine residues (positions 38–56).

Comparison of Chloroplast L12 with L12 Proteins of *E. coli* and Other Organisms. The L12 type acidic rprotein is a universal constituent of ribosomes. Primary structure data have allowed a distinction between a eukaryotic 80S type protein (which is also found in archaeobacteria) and a eubacterial 70S type protein (10). Although both these types show some common features—e.g., acidic pI and an alanine-rich region—whether or not the two types have a common ancestry is still an unresolved question (10).

The sequence of chloroplast L12 can be matched with that of the eubacterial 70S type L12 protein but not with that of the eukaryotic 80S type protein. Fig. 5 shows a comparison with *E. coli* L12. Several long stretches in the two proteins show identical amino acid sequences. Overall, 59 of the 120 residues of *E. coli* L12 (i.e., 49%) align with identical residues in chloroplast L12. An additional 14 residues (boxed with broken lines) are conservative replacements. This degree of homology would support a common evolutionary origin. Fig. 6 shows identical amino acid residues of chloroplast L12 compared with the known sequences of L12 from both Gram-negative and Gram-positive bacteria. The degrees of homology are similar. Thus, the comparison does not show a closer relationship of chloroplast L12 to the proteins of either class of bacteria.

The region of residues 66–76 of chloroplast L12 does not show homology to any of the bacterial proteins although the latter are homologous among themselves in this region. The homology between chloroplast L12 and the other L12 proteins is the least in the NH₂-terminal region (residues 1–32) and highest (70%) in the COOH-terminal quarter (residues 98–130). In the case of *E. coli* L12 the NH₂-terminal region is involved in ribosome binding; the COOH-terminal region is believed to be involved in the function of the molecule (10). It is possible that this difference in function caused different degrees of structural conservation during evolution.

Previous studies with antisera raised against *E. coli* and chloroplast ribosomes (31, 32) have shown only a small degree of immunological homology between them. In experiments with antisera against purified *E. coli* rproteins other than L7/12 (in

collaboration with Georg Stöffler), we have also detected only a few weak crossreactions. Further work should answer questions on the generality of rprotein homology between chloroplasts and bacteria and possible dependence of such homology on the compartmental location of chloroplast rprotein genes.

We thank Prof. H. G. Wittmann for his support and interest and Prof. L. Bogorad for comments on the manuscript. We also thank A. Lehmann for solid-phase peptide coupling, K. Ashman for computer homology search, and Dr. G. Stöffler for antisera.

1. Lyttleton, J. W. (1962) *Exp. Cell Res.* **26**, 312–317.
2. Vasconcelos, A. C. L. & Bogorad, L. (1971) *Biochim. Biophys. Acta* **228**, 492–502.
3. Boynton, J. E., Gilham, N. W. & Lambowitz, A. M. (1980) in *Ribosomes*, eds. Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore), pp. 903–950.
4. Ellis, J. R. (1976) in *The Intact Chloroplast*, ed. Barber, J. (Elsevier/North-Holland, Amsterdam), pp. 335–364.
5. Boardman, N. K., Francki, R. I. B. & Wildman, S. G. (1966) *J. Mol. Biol.* **17**, 470–489.
6. Tiboni, O., Di Pasquale, G. & Ciferri, O. (1976) *Plant Sci. Lett.* **6**, 419–429.
7. Bedbrook, J. R., Kolodner, R. & Bogorad, L. (1977) *Cell* **11**, 739–750.
8. Schwarz, Z. & Kössel, H. (1980) *Nature (London)* **283**, 739–742.
9. Bogorad, L., Davidson, J. N. & Hanson, M. R. (1977) in *Nucleic Acids and Protein Synthesis in Plants*, eds. Bogorad, L. & Weil, J. H. (Plenum, New York), pp. 135–154.
10. Matheson, A. T., Möller, W., Amons, R. & Yaguchi, M. (1980) in *Ribosomes*, eds. Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore), pp. 297–332.
11. Subramanian, A. R. (1975) *J. Mol. Biol.* **95**, 1–8.
12. Lake, J. A. (1980) in *Ribosomes*, eds. Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore), pp. 207–236.
13. Reeves, S. R. & Hall, D. O. (1980) *Methods Enzymol.* **69**, 85–94.
14. Walker, D. A. (1980) *Methods Enzymol.* **69**, 94–105.
15. Mets, L. & Bogorad, L. (1974) *Anal. Biochem.* **57**, 200–210.
16. Li, K. & Subramanian, A. R. (1975) *Anal. Biochem.* **64**, 121–129.
17. Stöffler, G. & Wittmann, H. G. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2283–2287.
18. Hamel, E., Koka, M. & Nakamoto, T. (1972) *J. Biol. Chem.* **247**, 805–814.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
20. Wittmann-Liebold, B. & Lehmann, A. (1980) in *Methods in Peptide and Protein Sequence Analysis*, ed. Birr, C. (Elsevier/North-Holland, Amsterdam), pp. 49–72.
21. Chang, J. Y., Brauer, D. & Wittmann-Liebold, B. (1978) *FEBS Lett.* **93**, 205–221.
22. Salnikow, J., Lehmann, A. & Wittmann-Liebold, B. (1981) *Anal. Biochem.* **117**, 433–442.
23. Grivell, L. A. & Groot, G. S. P. (1972) *FEBS Lett.* **25**, 21–24.
24. Subramanian, A. R. (1974) *Eur. J. Biochem.* **45**, 541–546.
25. Pettersson, I. & Liljas, A. (1979) *FEBS Lett.* **98**, 139–144.
26. Spahr, P. F. (1962) *J. Mol. Biol.* **4**, 395–406.
27. Terhorst, C. P., Möller, W., Laursen, R. & Wittmann-Liebold, B. (1972) *FEBS Lett.* **28**, 325–328.
28. Itoh, T. & Wittmann-Liebold, B. (1978) *FEBS Lett.* **96**, 392–394.
29. Itoh, T. (1981) *FEBS Lett.* **127**, 67–70.
30. Itoh, T., Sugiyama, M. & Higo, K. (1982) *Biochim. Biophys. Acta* **701**, 164–172.
31. Gualerzi, C., Janda, H. G., Passow, H. & Stöffler, G. (1974) *J. Biol. Chem.* **249**, 3347–3355.
32. Schneeman, R. & Surzycki, S. (1979) *Mol. Gen. Genet.* **176**, 95–104.